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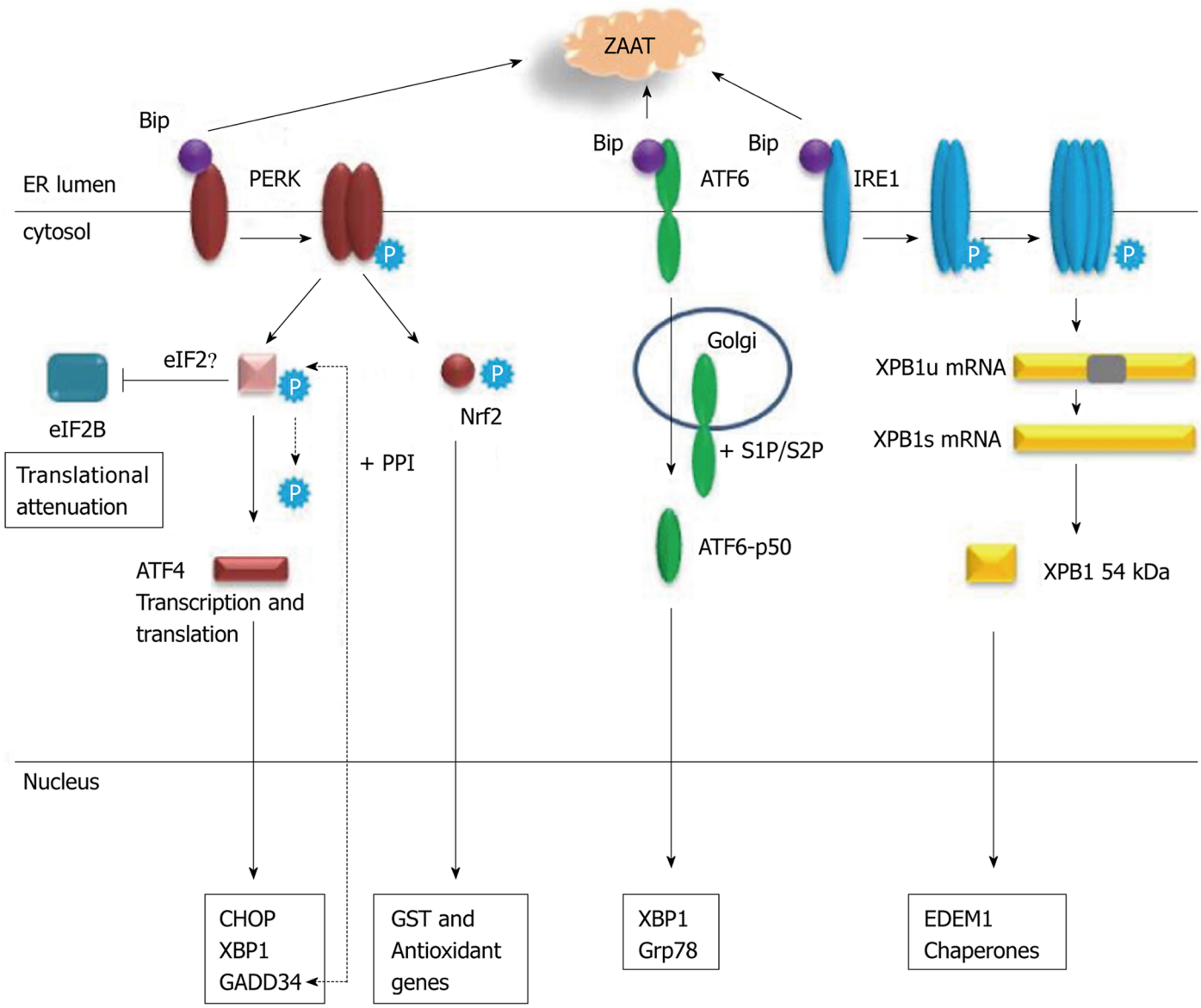

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AIM AND SCOPE

World Journal of Gastrointestinal Pharmacology and Therapeutics (*World J Gastrointest Pharmacol Ther*, *WJGPT*, online ISSN 2150-5349, DOI: 10.4292), is a bimonthly, open-access, peer-reviewed journal supported by an editorial board of 188 experts in gastrointestinal surgery from 36 countries.

The major task of *WJGPT* is to rapidly report the most recent results in basic and clinical research on gastrointestinal pharmacology and therapeutics, including the effects of drugs on the gastrointestinal, pancreatic and hepatobiliary systems, particularly with relevance to clinical practice. *WJGPT* accepts papers on the following aspects related to gastroenterology or hepatology: (1) Clinical pharmacological research articles on specific drugs, concerning with pharmacodynamics, pharmacokinetics, toxicology, clinical trial, drug reactions, drug metabolism and adverse reaction monitoring, *etc*; (2) Research progress of clinical pharmacology; (3) Introduction and evaluation of new drugs; (4) Experiences and problems in applied therapeutics; (5) Research and introductions of methodology in clinical pharmacology; and (6) Guidelines of clinical trial. Specifically, this journal welcome research and review articles associated with both Western medicine and Chinese herbs as well as their combinations in basic and clinical application.

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EDITORIAL

Novel topical therapies for distal colitis

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Abstract

Distal colitis (DC) can be effectively treated with topical 5ASA agents. Suppositories target the rectum while enemas can reliably reach the splenic flexure. Used in combination with oral 5ASAs, the control of the inflammation is even more effective. Unfortunately, resistant DC does occur and can be extremely challenging to manage. In these patients, the use of steroids, immunosuppressants and the anti-tumor necrosis factor α agents are often required. These, however, can be associated with systemic side effects and are not always effective. The investigation of new topical therapeutic agents is thus required as they are rarely associated with significant blood drug levels and side effects are infrequent. Some of the agents that have been proposed for use in resistant distal colitis include butyrate, cyclosporine and nicotine enemas as well as tacrolimus suppositories and tacrolimus, ecabet sodium, arsenic, lidocaine, rebamipide and Ridogrel® enemas. Some of these agents have demonstrated impressive results but the majority of the agents have only been assessed in small open-labelled patient cohorts. Further work is thus required with the investigation of promising agents in the context of randomized double-blinded placebo controlled trials. This review aims to highlight those potentially effective therapies in the management of resistant distal colitis and to promote interest in furthering their investigation.

Key words: Resistant proctitis; Tacrolimus; Treatment; Topical

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INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory condition that is characterised by a life-long course of clinical remissions and exacerbations. Up to 15% of patients suffer a severe attack of their disease that requires hospitalisation at some stage during their life. While the management of these exacerbations have traditionally been dependent upon steroid therapy, a not insignificant proportion of patients fail to respond[1,2] and even in those patients who do respond, 25% of them are dependent on the use of steroids to maintain disease control[3]. Inflammation confined to the rectum occurs in approximately 25% of UC patients and, although this results in distressing symptoms including stool frequency, tenesmus, urgency and bleeding, it can often be managed within the community. Resistant ulcerative proctitis, however, can be extremely challenging to manage. When topical rectal 5ASA and steroid medications fail, oral agents including the 5ASAs, azathioprine (AZA)/6-mercaptopurine (6MP) and steroids may be employed but they do not always help. Infliximab, a medication that binds the proinflammatory cytokine tumor necrosis factor (TNF) α, can also be

effective in these patients with a clinical response in 68% and remission in about a third[4-6]. There are still, however, a significant proportion of UC patients who do not obtain clinical improvement, let alone remission, with these agents. It is for these patients that new and novel therapies require investigation.

TACROLIMUS SUPPOSITORIES AND ENEMAS

Tacrolimus and cyclosporine are classical calcineurin inhibitors that are widely used as immunosuppressive medications with some promising results observed in UC[7,8]. Calcineurin, or protein phosphatase 2B (PP2B), is a ubiquitously expressed cytosolic Ser/Thr protein phosphatase that is highly conserved in eukaryotes[9]. It has the ability to dephosphorylate a broad range of proteins and can regulate interleukin (IL)-2, IL-4 and interferon (IFN) γ[10] expression as well as modulating the activity of transcription factors like NF-κB[11]. Enhanced NF-κB activity is well described in Crohn's disease (CD) and UC and induces the proinflammatory cytokines IL-1β, IL-6 and TNFα expression. It is primarily through the reduction in the levels of these cytokines that clinical remission may be achieved.

The efficacy of oral tacrolimus has been examined in the management of medication resistant CD and UC. Unfortunately, the majority of these studies have been open labelled with only one randomised controlled trial reported in UC[12]. This demonstrated a short-term clinical improvement but without a significant increase in the remission rate, potentially due to low patient numbers. Despite this, there are numerous open labelled studies in both UC and CD that suggest efficacy in the short term and with promising long-term data[13-17]. The evidence would suggest, however, that the blood trough level should be at least 10 ug/L in order to achieve the best efficacy (therapeutic range 5-20 ug/L), but the higher the trough level, the more likely a patient will suffer an adverse effect. These, unfortunately, can be numerous and include hypertension, nausea and diarrhea, hematological abnormalities and renal impairment[13]. Increased rates of skin cancers is also a concern[18] supported by animal studies[19]. Overall assessment of the current published data in inflammatory bowel disease (IBD), therefore, suggests some efficacy but it is unclear if tacrolimus will induce remission and it can be associated with serious adverse effects[20].

Topical tacrolimus has been effective in the treatment of perioral and perineal inflammation in paediatric CD patients with resolution of symptoms in 75%[21]. Work examining topical perianal tacrolimus therapy in adult CD patients also demonstrated clinical efficacy[22] and although tacrolimus is absorbed well transdermally[23], only low trough levels of tacrolimus are detected in the blood[22]. In these preliminary studies, the use of topical tacrolimus was associated with very few side effects. Long-term topical use, as with oral formulations, may be associated with an increased risk of skin cancer formation. Epidemiological evidence, however, would suggest that the risk is low and localised to the tacrolimus-treated sun-exposed skin[24-26].

Two recent studies have started to investigate the efficacy of rectal tacrolimus in resistant distal colitis. In the first, 8 UC patients with inflammation to a maximum of 30 cm from the anus were included. All patients had demonstrated disease resistant to numerous medications both standard and experimental[27]. Following 4 wk of topical tacrolimus, 75% (6/8) of patients achieved clinical remission with oral corticosteroids ceased in the majority of patients. The second study examined the use of topical tacrolimus in 19 patients with resistant distal colitis. Twelve patients received tacrolimus suppositories and 7 tacrolimus enemas. Clinical and histological improvement was observed in 10 of 12 patients treated with tacrolimus suppositories but there was no significant benefit in the majority of patient receiving the tacrolimus enemas[28], potentially due to the lower concentration of tacrolimus at the mucosal surface with the enema preparation. No major side effects were reported in either of the studies and the preparations were well tolerated. As these studies demonstrate encouraging results in a difficult-to-treat patient population, further randomised placebo controlled trials are warranted.

CYCLOSPORINE ENEMAS

The use of intravenous cyclosporine (CsA) has been well described as an effective rescue therapy in up to 80% of acute severe steroid-refractory UC patients[29,30]. The intravenous therapy is then followed by oral CsA for a period of 3 mo while the patients are transitioned onto long-term immunomodulator therapy with AZA/6MP[31]. Despite the use of these agents, however, many patients will relapse and require colectomy within 12 mo[30,32,33]. Concerns over the safety profile of CsA, even at a low oral dose[34] has, however, resulted in a reluctance for some clinicians to use this medication.

The topical use of CsA as an enema in distal UC was first described in 1989[35]. The bioavailability of CsA was not measurable for both the oil and water suspension enemas suggesting that the systemic absorption of CsA following retention enemas is negligible and unlikely to be associated with systemic side effects[36,37]. Two open labeled studies have been reported in the management of treatment-resistant left-sided UC but none has specifically investigated proctitis. In the first, of 10 patients with left-sided UC, 50% responded with 350 mg cyclosporine nightly enemas for 4 wk[37]. The second study observed that 7 of 12 UC patients improved with 250 mg CsA administered daily as a retention enema[38]. The single randomized placebo-controlled trial of CsA enemas in left-sided ulcerative colitis, however, demonstrated that at 4 wk, 40% of patients receiving CsA responded compared with 45% of those who received placebo[39]. This is similar to the findings for tacrolimus enemas and may also be related to the concentration of the medication at the mucosal surface. To date, the use of CsA suppositories has not been investigated.

BUTYRATE ENEMAS

NF-κB activation is important for the activation of inflammation in UC. Butyrate, a short chain fatty acid (SCFA), demonstrates anti-inflammatory effects through the decrease in the translocation of NF-κB into the nucleus of lamina propria macrophages[40]. Inflammation in UC may be due, in part, to a state of energy deficiency of the colonic mucosa secondary to impaired SCFA production, uptake or utilization, while butyrate appears to be the SCFA that is most actively metabolized by the colonic mucosa. The use of butyrate enemas may, therefore, potentially reverse any state of energy deficiency.

Examination of butyrate enemas in patients suffering distal UC demonstrated promising results in the initial open labelled studies. In the first of 2, 6 of 10 patients treated with nightly butyrate enemas responded while 4 obtained clinical remission[41]; in the second, out of 9 patients there was endoscopic and histological improvement in 7 following 2 wk of therapy[42]. In a single-blinded placebo-controlled study, 10 UC patients with distal colitis unresponsive or intolerant to standard therapy received 2 wk of butyrate enemas and then 2 wk of placebo in random order. Following butyrate irrigation, stool frequency decreased while the passage of blood ceased in 9 of 10 patients[43].

Unfortunately, the randomized, double blind, placebo-controlled studies have been less impressive. The first investigated 40 patients with mild to moderate distal colitis but there was no statistical difference detected between the number of patients who improved with butyrate enemas (n =14) compared to placebo (n = 5)[44]. A second study of 38 patients also failed to demonstrate a better clinical outcome with a clinical improvement observed in 37% of butyrate-treated compared to 47% of placebo-treated patients[45]. A third 6-wk double-blind, placebo-controlled trial of SCFA enemas that included sodium butyrate (40 mmol/L), in 91 patients only demonstrated an improvement in 33% of SCFA enemas-treated patients compared to 20% of those who received placebo. Again, these were not significantly different[46]. Thus, although all the studies commented that there was some efficacy with the use of butyrate in a subset of patients and to obtain as response there may be a need for prolonged mucosa contact, butyrate enemas do not appear to be superior to placebo in the treatment of distal colitis.

ECABET SODIUM ENEMAS

Ecabet sodium (ES) is a 12-sulfo dehydroabietic acid monosodium salt derived from an ingredient found in pine resin. It is primarily a non-absorbable protectant and following oral administration, the intestinal absorption rate is only between 3% and 7%[47]. ES appears to bind to proteins in a non-specific manner as the amount bound is almost constant regardless of the ES concentration. ES binding, however, does appear to be pH dependant with greater binding at low pH due to a higher hydrophobicity. Increased binding may also occur through the interaction between the negative charge of the dissociated sulfate moiety of ES at low pH and the positive charge of the proteins[48].

Clinical studies have demonstrated efficacy for ES in the management of gastritis and gastric ulceration due to its affinity for adherence to the gastric mucosa and to fibrinogen located on the gastric ulcer base[47]. This was also observed to be the case for the rat model of colitis [following 9 d ingestion of dextran sodium sulphate (DSS) added to the drinking water]. In this model, rectally administered ES bound at greater rates to damaged mucosa than to the normal intestinal lining[49]. Two open labelled studies have also investigated the utility of ES in the management of distal UC. In the original study, 7 patients demonstrated clinical, endoscopic and histological remissions following twice daily rectal administration for 2 wk[50]. In the second study the findings were less impressive with all six patients responding to ES administration following up to 7 wk of therapy but none achieved remission[51]. High binding of ES to sites of intestinal inflammation was again demonstrated in the first of these studies suggesting that, as for its proposed primary mode of action in gastric inflammation, the clinical benefit of ES in colonic inflammation can be attributable to its role as a coating agent.

Mucin is the major component of the intestinal mucus barrier and is produced by intestinal goblet cells. Goblet cell loss, diminished mucin production and epithelial cell damage accompany the histological changes observed with the active inflammation associated with UC. Loss of goblet cells and attenuation of the mucus protective barrier has also been observed in murine models of colitis, including mice with mutations in the *MUC2* gene that have a sub-optimal mucosal barrier and are more susceptible to the colitis induced by luminal toxins[52]. These animal models develop chronic transmural enterocolitis due to an aberrant immune response against normal enteric pathogens. When animals, however, are maintained in germ-free conditions, colitis does not develop[53-55]. In these animal models, it is the combination of a breakdown in the protective barrier between the colon luminal contents and intestinal mucosa with the presence of an intact colonic flora that promotes intestinal inflammation. As ES has the ability to provide a barrier against the translocation of luminal antigens into the intestinal wall, it is thus not unreasonable that a beneficial effect following its use may be observed in patients with resistant proctitis. Further studies, however, are still required to adequately assess the role, function and efficacy of ES in the topical management of distal colitis.

LIDOCAINE ENEMAS

Lidocaine was first proposed in 1988 as a treatment of DC based on the hypothesis that hyper-reactivity of the autonomic nerves may play a role in the pathogenesis of UC[56]. Efficacy has since been shown to reduce the level of acute inflammation in the trinitrobenzene sulfonic acid (TNBS) and DSS rat models of colitis[57,58]. The initial open-labelled study into UC investigated the use of 2% lidocaine gel (400 mg twice daily) and included 28 patients

with proctitis, all of who responded clinically within 3-12 wk. The cohort also included 49 patients with DC and of these, 41 responded following 6-34 wk of therapy. Despite these impressive results, however, no further studies have been published.

EPIDERMAL GROWTH FACTOR ENEMAS

Epidermal growth factor (EGF) is a 1207-amino-acid precursor that is found in the gastric juices (500 ng per liter)[59]. As it can stimulate healing[60], it has warranted investigation with preliminary human studies suggesting that the topical use of EGF can enhance skin wound healing[61] while systemic EGF can be beneficial in the management of necrotizing enterocolitis[62]. In the proximal gastrointestinal tract, however, EGF is cleaved to a less active form and under physiological conditions very little luminal EGF ever reaches the colon. Circulating levels of EGF are also low and not readily available to the gastrointestinal mucosa.

The use of EGF enemas (5 mg in 100 mL) in the management of left-sided UC was assessed in a randomized, double-blind placebo-controlled trial in 24 patients. After 2 wk of therapy, all patients who received EGF had improved with 10 of 12 (83%) in remission compared with 1 of 12 in the control group (8%, $P < 0.001$). The endoscopic and histological scores were all significantly better in the EGF than placebo group[63]. Unfortunately, despite these impressive results no further investigations into the use of EGF in distal colitis have been undertaken or have not yet been published.

REBAMIPIDE ENEMAS

Rebamipide [2-(4-chlorobenzoylamino)-3-[2-(1H)-quinolinon-4-yl]-propionic acid] is able to stimulate the production of endogenous prostaglandins and accelerate the healing process[64]. It also reduces the intestinal inflammation in both the TNBS and DSS rat models of colitis[65,66]. The first open-labelled study investigating its use included 11 patients with steroid resistant/dependant proctitis or DC[67]. Histological improvement and clinical remission in 9 patients was demonstrated after 12 wk of twice daily administration of 150 mg rebamipide in 1.5% carboxymethylcellulose at pH 6.34. A further open-labelled study demonstrated clinical remission in 5 of 16 patients while another 2 demonstrated a marked improvement after 4 wk of therapy[68]. The final open-labelled study treated 20 patients for 3 wk with 11 achieving clinical remission and 16 responding endoscopically[69]. As yet, however, no randomized double-blind, placebo-controlled studies have been undertaken.

NICOTINE ENEMAS

As UC is largely a disease of non-smokers, the use of nicotine in its management has been investigated. It has several modes of action that could potentially reduce intestinal inflammation including effects on the gut motility[70] and immune function[71]. The open labelled use of a nightly enema containing 6 mg of nicotine for 4 wk was examined in 17 UC patients. All were non-smokers and 16 of 17 improved their St Mark's score, stool frequency and urgency improved in 12 patients and the endoscopic and histological scores improved in 10[72]. The only randomized placebo-controlled study that investigated the use of 6 mg nicotine enemas for 6 wk in 104 patients with active UC, however, demonstrated no significant benefit with nicotine over placebo enemas with clinical remission achieved in 27% patients on active treatment and 33% on placebo[73].

ARSENIC ENEMAS

The use of arsenic suppositories for the management of resistant proctitis was first described over 30 years ago[74] but the mechanism of action remains unknown. However, there has only been a single small open labeled study that investigated the use of Acetarsol® suppositories twice a day for 4 wk in 10 patients. These suppositories contain 68 mg of 3-acetamido-4-hydroxyphenylarsonic acid which is organic arsenic. In 9 of these patients, the symptoms and endoscopic signs of proctitis resolved within 2 wk. Despite the promising findings of efficacy, in 6 patients the arsenic was absorbed systemically with the total inorganic arsenic blood level considered to be in the hazardous range[75]. Unfortunately, despite anecdotal reports of efficacy, no further studies have been published on the use of this agent in distal UC.

THROMBOXANE ENEMAS

Thromboxanes are produced in excess in the inflamed intestinal mucosa of IBD patients and in isolated intestinal cells and peripheral blood mononuclear cells in patients with CD. Inhibitors of thromboxane synthesis have also been shown to reduce the release of TNFα by human macrophages. The open labeled use of the thromboxane synthase inhibitor and receptor antagonist, Ridogrel®, has been investigated in 11 patients as an enema in left-sided UC. Mucosal thromboxane levels were reduced in all patients but the level of the anti-inflammatory mediators IL-6 and TNFα were unchanged. Five patients responded clinically to the treatment but this was not always associated with a decrease in the endoscopic or histological scores of inflammation[76]. This preliminary study may suggest some efficacy to this therapy but as yet no further studies have been undertaken.

CONCLUSION

When topical 5ASA and steroid medications fail, distal ulcerative colitis and proctitis can be extremely challenging to manage. Oral agents and anti-TNFα therapy may be employed but they do not always help. The use of oral medications is also frequently associated with systemic

side effects while the use of topical agents is rarely associated with significant systemic drug levels. Unfortunately, despite there being a number of potentially useful topical therapeutic agents reported in the literature, medications like tacrolimus suppositories and tacrolimus, ecabet sodium, arsenic, lidocaine, rebamipide and Ridogrel® enemas have only demonstrated clinical efficacy in open-labelled studies. In those novel agents that have undergone randomised studies, butyrate, cyclosporine and nicotine enemas did not demonstrate efficacy above that observed for placebo, while, despite impressive evidence for epidermal growth factor enemas, there has only been a single small study. It does appear, however, that the mucosal medication concentration and/or contact time may be important for these agents to work suggesting that perhaps enemas are not the best method of administration and that suppositories could be more appropriate. It is, however, more than obvious that further investigation is required before any of these agents can be considered as routine in the management of resistant ulcerative proctitis and distal colitis.

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EDITORIAL

Z α-1 antitrypsin deficiency and the endoplasmic reticulum stress response

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Abstract

The serine proteinase inhibitor α-1 antitrypsin (AAT) is produced principally by the liver at the rate of 2 g/d. It is secreted into the circulation and provides an antiprotease protective screen throughout the body but most importantly in the lung, where it can neutralise the activity of the serine protease neutrophil elastase. Mutations leading to deficiency in AAT are associated with liver and lung disease. The most notable is the Z AAT mutation, which encodes a misfolded variant of the AAT protein in which the glutamic acid at position 342 is replaced by a lysine. More than 95% of all individuals with AAT deficiency carry at least one Z allele. ZAAT protein is not secreted effectively and accumulates intracellularly in the endoplasmic reticulum (ER) of hepatocytes and other AAT-producing cells. This results in a loss of function associated with decreased circulating and intrapulmonary levels of AAT. However, the misfolded protein acquires a toxic gain of function that impacts on the ER. A major function of the ER is to ensure correct protein folding. ZAAT interferes with this function and promotes ER stress responses and inflammation. Here the signalling pathways activated during ER stress in response to accumulation of ZAAT are described and therapeutic strategies that can potentially relieve ER stress are discussed.

Key words: α-1 antitrypsin; Unfolded protein response; Endoplasmic reticulum stress; Apoptosis; Autophagy; NFκB

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INTRODUCTION

Conformational disorders are characterised by protein misfolding and are dependent on the accumulation or localisation of specific misfolded proteins to various cellular organelles. These disorders are caused by inherited or acquired modifications in protein folding culminating in accumulation of aberrant protein conformers and include metabolic disorders such as diabetes, the neurological disorders Alzheimer's and Huntington's diseases as well as a number of pulmonary disorders such as cystic fibrosis and α-1 antichymotrypsin deficiency. α-1 antitrypsin (AAT) deficiency is a genetic conformational disease associated with accumulation of misfolded AAT in the endoplasmic reticulum (ER) of hepatocytes and other AAT-producing cells[1]. It predisposes sufferers to early onset emphysema or liver disease. It is the only known cause of genetic emphysema and is the most common genetic cause of liver disease in children.

α-1 ANTITRYPSIN DEFICIENCY

AAT deficiency is a hereditary disorder associated with mutations in the *SERPINA1* gene that can affect the liver and the lungs. The most common disease-causing mutation is a single nucleotide polymorphism encoding a glutamic acid to lysine substitution at position 342 of the mature protein (Glu342Lys) that leads to misfolding and intracellular polymerisation of AAT. This "Z" mutation, as it is called, modifies the protein's reactive centre loop enabling it to interact with another ZAAT molecule in a process that can continue until large polymers form[2,3]. The normal AAT protein is referred to MAAT; it and all other conformers are named based on their electrophoretic mobility in isoelectric focusing gels and in some cases on the geographical location where they were first identified e.g. M_{Malton}. MAAT is a serine proteinase inhibitor with activity against neutrophil elastase (NE), a powerful neutrophil-derived protease capable of degrading a wide range of soluble proteins and most connective tissue components of the lung. Although up to 100 different AAT alleles have been identified it is the Z allele that is predominantly associated with disease.

In the 1970s the largest unbiased nationwide AAT deficiency screen of newborns was carried out by Laurell and Sveger in Sweden[4]. Of 200 000 individuals studied 127 were identified as being homozygous for the Z allele and have been studied since. Notwithstanding Sveger's study, globally ZAAT deficiency is an under-diagnosed condition; many being diagnosed as non-responsive asthmatics or chronic obstructive pulmonary disease (COPD) sufferers. Since 1997 the World Health Organization together with the American Thoracic Society and European Respiratory Society have advocated targeted detection programmes for AAT deficiency in all COPD, non-responsive asthma and cryptogenic liver disease patients and also for first degree relatives of AAT deficient individuals[5]. In Ireland emerging data from the national targeted detection programme, the only government funded AAT deficiency targeted detection programme, indicates that AAT deficiency is twice as prevalent as previously estimated with one of the highest frequencies in Europe. These findings support the concept that AAT deficiency is not a rare disease but a disease that is rarely diagnosed.

Pathophysiology

Although ZAAT deficiency is associated with lung and liver manifestations, both organs are rarely affected in the same individual. AAT deficiency is the most common genetic diagnosis in children undergoing liver transplantation[6,7]. It is also an important cause of hepatocellular carcinoma in adults[8]. Even so there is a marked heterogeneity in the liver disease phenotype. The histological hallmark of the disease is the presence of ZAAT-containing globules in some but not all hepatocytes. These glycoprotein-containing globules stain positive with periodic acid-Schiff (PAS) after treatment with diastase[9]. Transgenic ZAAT mice (PiZ mice) can develop hepatic inflammation and carcinomas that are associated with evidence of these characteristic intra-hepatocytic globules[10,11]. Although relatively little is known about the pathogenesis of hepatocellular carcinoma in ZAAT deficiency, based on their observations in ZAAT mouse livers and studies on biopsies from ZZ homozygous individuals Perlmutter *et al*[12] have proposed a model whereby increased hepatocellular proliferation occurs in resting globule-devoid hepatocytes in response to an unidentified "*trans*" signal from adjacent globule-containing cells. It is these globule-devoid cells that proliferate and in which adenomas and later carcinomas develop[13,14].

In contrast to the liver disease which is believed to arise due to a toxic gain of function resulting from intracellular accumulation of ZAAT, the lung manifestations occur due to a loss of function. The liver is the body's major source of AAT. Decreased AAT secretion from the liver, decreases circulating AAT levels and markedly diminishes the lungs' anti-NE protective defences. This can lead to a number of deleterious events. In addition to causing direct damage to airway epithelial cells, unchecked NE activity can promote goblet cell hyperplasia and enhance mucus secretion. Impaired mucociliary clearance, cleavage of complement, immunoglobulins and cell surface receptors occurring as a direct result of too much NE on the respiratory epithelial surface also has important consequences for innate immunity[15]. Efficient neutrophil killing of microorganisms is also impaired. Clinically, ZAAT-related lung disease is associated with aggressive emphysema in the 4th and 5th decades which is aggravated by cigarette smoking[16].

Modifiers of liver disease

The variable clinical presentation among AAT deficient individuals suggests that genetic and environmental disease modifiers make an important contribution to disease severity and outcome. In Sveger's study only 8%-10% of ZZ homozygotes developed clinically significant liver disease[17]. A study by Wu *et al*[18] demonstrated that there is a delay in intracellular disposal of ZAAT after gene transfer into fibroblast cell lines from ZZ homozygotes with liver disease compared to cell lines from homozygotes without liver disease. This suggests that differences exist in the quality control recognition and/or disposal mechanisms for misfolded proteins in cells of ZZ liver disease sufferers. ER mannosidase I (ERManI) is a quality control factor that plays a key role in the sorting and targeting of misfolded glycoproteins for proteosomal degradation. ZAAT is a misfolded asparagine-linked secretory glycoprotein and as such, the removal of mannose units from its *N*-linked glycans represents a crucial early event in its disposal. ERManI plays a stochastic and rate limiting role in distinguishing and targeting ZAAT for degradation. Pan *et al*[19] have identified a G/A single nucleotide polymorphism (SNP) at position 4567 of ERManI that mediates translational suppression of ERManI and have proposed that the "A" SNP can accelerate the onset of end stage liver disease in AAT deficiency. They showed that substitution of "A" for "G" disrupts a potential microRNA (miRNA) binding site for miR-205 in ERManI, and suggest that this may facilitate negative regulation by other miRNAs and concomitant translational repression of ERManI. Another potential gene modifier associated with ZAAT-related liver disease is regulator of G signaling 16 (RGS16) which is up regulated in the liver of ZAAT-deficient individuals[20]. Interestingly the degree

of up regulation of RGS16 *in vivo* correlates with hepatic levels of insoluble ZAAT.

ENDOPLASMIC RETICULUM STRESS

A major area of interest in the biology of ZAAT deficiency is ER stress. The ER is an organelle which extends from the nuclear membrane. It is the site of translation, folding, modification and transport of membrane and secreted proteins and consists of an extensive membranous network of tubes and cisternae. Correct folding of newly made proteins is made possible by several ER-resident chaperones, foldases and enzymes including, for example, Hsc proteins, calnexin, calreticulin and protein disulfide isomerases. Properly folded proteins are transported from the ER to the Golgi apparatus *via* COPII vesicles. Incorrectly folded proteins become complexed with Bip/Grp78 and are targeted to the Sec61/Derlin/p97 VCP channel located in the ER membrane for extrusion into the cytosol and degradation by proteosomal and non-proteosomal mechanisms[21]. Intracellular conditions such as increased temperature, high salt concentrations, unchecked protease activity and the presence of other unfolded proteins can lead to intracellular protein damage. Together with aberrant oxidative, glycation, nitrosylation or deamination events, these conditions promote protein damage and possible misfolding in the ER. Chaperone proteins facilitate the recognition of these incorrectly folded proteins. Misfolded ZAAT protein accumulation in the ER of hepatocytes and other AAT-expressing cells is a hallmark of ZAAT deficiency[6,22,23].

Perturbation of ER homeostasis can induce ER stress and a number of discrete signalling pathways can be activated as a result[1]. These include the unfolded protein response (UPR) which is a conserved signalling pathway that measures unfolded protein levels in the ER and adjusts the production of ER chaperones, foldases and degradation factors appropriately to keep levels of misfolded proteins in the ER lumen acceptably low. ER stress can also lead to apoptotic cell death mediated by the activation of specific caspases. A third signalling pathway activated in response to ER stress in the ER overload pathway (EOR). This is characterised by activation of the transcription factor NFκB which regulates proinflammatory gene expression. Much is now known regarding the effect of ZAAT expression on ER stress responses from studies done with cell culture models, human monocytes and airway epithelial cells, and human and animal liver biopsies[6,20, 22-30].

Endoplasmic reticulum overload response

The transcription factor NFκB is activated by a range of diverse stimuli. Many of these engage cell surface receptors and initiate intracellular signal transduction pathways that converge at IκB kinase (IKK). IKK can also be activated in response to accumulation of misfolded proteins in the ER. This enzyme complex phosphorylates the NFκB inhibitory proteins IκB-α, -β and -γ on key serine residues, leading to their ubiquitination and ultimate recognition and degradation by the proteasome. After removal of IκB protein, NFκB is free to translocate to the nucleus due the exposure of its nuclear localisation sequences, where it binds to NFκB recognition elements in the promoter of target genes and promotes their transcription.

We and others have investigated NFκB activation in the context of ER accumulation of misfolded ZAAT in Chinese hamster ovary (CHO) and 16HBE14o- human bronchial epithelial cell lines and in human monocytes[27,30]. In all cell types studied expression of ZAAT is associated with activation of NFκB. In liver cells in cell culture, animal models of ZAAT deficiency and also *in vivo* in liver biopsies from ZZ homogygous individuals, NFκB activation has also been detected[26,29]. In liver cells it is feasible that activation of NFκB in response to accumulation of ZAAT could mediate inflammation and neutrophil infiltration *via* up regulation of interleukin-8. Given that NFκB is involved in inflammation-associated carcinogenesis, EOR is likely to have important role in the pathogenesis of hepatocellular carcinoma in ZAAT-deficient individuals[31].

Apoptosis

Although NFκB is largely cytoprotective, when the balance between the misfolded protein load in the ER and the ability to correct ER homeostasis cannot be restored, cell death may occur *via* apoptosis. This is the second ER stress-induced response. Apoptosis induced by ER stress differs from conventional apoptosis by the involvement of an ER-resident caspase, caspase-4[32]. Two other mechanisms of ER stress-induced apoptosis are associated with the UPR (see below) and involve PERK-CHOP-GADD34 and IRE1-TRAF2-JNK.

We evaluated ER stress-induced apoptosis in HEK293 cells using the ER stress inducer thapsigargin and MAAT and ZAAT transgenes[28]. Our studies showed that, similar to thapsigargin treatment, expression of ZAAT, but not MAAT, induced cleavage of both procaspase-4 and downstream procaspase-7. However the role of caspase-4 in ER stress-induced apoptosis appears to be stimulus- and cell type- specific. In order to evaluate its role in ZAAT-induced cell death we performed gene knock-down studies using caspase-4 or GAPDH siRNAs. The caspase-4 siRNA resulted in greater than 70% inhibition of caspase-4 protein production. However, surprisingly this did not promote cell survival in ZAAT expressing cells, nor did it inhibit ZAAT-induced caspase-7 activation. We concluded that caspase-4 is not at the apex of the ZAAT/ER-induced apoptotic signalling pathway, nor is it essential for ER-mediated apoptosis in HEK293 cells and it is unlikely to represent a useful target for interfering with ER stress-induced apoptosis by ZAAT.

Using TUNEL staining, terminal apoptosis could be detected in HEK293 cells expressing ZAAT compared to mock-transfected cells[28]. In contrast to these *in vitro* studies, increased apoptosis has not been detected histologically or by TUNEL staining in the livers of ZAAT mice. This may be related to the robust regenerative and cell survival properties of hepatocytes and emphasizes once again the highly specific stimulus- and cell type-specific nature of ER stress responses. The mechanism by which the caspase pathway is inhibited in globule-containing hepatocytes is not known but it could be related to the known antiapoptotic effects of MAAT and ZAAT[30,33] which, in the case of MAAT, are

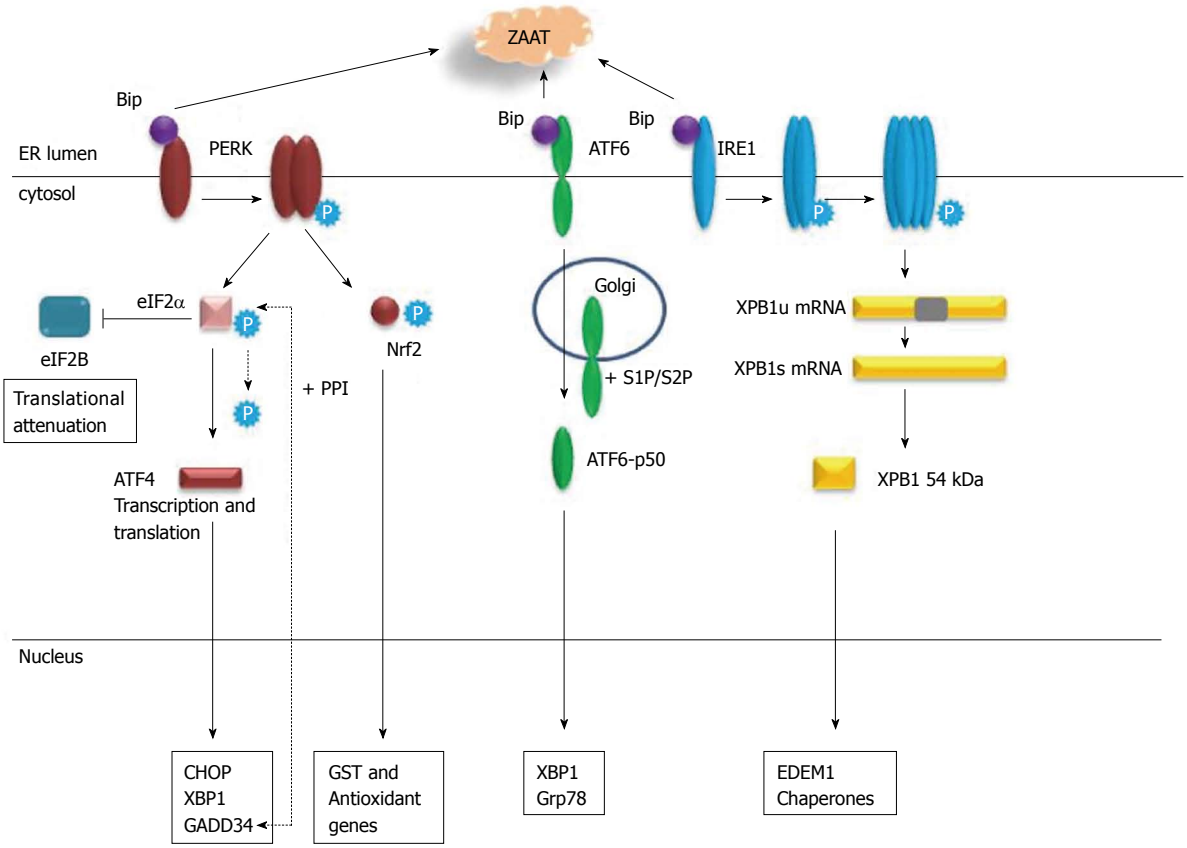

Figure 1 Unfolded protein response. Accumulation of misfolded proteins in the endoplasmic reticulum lumen (e.g. Z α-1 antitrypsin) titrates Bip/Grp78 away from PERK, ATF6 and IRE1. PERK dimerises, becomes phosphorylated and inactivates eIF2α by phosphorylation which blocks translation *via* eIF2B. ATF4 gene and protein expression is induced leading to ATF4-regulated gene expression, including GADD34 which stimulates protein phosphatase-1 (PP1) and triggers dephosphorylation of eIF2α. Nrf2 is phosphorylated and activated by PERK, translocates to the nucleus and induces antioxidant gene expression. ATF6 is transported to the Golgi and cleaved by the proteases S1P and S2P to generate the active ATF6p50 transcription factor which can activate expression of XBP1 and chaperone genes. IRE1 is phosphorylated, assembles into multimers and *via* its RNAse activity uses unspliced XBP1 (uXBP1) mRNA as a substrate to generate spliced XBP1 (XBP1s) mRNA which encodes a transcription factor that regulates expression of EDEM1 and chaperones.

related to direct binding and inhibition of caspase-3. How ZAAT might gain access to caspase-3 in liver cells and in which cellular compartment this may occur are not known.

Unfolded protein response

The third important pathway activated by ER stress is the unfolded protein response[21,34]. This is an integrated signalling network activated by ER stress. UPR governs repression of translation and results in the synthesis of molecular chaperones, degradation factors and ER-resident enzymes, which co-operate to restore correct protein folding in the ER. If this response fails, UPR activation may lead to induction of ER-associated degradation (ERAD) to effectively remove terminally misfolded proteins.

Figure 1 summarises the major events that occur during UPR. Signalling involves three key ER membrane-localised proteins PERK, ATF6 and IRE1 which are normally maintained in an inactive state by association with Bip/Grp78. In the presence of misfolded proteins in the ER lumen, Bip is titrated away enabling oligomerization and activation of each of these factors. Activation of PERK results in phosphorylation and inactivation of the eukaryotic initiation factor eIF2α which interferes with global protein synthesis *via* inhibiting eIF2B. Paradoxically this event actually enhances transcription and translation of the ATF4 transcription factor[35]. ATF4 induces expression of the transcription factor CHOP (also called GADD153) which can trigger the dephosphorylation of eIF2α *via* induction of GADD34 which in turn stimulates protein phosphatase-1 (PP1)[36]. Prolonged CHOP activation can also lead to apoptosis characterised by a decrease in Bcl-2 protein and translocation of Bax from the cytosol to the mitochondria[37].

PERK can also directly phosphorylate Nrf2[38], a transcription factor that together with Maf binds to antioxidant response elements in the promoters of many antioxidative genes, and promotes their transcription[39]. Nrf2 also has a role in regulating the proteasome system that degrades damaged and misfolded proteins, in particular the proteosomal subunit PSMB6[40]. Impaired Nrf2 signaling can significantly impair proteosomal activity and heighten ER stress responses in the airways. Malhotra *et al*[40] suggest that pharmacological approaches which augment Nrf2 activity may up-regulate antioxidant defenses, relieve ER stress, and could be important for treatment of COPD. How this might affect the liver manifestations of ER stress in the context of intracellular accumulation of ZAAT remains unclear.

Another cascade activated in the UPR involves ATF6 (Figure 1). Full length ATF6p90 is transported to the

Golgi apparatus where it is cleaved by two proteases S1P and S2P[41] to generate a basic leucine zipper transcription factor ATF6p50 that migrates to the nucleus and activates transcription of genes under the control of ER stress response elements (ERSE) e.g. Grp78, XBP-1.

IRE1 is an endoribonuclease that becomes activated by phosphorylation when it forms a dimer. Following its subsequent assembly into multimers IRE1 acquires RNAse activity and acts on XBP1 mRNA as a substrate[42]. The resulting alternatively spliced transcript encodes a transcriptionally active 54kDa XBP1 protein which regulates the transcriptional induction of important factors involved in degradation of misfolded proteins e.g. ER degradation-enhancing α-mannosidase-like protein (EDEM) 1. Mulimerisation of IRE1 can also lead to JNK activation *via* TRAF2 and ASK1 and potentially promote apoptosis[34].

Although we know that all three arms of the UPR can be activated in response to overexpression of ZAAT in CHO, HEK293, HepG2 and 16HBE14o- cells[27-30] and basally in human peripheral blood monocytes isolated from ZZ homozygous individuals[23], these pathways do not appear to be activated in inducible models of ZAAT deficiency liver disease or in liver cells *in vivo*. Indeed a number of studies have failed to detect activation of the UPR in cell culture and animal liver models of ZAAT deficiency[20,26] and it has been speculated that the absence of UPR signalling permits the survival of cells that have accumulated high levels of ZAAT i.e. globule-containing hepatocytes.

Recently it has emerged that ZAAT deficiency is also associated with aberrant immune cell function[23]. Peripheral blood monocytes express AAT. Carroll *et al*[23] have generated evidence for UPR activation in monocytes from individuals with ZAAT deficiency and link this phenomenon to an altered inflammatory response. Specifically ATF4, XBP-1 and a subset of genes involved in the UPR are all increased in monocytes from ZZ compared to MM individuals. Expression of these genes could be induced in MM monocytes by treatment with the ER stress inducer thapsigargin, linking the observed ZZ monocyte changes to ER stress. Confocal microscopy demonstrated that not only is ZAAT retained in the ER in ZZ monocytes but that GRP78 is also increased even in resting cells. These altered gene expression patterns contribute to enhanced basal and agonist-induced cytokine production by ZZ monocytes and activation, again, of the NFκB pathway. Thus our current understanding of the mechanisms regulating ZAAT-related lung and liver disease should now be expanded to include of a role for exaggerated inflammatory responses by circulating blood cells.

REMOVAL OF MISFOLDED α-1 ANTITRYPSIN

There are at least two pathways for degradation of ZAAT that accumulates in the ER, the proteosomal and autophagic degradative pathways. Soluble ZAAT is degraded by the proteasome whilst polymerized ZAAT is degraded by autophagy[9].

Endoplasmic reticulum associated degradation

ERAD is the process which monitors the production of mutant glycosylated secretory proteins and directs them to the ubiquitin-proteasome system to be degraded[43]. The ER luminal and transmembrane proteins EDEM1, EDEM2 and EDEM 3 extract misfolded AAT from ER chaperones and facilitate enzymatic mannose trimming of its carbohydrate side-chain by ERManI[9]. The misfolded proteins are retro-translocated to the cytosol, modified with polyubiquitin and degraded by the proteasome. Up to 30% of all newly synthesised proteins undergo degradation by this process within minutes of formation most likely because they are products of unsuccessful folding or failures of multimer assembly. When the level of abnormal proteins accumulating surpasses the ability of a cell to degrade and dispose of them, ER accumulation and ER stress ensue. This is classically seen in ZAAT deficiency where terminally misfolded ZAAT proteins and unassembled complexes are eliminated by ERAD.

Autophagy

The proteosomal pathway does not fully account for disposal of all ZAAT. In addition to ERAD ZAAT can also be degraded by autophagy[25], a cellular mechanism for the degradation of cytoplasmic constituents within lysosomes. Autophagy is the process by which cytoplasmic constituents and intracellular organelles are sequestered within double membraned vesicles called autophagosomes that fuse with lysosomes to form autophagolysosomes, inside which their constituents are degraded. Three autophagy gene products ATG5, ATG6 and ATG16 are particularly important for this process and are necessary for the piece-by-piece digestion of aggregated ZAAT[44]. The presence of aggregated rather than soluble ZAAT appears to specifically activate autophagy. Expansion and dilatation of the ER and increased numbers of autophagosomes, two morphological changes characteristic of autophagy, are evident in fibroblasts overexpressing ZAAT, in ZAAT mouse liver cells and liver cells from ZAAT individuals[6,22,24,25]. Autophagy also plays a role in the removal of senescent or damaged mitochondria in a process termed 'mitophagy'. This is likely to be an important process in the ZAAT deficient liver given that mitochondrial dysfunction and mitophagy have been observed in patient liver samples and mouse models[24].

Thus the current dogma regarding degradation of ZAAT involves two major mechanisms, ERAD and autophagy. ERAD deals with removal of soluble ZAAT that accumulates in the ER presumably bound to chaperones whilst autophagy is specialised for the polymerised and aggregated forms of ZAAT that accumulate constitutively but that become more abundant during the acute phase response when expression of AAT is induced.

POTENTIAL THERAPIES

Notwithstanding the fact that a small percentage of ZAAT can be secreted from hepatocytes and fulfils a functional

role as an antiprotease in the body[45], there is a pressing need to design strategies to impair or prevent expression of mutant ZAAT to counteract its deleterious effects on ER accumulation in hepatocytes and monocytes in particular where ZAAT ER accumulation has known effects. This is particularly important during episodes of elevated AAT expression such as during infection or fever. A number of potential interventions likely to have therapeutic potential for the liver disease in ZAAT deficiency are under investigation. These include a variety of approaches.

Surgical intervention

Liver transplantation for ZAAT deficiency is confounded by a number of factors including donor availability and poor, but improving, survival rates. Hepatocyte transplantation now offers hope for some ZAAT deficient liver patients and has been developed as an alternative to whole liver transplantation. Successful hepatocellular transplantation requires transplantation of up to 5% of the liver mass and can achieve 1%-5% repopulation of the liver[9].

Endoplasmic reticulum stress relieving agents and inducers of apoptosis

A selection of compounds that have the ability to alleviate ER stress by a variety of different mechanisms may be worth considering for the treatment of ZAAT-related liver disease and to reverse the hyperinflammatory state of ZAAT monocytes. Selenium is a trace mineral that can relieve a number of indices of ER stress in a HepG2 liver cells model over expressing ZAAT[29]. It mediates its effect in two ways; *via* inhibition of NFκB activation through up regulated expression of 15deoxy-prostaglandin J2 and PPARγ, and by enhancing expression of selenoproteins such as selenoprotein S which can participate in ERAD.

α-linolenic acid and palmitoleate are agents that inhibit UPR signals[46,47]. α-linolenic acid, for example can inhibit eIF2α phosphorylation and expression of CHOP and GRP78 in rat renal cells. These agents are likely to be useful for treating the immune cell defects in ZZ individuals. However, since UPR is not activated *in vivo* in ZAAT deficient liver disease patients these agents are unlikely to have therapeutic benefit. Similarly, agents such as tauroursodeoxycholic acid (TUDCA) and salubrinal may prove to have more use in modulating ER stress in monocytes and possibly airway epithelial cells rather than liver cells. TUDCA is a bile acid that can inhibit ER stress-induced apoptosis[28]. Salubrinal can block cell death in response to UPR induction by preventing GADD34/PP1-mediated dephosphorylation of eIF2α[48]. Given that it is desirable to induce rather than inhibit apoptosis in globule-containing hepatocytes, TUDCA and salubrinal have little relevance to liver disease in this context. However, at lower doses than are used for induction of apoptosis they may have other desirable ER stress relieving properties.

Gene therapies

Ribozymes, peptide nucleic acid (PNA) and small-interfering RNAs (siRNA) are all potential tools to inhibit gene expression in ZAAT deficient individuals with liver disease[49]. Ribozymes are naturally occurring catalytic RNA molecules that can cleave target RNA molecules with high specificity[50]. Synthetic ribozymes targetting AAT have been designed[51]. These contain a catalytic RNA domain that cleaves mRNA and a substrate-binding domain specific for AAT mRNA. Ozaki *et al*[51] successfully transduced human hepatoma cells with ribozymes to inhibit the expression of a mutant AAT gene and co-express a modified AAT gene that was not sensitive to the ribozyme. This gene therapy approach led to inhibition of the mutant gene and expression of the modified AAT gene; unfortunately the overall efficiency of this method was low. This is a major problem associated with ribozyme use *in vivo;* their activity rarely achieves the desired effect.

PNA are synthetic DNA analogues comprising repetitive units of the pseudo-peptide polymer *N*-(2-aminoethyl) glycine to which purine and pyrimidine bases are attached by a methyl carbonyl linker[52]. PNAs hybridize to complementary DNA or RNA in a sequence-dependent manner leading to anti-gene or antisense inhibition, and blockage of transcription or translation, respectively. As PNA oligomers are stable and have high binding affinities they constitute potentially efficient compounds for gene therapy. Moreover they are non-toxic, even at relatively high concentrations although their uptake by living cells is slow. Modification with cell penetrating peptides can significantly improve their uptake. AAT-directed PNAs can inhibit MAAT expression in HepG2 cells and human MM monocytes (Greene C, unpublished data). They are also capable of interfering with ZAAT transgene expression in HEK293 cells or endogenous ZAAT gene expression in peripheral blood monocytes isolated from ZZ homozygous individuals. Unfortunately only partial inhibition can be achieved and the approach is considerably less effective than using siRNA.

We have effectively knocked down expression of MAAT and ZAAT genes in model systems over-expressing these genes and also in human monocytes from MM and ZZ homozygous individuals[53]. Others have similarly reported successful knockdown of AAT in cell lines, and have taken the technology further by successfully knocking down AAT expression in ZAAT overexpressing transgenic mice[54].

Inhibition of polymerisation and enhanced secretion

Peptides designed to block polymerization of ZAAT represent important therapies for the treatment ZAAT-related liver disease. Mahadeva *et al*[55] designed a 6-mer peptide Phe-Leu-Glu-Ala-Ile-Gly (FLEAIG) that selectively targets the reactive centre loop of ZAAT and blocks its polymerization. A second generation peptide which has the N-acyl terminus removed is as effective at inhibiting polymerisation and yields an active inhibitor[56]. Two additional peptides Ac-FLAAIG-OH and Ac-FLEAAG-OH and their daughter 4-mers Ac-FLEAA-NH(2) and Ac-FLAA-NH(2) can also bind avidly to ZAAT and prevent polymerization of the protein[57]. In further study using a combinatorial approach based on the inhibitory mechanism of A1AT, another peptide developed by Chang *et al*[58], Ac-TTAI-NH(2), acts as a tight-binding ligand for ZA1AT. It effectively blocks ZAAT polymerization but can also promote dissociation of the oligomerized serpin.

4-phenylbutyric acid (4PBA) and glycerol are reagents that that can potentially reverse misfolding and enhance secretion of ZAAT. These so-called chemical chaperone reagents have potential for targeting ZAAT. 4PBA can increase secretion of ZAAT although the mechanism remains unclear[59], whilst glycerol (and erythritol, trehalose and glucose) can interfere with polymerisation of ZAAT, by slowing down conformational transitions of the protein. Unfortunately, refolding of the misfolded conformer is usually not possible[60]. Imino sugar compounds including castanospermine, kifunensine and deoxymannojirimycin have also been suggested to be useful for enhancing ZAAT secretion from cells[61].

CONCLUSION

Accumulation of ZAAT in the ER has the potential to induce multiple signalling events related to ER stress. Interestingly, although perturbation of the ER can be induced by ZAAT expression in most cells, important differences exist in the responses induced in different cell types. The next steps in enhancing our understanding of the mechanisms of ER stress will be to identify these stimulus- and cell-specific responses that occur. Research within the next decade is also likely to advance our understanding of the role of gene modifiers in determining the susceptibility to and pathogenesis of ZAAT-related liver and lung disease. This knowledge we will bring us closer to a point where we can tailor make specific therapeutics for ZAAT deficient individuals.

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EDITORIAL

Which is the best choice for gastroesophageal disorders: Melatonin or proton pump inhibitors?

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Abstract

Melatonin is used in many countries to improve sleep disorders. Melatonin is a hormone produced by the pineal gland and enterochromaffin cells which control sleep and gastrointestinal motility. Low levels of melatonin lead to gastroesophageal reflux disease (GERD). Most of patients with GERD have a sleep disorder. So, low melatonin levels is the main cause of insomnia. Beyond this, it has an inhibitory action on gastric acid secretion and seems to control the lower esophageal sphincter. Proton pump inhibitors (PPIs) are a group of drugs whose main action is a pronounced and long-lasting reduction of gastric acid production. They are the most potent inhibitors of acid secretion available today. Omeprazole (one of the PPIs) and melatonin have similarities in their chemical structures. Therefore, we could consider omeprazole as a rough copy of melatonin. In this paper, we compare the advantages and disadvantages of the clinical use of melatonin and PPIs.

Key words: Melatonin; Sleep disorder; Gastroesophageal reflux disease; Omeprazole; Ulcers; Gastritis

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INTRODUCTION

Melatonin, also known chemically as N-acetyl-5-methoxytryptamine, is a hormone found in animals, plants and microorganisms[1,2]. In animals, it regulates sleep (circadian rhythms)[3] and gastrointestinal (GI) functions[4].

Proton pump inhibitors (PPIs) (pantoprazole, lansoprazole, rabeprazole, esomeprazole and omeprazole) are a group of drugs whose main action is a pronounced and long-lasting reduction of gastric acid production[5].

SYNTHESIS OF MELATONIN

Melatonin is synthesized from tryptophan in a sequence of reactions depicted in Figure 1.

The gastrointestinal tract represents the most important extra pineal source of melatonin (it secretes 400 times

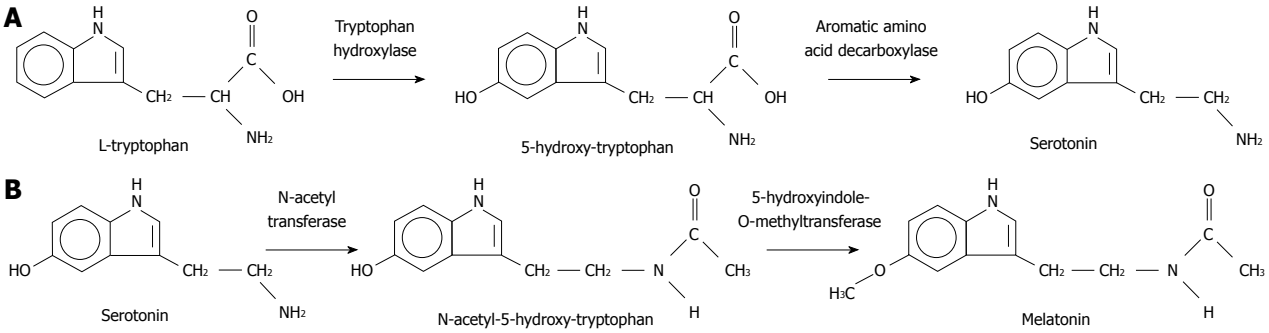

Figure 1 A schematic illustration of the synthesis of melatonin from tryptophan and serotonin.

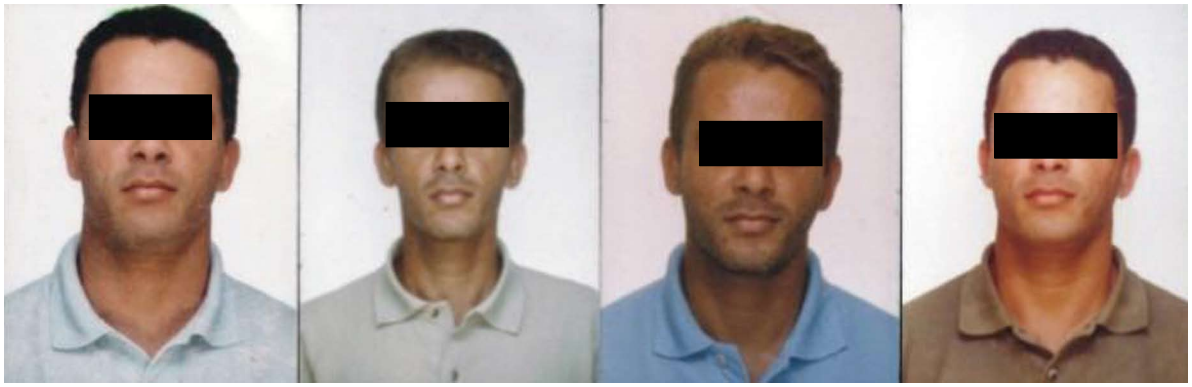

Figure 2 Photographs of the face of a HIV positive patient with an esophageal ulcer of 6 cm (about 3.2 inches) who used the dietary supplementation with melatonin, vitamins and amino acids in four different time periods (with permission)[8,9]. A: March 2002: Before the ulcer appeared (patient weighed 80 kg); B: October 12, 2003: During the period of the ulcer (patient weighed 40 kg); C: November 14, 2003: After 32 d of treatment (patient weighed 70 kg); D: July 2, 2004: after 9 mo of treatment. The patient now has body weigh as of March 2002 (80 kg).

than pineal gland). The presence of melatonin suggests that this hormone is somehow involved in digestive pathophysiology. Release of GI melatonin from serotonin-rich enterochromaffin cells of the GI mucosa suggest a close antagonistic relationship with serotonin and seems to be related to periodicity of food intake[4,6].

CLINICAL USE

Bubenik *et al*[7] demonstrated that a 4-wk administration of melatonin in the diet significantly reduced the incidence of spontaneous gastric ulcers in young pigs. The ulcers in this case may have been due to a local deficiency of the melatonin synthesis[7].

Recently, melatonin (combined with other natural supplements) was successfully used to heal a patient with a giant ulcer of 6 cm (about 3.2 inches). This type of ulcer is typical of HIV positive patients. This patient took omeprazole and other PPIs prescribed by gastroenterologists for months. Neither treatment produced even partial relief of his symptoms. Subsequently, he started to lose weight because he could no longer eat properly because of the acute pain, impaired swallowing and massive hematemesis. Even when he tried to drink an apple blended with water he vomitted blood. As a consequence, he lost 40 kg in 6 mo. After he consulted five gastroenterologists, he was recommended to our research group. We prescribed him the above mentioned formula with melatonin, vitamins and amino acid. In 32 d of treatment, he regained 30 kg (Figure 2) and the ulcer was cured (Figure 3)[8]. Figures 3 and 4 show his endoscopy biopsy records before and after treatment[8,9].

The same formula mentioned above (melatonin combined with other natural supplements) was also utilized in a randomized single-blind clinical trial of 351 human subjects with gastroesophageal reflux disease (GERD) and compared with omeprazole (group B)[9]. In this case, melatonin (combined with amino acids and vitamins) is better than omeprazole alone[9].

All patients with GERD reported that the symptoms started after a strong period of stress such as death of a beloved person, robbery, bankruptcy, financial loss, rape, assault, kidnapping, fighting in war, imprisonment, natural catastrophes and car, bus, train, airplane accidents *etc*. (data not shown).

These results with this formula (melatonin, vitamins and amino acids) were confirmed by other research group in a case report[4].

Kandil *et al*[10] confirmed that melatonin has a role in the improvement of GERD in human subjects when used alone or in combination with omeprazole. Meanwhile, omeprazole alone is better in the treatment of GERD than melatonin alone. They treated 36 human subjects with GERD[10].

PPIs are utilized in the treatment of many conditions such as dyspepsia, peptic ulcer disease, prevention of stress gastritis, gastroesophageal reflux disease, extraesopha-

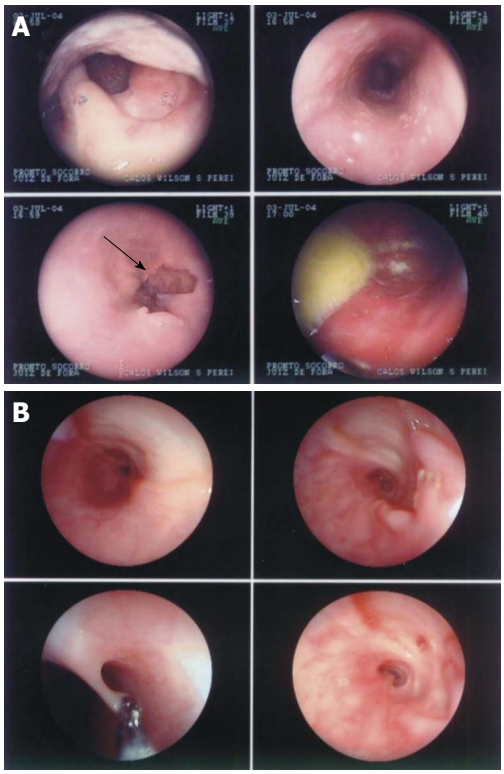

Figure 3 Endoscopy records performed on patient (of the group A)[8,9]. A: July 2003: Showing the ulcer (indicated by an arrow); B: July 2004: Showing that the ulcer had practically disappeared after 9 mo of treatment.

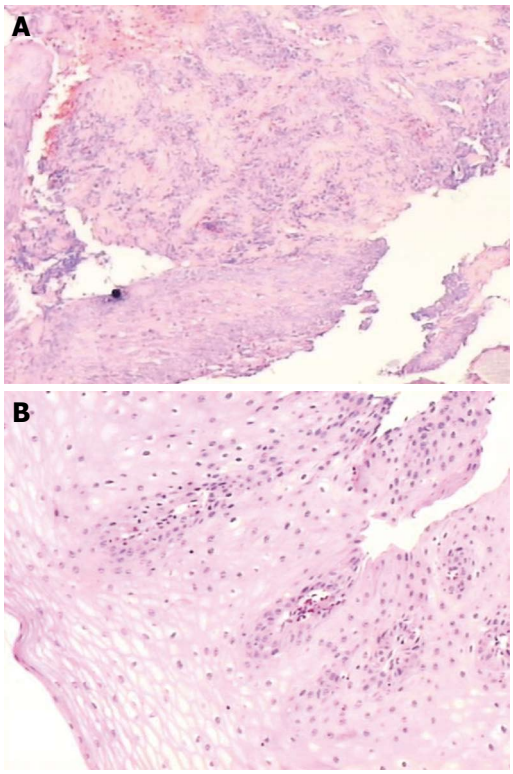

Figure 4 Histological cuttings of a biopsy from a patient with an ulcer of 6 cm (3.2 inches)[8,9]. A: During the period of ulcer; B: After the treatment with melatonin, vitamins and tryptophan.

geal reflux disease, Barrett's esophagus, gastrinomas and other conditions that cause hypersecretion of acid such as Zollinger-Ellison syndrome. The effectiveness of PPIs has not been demonstrated in every case despite their widespread use for these conditions. For example, PPIs do not change the length of Barrett's esophagus[5,11].

Omeprazole and melatonin have similarities in their chemical structures and, in this case, we could consider omeprazole as a rough copy of melatonin (Figure 5).

MECHANISM OF ACTION

Melatonin stimulates HCO_3^- secretion which protects duodenal epithelium against gastric H^+[12]. Melatonin presumably stimulates secretomotor neurons of the ENS thereby increasing HCO_3^- secretion from enterocytes. This action of melatonin involves MT2 membrane receptors on duodenal enterocytes and increases intracellular calcium which in turn activates apical electroneutral HCO_3^-/Cl^- exchange[12]. Experiments indicate that this indole has an inhibitory action on gastric acid secretion[13] (Figure 6).

Transient lower esophageal sphincter relaxation (TLESR) is a major mechanism of reflux in patients with GERD. Nitric oxide has an important role in TLESR[14]. Melatonin inhibits nitric oxide biosynthesis[13] which may explain the regression of GERD symptoms (Figure 6)[9].

PPIs abolish acid production. PPIs act by irreversibly blocking the hydrogen/potassium adenosine triphosphatase enzyme system (the H^+/K^+ ATPase or, more common, gastric proton pump) of the gastric parietal cell. The proton pump is the terminal stage in gastric acid secretion and is directly responsible for secreting H^+ ions into the gastric lumen, making it an ideal target for inhibiting acid secretion[5,15].

SIDE EFFECTS

Synthetic melatonin (2 mg) has no significant side effects even if taken during 12 mo at low doses[8,9].

PPIs have potentially serious adverse side effects. They abolish acid production so completely that serum gastrin levels rise. In rodents, enterochromaffin-like cell tumors and carcinoid tumors have developed. It is not known whether these drugs are carcinogenic in humans by a similar mechanism[5,15]. Melatonin is secreted by enterochromaffin cells[4,6] and if PPIs can develop enterochromaffin-like cell tumors and carcinoid tumors in rodents, we assume that therapy with PPIs can be dangerous.

Moreover, bacterial overgrowth may develop in the stomach in the absence of acid. Bacterial metabolism of dietary nitrites may then lead to the production of N-nitroso compounds that are carcinogenic[5,16]. This risk is not limited to chronic omeprazole treatment; it can theoretically occur with any effective long-term antacid regimen. Moreover, omeprazole appears to affect cytochrome P450. Although initial studies suggested an inhibitory effect, more recent studies indicate that omeprazole may induce the cytochrome P450 1A subfamily that is associated with

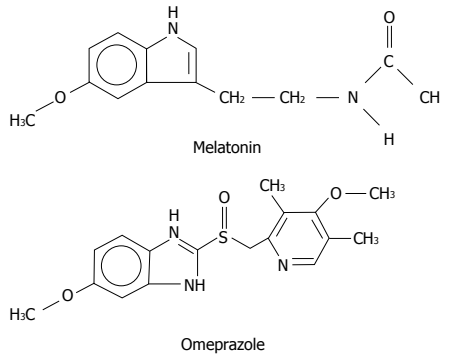

Figure 5 Chemical structure of melatonin and omeprazole.

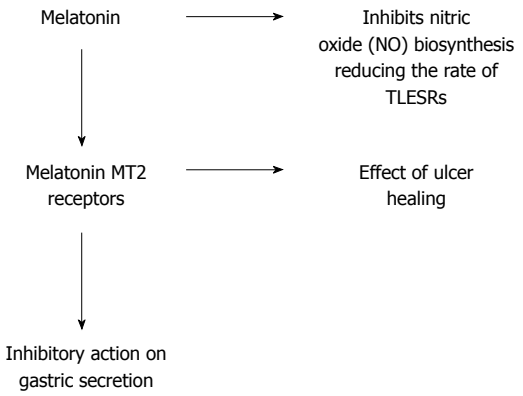

Figure 6 Action mechanism of melatonin.

activation of certain chemical procarcinogens such as polycyclic aromatic hydrocarbons[5].

Patients who receive PPIs have a higher incidence of hospital-acquired pneumonia, *Clostridium difficile* infection and other less severe side effects[17,18].

Recently, a study found that healthy volunteers taking PPIs had significantly greater acid-related symptoms following discontinuation of therapy compared with those taking placebo (44% *vs* 15%)[19].

POST-TRAUMATIC STRESS DISORDER

Post-traumatic stress disorder (PTSD) is an anxiety disorder associated with serious traumatic events (death of a beloved person, robbery, bankruptcy, financial lost, rape, assault, kidnapping, fighting in war, imprisonment, natural catastrophes and car, bus, train, airplane accidents, *etc*.) and is characterized by symptoms such as survivor guilt, reliving the trauma in dreams, numbness and lack of involvement with reality or recurrent thoughts and images[20].

Ethological research suggests that the experience of trauma during key development periods can result in persistent changes in brain morphology and function and lead to increased vulnerability to subsequent adversities[20]. PTSD may cause tryptophan depletion[20-22]. Melatonin secretion decreases in patients with tryptophan depletion[23]. Melatonin low level leads to insomnia and gastrointestinal disorders such as ulcers and GERD[6,8,9].

CONCLUSION

PTSD causes tryptophan depletion and, as a consequence, low levels of melatonin. The latter is responsible for the control of the lower esophageal sphincter and gastric acid secretion. This hormone is produced by the pineal gland and enterochromaffin cells and it seems that these cells are damaged by PPIs. Based on this rationale, how could we say that therapy with PPIs is safe?

At the present moment, three hospitals in the USA are starting a stop order policy for all PPIs. They are interested in conducting further studies with melatonin (associated with amino acids and vitamins) in order to replace the therapy with PPIs. The formula with melatonin associated with amino acid and vitamins is a very promising therapy with no significant side effects and very effective in terms of cure of giant ulcers (which are typical of HIV positive patients). PPIs can not regress or heal this type of ulcer. On the other hand, melatonin alone in human beings is less effective than omeprazole.

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TOPIC HIGHLIGHT

Maria D Yago, PhD, *Series Editor*

Role of macrophages in the progression of acute pancreatitis

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Abstract

In addition to pancreatic cells, other inflammatory cell populations contribute to the generation of inflammatory mediators during acute pancreatitis. In particular, macrophages could be activated by mediators released during pancreatitis by a damaged pancreas. It has been reported that peritoneal macrophages, alveolar macrophages and Kupffer cells become activated in different stages of severe acute pancreatitis. However, macrophages display remarkable plasticity and can change their physiology in response to environmental cues. Depending on their microenvironmental stimulation, macrophages could follow different activation pathways resulting in marked phenotypic heterogeneity. This ability has made these cells interesting therapeutical targets and several approaches have been assayed to modulate the progression of inflammatory response secondary to acute pancreatitis. However, despite the recent advances in the modulation of macrophage function *in vivo*, the therapeutical applications of these strategies require a better understanding of the regulation of gene expression in these cells.

INTRODUCTION

Acute pancreatitis (AP) is an inflammatory process of the pancreatic gland that exhibits a broad clinical spectrum and its severity may vary from a mild, edematous to a severe, necrotizing disease with high morbidity and mortality. In the most severe forms, the process involves remote organ systems. In fact, systemic inflammatory response syndrome (SIRS) is one of the major pathobiological processes underlying severe acute pancreatitis. This is of major importance because half of deaths in the first week of the process are attributed to organ failure and, in particular, the acute respiratory distress syndrome associated with SIRS[1]. Despite advances in diagnosis and treatment of inflammatory pancreatic disease, to date, supportive care remains the only treatment for patients with pulmonary complications.

It is widely accepted that the premature activation of digestive enzymes (trypsin, elastase and lipase) within the pancreatic acinar cells is a critical initiating event that leads to autodigestion of the pancreas[2]. However, acute pancreatitis is also an inflammatory disorder which develops a

complex cascade of immunological events which not only affect the pathogenesis but also the course of the disease. Although intra-acinar or interstitial activation of trypsinogen is most probably the trigger of acute pancreatitis[3], in recent years much emphasis has been put on the role of leukocytes[4]. In addition, a number of proinflammatory mediators have been identified to play a role in the progression of local pancreatic damage to systemic inflammation. This includes tumor necrosis factor α (TNFα), interleukin (IL)-1β, IL-6, MCP-1 and Platelet activating factor[5]. Some of these mediators are initially released by pancreatic acinar cells and results in the recruitment of neutrophils and monocytes. Numerous experimental and clinical data indicate that more pro-inflammatory mediators including cytokines, arachidonic acid derivatives, activated oxygen species and proteases are released locally by over activated neutrophils and monocytes/macrophages among other cells[6]. When released, these mediators gain access to the systemic circulation and play a central role in the progression of multisystem organ failure[7].

MACROPHAGES

In addition to pancreatic cells, other cell populations contribute to the systemic generation of inflammatory mediators. In particular, it has been reported that peritoneal macrophages, alveolar macrophages and Kupffer cells become activated in different stages of severe acute pancreatitis[8-10].

Macrophages display remarkable plasticity and can change their physiology in response to environmental cues[11]. Depending on their microenvironmental stimulation, macrophages could follow different activation pathways resulting in marked phenotypic heterogeneity (Figure 1)[12]. These changes can give rise to different populations of cells with distinct functions.

During the initial stages of the inflammatory response, the presence of pro-inflammatory stimulus induces classically activated macrophages (M1 macrophages). Under this activation, M1 macrophages are characterized by the secretion of proinflammatory cytokines including TNFα, IL1β and IL6 and the induction of enzymes, such as iNOS or COX2, involved in the generation of other pro-inflammatory mediators as nitric oxide or arachidonic acid metabolites[11].

In addition to classical M1 activation, a second population of macrophages was identified in the presence of the Th2 cytokines IL4 and IL13. These macrophages are termed alternative, wound healing or M2a. Under this activation, these macrophages fail to produce NO and to present antigens to T cells but they up-regulate mannose receptor expression and arginase II [13,14]. These cells also contribute to the production of the extra-cellular matrix.

Finally, a third population of activated macrophages termed regulatory or M2b have been described in the later stages of immune responses. The primary role of these macrophages seems to be to limit the inflammatory response. The production of the regulatory cytokine TGFβ and IL10 can inhibit the production of proinflammatory

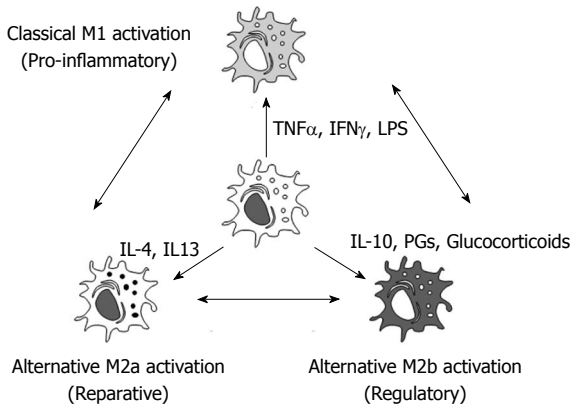

Figure 1 Depending on the microenvironment, macrophages could follow different activation processes. Classical M1 activation is induced by bacterial products or pro-inflammatory cytokines as tumor necrosis factor α or IFNγ. Regulatory M2b activation is induced by glucocorticoids, prostaglandins or interleukin (IL)-10. Finally, reparative M2a activation depends mainly on IL-4 and IL-13. Since these phenotypes could be redirected, the modulation of macrophage phenotype could be a promising therapeutical approach for the treatment of the systemic effects of acute pancreatitis. TNFα: Tumor necrosis factor α.

mediators by the classical M1 macrophages[15]. These regulatory macrophages do not contribute to the production of the extracellular matrix but express high levels of co-stimulatory molecules (CD80 and CD86) and can present antigens to T cells[11].

Macrophages seem to retain their plasticity and respond to environmental signals[16]. Several *in vitro* studies indicate that the phenotype of a macrophage population can change in response to different stimuli[17]. On the other hand, *in vivo* there are some cases in which a phenotypic switch in the macrophage population occurs over time and is associated with pathology[18]. However, it is less clear whether these changes in the phenotype are the result of differentiation of the original macrophages or of the migration of a new population of macrophages into the tissue site where they replace the original cells[11].

Since macrophages orchestrate both the initiation and the resolution of inflammation, it is clear that the degree of macrophage activation could be one of the factors that finally determine the severity of the inflammatory process.

MACROPHAGE ACTIVATION DURING ACUTE PANCREATITIS

The fact that macrophages could generate both pro and anti-inflammatory mediators confers a pivotal role to these cells in the progression of acute pancreatitis. Several reports demonstrate that macrophages could be activated by mediators released during pancreatitis by a damaged pancreas. In particular, pancreatic enzymes such as trypsin, elastase, carboxypeptidase A and lipase induce the generation of TNFα in cultured peritoneal macrophages or in macrophage cell lines[19,20]. The fact that these effects are mediated by IκB degradation and NFκB activation indicates that these enzymes trigger macrophage activation through specific membrane-bound receptors[21].

In vitro activation of macrophages could also be observed by treating these cells with supernatants of pancreatic acinar cells cultures incubated with cerulein[22]. Similarly, ascitic fluid collected from rats after the induction of experimental models of pancreatitis activates macrophages *in vitro*[23,24]. This activation could also be blocked by treating the cells with NFκB inhibitors as pyrrolidine dithiocarbamate. Interestingly, lipids present in ascitic fluid also have an effect on the activation of macrophages. It has been observed that lipid fraction of ascitic fluid does not activate macrophages but interferes in the inhibitory activity of PPARγ nuclear receptor, thus resulting in an increased activation of these inflammatory cells[24].

Direct measurement of circulating monocytes also revealed a significant degree of activation. Increased expression of iNOS[25] and TLR4[26] was reported in blood monocytes obtained from human patients with severe acute pancreatitis. In an experimental model of taurocholate-induced pancreatitis, the activation of NFκB and p38 MAPK signalling pathways in circulating monocytes has also been observed[27].

These results indicate that during acute pancreatitis there are mediators released with the capability to activate these inflammatory cells. When activated, macrophages could act to amplify the inflammatory response triggered in the pancreas through the generation of more cytokines and inflammatory mediators in systemic organs[28]. The importance of this activation on the progression of the disease was shown by the use of different inhibitors.

In this line, the administration of drugs as the macrophage-pacifying compound CNI-1493 prior to the induction of severe acute pancreatitis in rats results in an increased survival and a reduction in the severity of the process characterized by lower levels of circulating enzymes, cytokines as well as transaminases[29,30]. A protective effect was also observed by depleting macrophages with the injection of liposome-encapsulated dichloromethylene-diphosphonate, a macrophage-depleting agent, in a mice model of virus -induced pancreatitis[31]. However, in this case, a reduction in the levels of potentially protective cytokines as IL-10 was also observed.

MACROPHAGE POPULATIONS IN PANCREATITIS: PERITONEAL MACROPHAGES, KUPFFER CELLS AND ALVEOLAR MACROPHAGES

During the progression of acute pancreatitis from local pancreatic damage to systemic organ inflammation, several macrophage populations are of particular importance. Peritoneal macrophages are in direct contact with ascitic fluid secreted by the pancreas. This fluid generated in severe acute pancreatitis contains pancreatic enzymes and cytokines in a concentration that exceeds that observed in plasma in an order of magnitude[32]. Consequently, peritoneal macrophages are exposed to an intense pro-inflammatory environment and strong activation could be expected. The importance of this activation is the fact that mediators released by these macrophages to the peritoneal cavity could easily achieve the bloodstream through mesenteric absorption, thus contributing to the inflammatory response associated with acute pancreatitis[32].

A number of studies using different models of acute pancreatitis confirmed an early and intense M1 activation of peritoneal macrophages, reflected in the high expression of proinflammatory cytokines such as TNFα, IL1β, IL6 and enzymes such as iNOS[33]. The contribution of peritoneal macrophage-derived mediators to the toxicity of ascitic fluid was shown when a peritoneal lavage was carried out before the induction of pancreatitis in order to remove the macrophages[34]. In these conditions, ascitic fluid was generated by the effect of acute pancreatitis but the cytotoxic effects of this fluid, and in particular its apoptosis-inducing activity, was significantly reduced.

Another population of macrophages that has been involved in the pathogenesis of acute pancreatitis are Kupffer cells. They are the resident macrophages in the liver and participate in the acute response of this organ to toxic compounds. Since mediators released by a damaged pancreas or present in ascitic fluid are carried to the systemic circulation *via* the portal vein, the Kupffer cells could interact with all these products before they become diluted into the systemic circulation[26]. *In vitro* analysis of Kupffer cell activity revealed that these macrophages could also be activated by pancreatic enzymes[35,36].

Several works reported on the effect of gadolinium chloride administration to inhibit Kupffer cells activity before the induction of acute pancreatitis[37-41]. This inhibition results in lower levels of circulating cytokines and the pathological injury of the lung was ameliorated. By contrast, pancreatic damage was not affected by Kupffer cell blockage. These results indicate that the liver acts to amplify the inflammatory signal triggered by the pancreas in a process that is mediated by the activation of hepatic macrophages. Interestingly, the liver itself is not affected by this process and hepatic damage is not evident in early stages of pancreatitis.

The third family of macrophages involved in the progression of acute pancreatitis is alveolar macrophages. The capacity of alveolar macrophages to mobilize a large amount of leukocytes and release secretory products such as cytokines, arachidonic acid metabolites and nitric oxide (NO) after their activation in the course of different pulmonary inflammatory diseases suggests that these cells can be involved in the lung damage associated with AP. These macrophages exhibit particular characteristics, probably as a consequence of their anatomical situation, in direct contact with the environmental pollutants present in the breathing air.

A number of works reported on the changes presented by these cells during the acute lung injury secondary to pancreatitis. In particular, increased NO synthesis related to the induction of iNOS has been shown[42,43]. The use of phospholipase A2 inhibitors indicates that this enzyme could be involved in the activation of alveolar macro-

phages to generate nitric oxide[44]. However, the role of alveolar macrophages in the progression of acute lung injury during pancreatitis remains controversial. The use of inhibitors could affect other pulmonary cells involved in the generation of cytokines and the level of activation observed in alveolar macrophages seems to be lower than that observed in peritoneal or hepatic macrophages.

MACROPHAGES: A THERAPEUTIC TARGET?

The role of macrophages in the progression from local inflammation of the pancreas to a systemic inflammation and multiple-organ dysfunction made these cells interesting therapeutical targets. In particular, the capacity of macrophages to sequentially exhibit pro- and anti- inflammatory properties is of interest. This capacity suggests that macrophages not only are mediators in the inflammatory injury but can be induced to modify the sequence of events that occurs during acute pancreatitis.

Initial strategies were focused on the inhibition of macrophages. In experimental models, the use of gadolinium chloride to inhibit Kupffer cells[28,35], liposome-encapsulated dichloromethylene-diphosphonate to act on peritoneal macrophages[26] or PAF antagonists to block the activation of alveolar macrophages[45] resulted in the modulation of the systemic inflammatory response. Unfortunately, in these studies macrophage inhibitors were administered before the induction of pancreatitis and the application of this approach in clinical practice is difficult. Another problem is the long time needed to effectively inhibit macrophage activity or to deplete the macrophage population. Consequently, other approaches have been assayed based on the capability of macrophages to modify its phenotype.

The administration of IL-4 and IL-13 has been evaluated in order to derivate the pancreatitis-activated M1 peritoneal macrophages to a reparative M2a phenotype. However, despite this treatment effectively reverting the M1 pro-inflammatory macrophages to M2b reparative cells *in vitro*, it failed when assayed *in vivo*[33]. The reason for this failure seems to be the related to the ascitic fluid present in the peritoneal cavity. The high concentration of hydrolytic enzymes in this fluid result in the degradation of cytokines and its activity was lost before any effect on the macrophages could be observed.

Another interesting strategy was to programme macrophages *ex vivo* with anti-inflammatory or protective characteristics and to transfer these cells into pancreatitis-induced animals. This has been carried out using an experimental model of diet- induced pancreatitis in mice and transferring heme-activated macrophages before starting the diet[46]. Heme activated macrophages express high amount of heme-oxygenase-1 that unveil several potential protective mechanisms mediated by IL-10 and p38 MAPK activity. This approach results in a reduction of histological score and in the levels of circulating amylase. However, despite these data confirming the role of inflammatory cells on the progression of local pancreatic damage during pancreatitis, the long time needed to obtain heme-activated macrophages is a challenge to apply this approach as a therapeutical strategy.

CONCLUSION

Different populations of resident macrophages are involved in the progression of acute pancreatitis from local pancreatic damage to a multiple organ failure response. The plasticity of these cells makes them an attractive target to manipulate the systemic inflammatory response associated with acute pancreatitis. Therefore, future studies are needed to improve the manipulation or selective depletion of macrophages.

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BRIEF ARTICLE

An open-label, randomized, cross-over bioequivalence study of lafutidine 10 mg under fasting condition

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Abstract

AIM: To assess the relative bioavailability and pharmacokinetic properties of two formulations (test and reference) of Lafutidine 10 mg.

METHODS: The study was performed as an open label, randomized, two-way, two-period, two-treatment, single dose cross-over bioequivalence study, under non-fed condition to compare the pharmacokinetic profiles of the lafutidine formulation manufactured by Emcure Pharmaceuticals Ltd., India using an indigenously developed active pharmaceutical ingredient (API) and the commercially available Stogra® formulation, of UCB Japan Co., Ltd., Japan. The two treatments were separated by a washout period of 5 d. After an overnight fasting period of 10 h, the subjects were administered either the test or the reference medication as per the randomization schedule. Blood samples were collected at intervals up to 24 h, as per the approved protocol. Concentrations of lafutidine in plasma were analyzed by a validated liquid chromatography/tandem mass spectrometry (LC/MS/MS) method, and a non-compartmental model was used for pharmacokinetic analysis. The pharmacokinetic parameters were subjected to a 4-way ANOVA accounting for sequence, subjects, period and treatment. Statistical significance was evaluated at 95% confidence level ($P \geqslant 0.05$).

RESULTS: The mean (± SD) values of the pharmacokinetic parameters (test *vs* reference) were C_{max} (265.15 ± 49.84 ng/mL *vs* 246.79 ± 29.30 ng/mL, $P < 0.05$), Area under the curve $(AUC)_{(0\text{-}t)}$ (1033.13 ± 298.74 ng.h/mL *vs* 952.93 ± 244.07 ng.h/mL, $P < 0.05$), $AUC_{(0\text{-}\infty)}$ (1047.61 ± 301.22 ng.h/mL *vs* 964.21 ± 246.45 ng.h/mL, $P < 0.05$), and $t_{½}$(1.92 ± 0.94 h *vs* 2.05 ± 1.01 h, $P < 0.05$). The 90% confidence intervals (CI) for the test/reference ratio of mean C_{max}, $AUC_{(0\text{-}t)}$, and $AUC_{(0\text{-}\infty)}$ were within the acceptable range of 80.00 to 125.00. The mean times (± SD) to attain maximal plasma concentration (t_{max}) of lafutidine were 0.95 ± 0.24 h *vs* 1.01 ± 0.29 h ($P < 0.05$) for the test and the reference formulations respectively. Both the formulations were well tolerated.

CONCLUSION: In summary, this study has demonstrated the bioequivalence of the two formulations of lafutidine 10 mg. Hence it can be concluded that the two formulations can be used interchangeably in clinical settings.

Key words: Liquid chromatography/tandem mass spectrometry; Lafutidine; Gastroprotective; Pharmacokinetics; Bioequivalence

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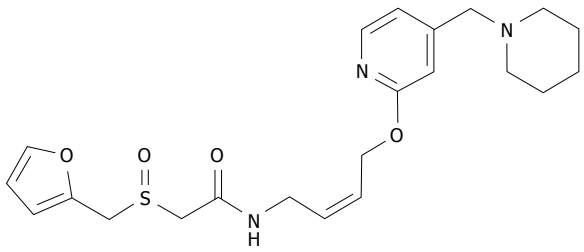

Figure 1 Chemical structure of Lafutidine[28].

INTRODUCTION

Lafutidine, (±)-2-(furfuryl sulfinyl)-N-[4-[4-(piperidinomethyl)-2-pyridyl]oxy-(Z)-2-butenyl] acetamide (Figure 1), is a newly developed second generation histamine H_2-receptor antagonist[1]. It is absorbed in the small intestine, reaches gastric cells *via* the systemic circulation, and then directly and rapidly binds to gastric cell histamine H_2 receptors, resulting in immediate inhibition of gastric acid secretion[2]. Lafutidine is used in the treatment of gastric ulcers, duodenal ulcers, and gastric mucosal lesions associated with acute gastritis and acute exacerbation of chronic gastritis[3]. It has been shown to have mucosal protective action in the gastrointestinal tract, including the esophagus, stomach, small intestine, and large intestine[3-5].

In clinical studies, lafutidine has been shown to inhibit gastric acid secretion during the daytime (i.e. postprandial) as well as during the night[6]. Lafutidine possesses a potent and long lasting gastric antisecretory effect mediated by H_2-receptor blockade in animals. Lafutidine has a receptor-binding affinity which is 2-80 times higher than other representative H_2-receptor antagonists (e.g. famotidine, ranitidine, and cimetidine)[7]. In addition, lafutidine exerts gastroprotective effects independent of its antisecretory action[8,9]. Lafutidine has been shown to increase the gastric mucosal blood flow[9] and gastric mucus secretion[10,11] and to accelerate epithelial restitution in rats.

The gastroprotective effects and intestinal protective effects of lafutidine are due to the activation of capsaicin-sensitive calcitonin gene related peptide (CGRP) containing vasodilator nerves (CGRPergic nerves) *via* modulation of presynaptic vanilloid-1 receptors[12,13]. Lafutidine causes a sustained increase in intracellular Ca^{2+} ion concentration in endothelial cells, which induces the release of neurotransmitters including CGRP. Lafutidine induced CGRP release stimulates nitric oxide (NO) production in endothelial cells, where NO participates in the regulation of gastric mucosal blood flow through vasodilation in the gastric microvasculature[14,15].

CGRP released from afferent neurons in the gastric mucosa stimulates D cells in the antral and fundic glands and increases somatostatin secretion from D cells. Somatostatin inhibits gastric acid secretion, acting directly on somatostatin receptors on parietal cells and indirectly by decreasing gastrin from antral G cells[16]. Lafutidine has been shown to significantly increase plasma somatostatin levels 0.3-2 h after a dose has been taken[17].

Lafutidine promptly suppresses gastric acid secretion, hence it is considered to be a useful drug for the on-demand treatment of mild gastroesophageal reflux disease[16]. Studies have shown that a triple therapy with lafutidine, clarithromycin and amoxicillin shows equivalent effect to that of lansoprazole, clarithromycin and amoxicillin in terms of *Helicobacter pylori* eradication rates, improvements in gastroesophageal reflux and abdominal symptoms after treatment, although the eradication rate with a triple therapy including lafutidine is not influenced by genetic polymorphism of CYP2C19 activity[18,19]. Lafutidine is used as a preanesthetic medication to decrease gastric fluid acidity and volume[20,21].

Lafutidine is presently approved in Japan as a tablet[3]. This product is not available in Europe, USA or India. The objective of the present study was to compare the pharmacokinetic profiles of lafutidine formulation manufactured by Emcure Pharmaceuticals Ltd., India using an indigenously developed active pharmaceutical ingredient (API) and the commercially available Stogra® formulation of UCB Japan Co., Ltd., Japan.

MATERIALS AND METHODS

Drugs

The API along with the test formulation (batch number FD/388/09; manufacturing date March 2009) were indigenously manufactured by Emcure Pharmaceuticals Ltd., India. The reference product Stogra® (batch number 9456, expiry date February' 2012) was manufactured by UCB Japan Co., Ltd., Japan. Each film coated tablet of both formulations contained lafutidine equivalent to 10 mg. The study was conducted at Therapeutic Drug Monitoring (TDM) Laboratory, Mumbai, India and it was sponsored by Emcure Pharmaceuticals Ltd., India.

Study subjects

Guidelines drawn up by the Institutional Review Board (IRB), which met the requirements of the U.S. code of Federal Regulations, the Canadian MRC guidelines and Declaration of Helsinki, Tokyo 2004 as well as the ethical norms laid down by the Indian Council of Medical Research (ICMR), New Delhi, India, 2006 were followed during the study[22-24]. The protocol was approved by the institutional ethics committee.

Twenty eight healthy male subjects, including 4 subjects as standby to replace dropouts, were included in the study. All participants gave a written informed consent prior to participation, which had the approval of the institutional

ethics committee, after they had been informed of the nature and details of the study in a language (both written and verbal) which they understood. Subject inclusion criteria included age between 18-45 years, non-smoker and Asian adult male of Indian origin with no evidence of underlying disease, medical disorders/impairments (hepatic, renal, cardiac, gastrointestinal tract and psychiatric), no vital sign abnormalities, no clinically significant abnormal values during pre-study screening, acceptable ECG, no consumption of drugs for 2 wk prior to the study, and no participation in any bioavailability or bioequivalence study at least 3 mo prior to the present study.

The exclusion criteria included history of hypersensitivity to the study product or related products, significant medical illness or conditions known to interfere with absorption, distribution, metabolism and excretion of the study drugs, significant history of medical illness like asthma, chronic bronchitis or other bronchospastic condition, glaucoma, cardiovascular or hematological disease, diabetes, metabolic acidosis or a known food allergy, significant clinical illness during 4 wk prior to day one of the study or hospitalization during 3 mo prior to the commencement of the study, maintenance therapy with any drug, alcohol abuse, drug dependency, use of enzyme modifying drugs within 30 d prior to day one of the study or use of any systemic medications including over the counter (OTC) drugs within 14 d prior to day one of the study, subjects who had a depot injection or an implant of any drug 3 mo prior to the commencement of the study, HIV or hepatitis positive subjects, and subjects who had donated blood (350 mL) within last 3 mo prior to the study.

Study design

The study was performed as an open label, randomized, two-way, two-period, two-treatment, single dose cross-over bioequivalence study, under non fed condition, and the treatments were separated by a wash-out period of 5 d. Each subject was assigned a unique identification number.

All the subjects arrived at the study center at least 13 h prior to the start of the study. They were housed in an air-conditioned facility and were given a standard dinner, which was finished at least 10 h before dosing in each period of the study. After an overnight fasting period of 10 h, the subjects were administered the medications as per the randomization schedule, for the test or the reference products, with 240 mL of plain drinking water. The intake of the study formulations was closely monitored by a physician and the oral cavity was checked properly to ensure completion of the administration process. Subjects were instructed to remain inclined on the bed for the first 2.0 h after dosing.

No meal was allowed until 4 h after dosing. Drinking water was restricted from 1 h before dosing till 2 h after dosing and *ad libitum* thereafter. Excess fluid intake (> 120 mL/h) was not allowed. Lunch, snacks and dinner were served as per the scheduled time.

All the subjects were abstained from any xanthine-containing food or beverages or alcoholic products for 72 h prior to formulation administration and throughout the sampling schedule during each period.

Subjects were informed not to take any drug at least 14 d prior to the study, especially cold preparations, aspirin, vitamins and antacid preparations. No concomitant medication was permitted during the study period.

Blood sampling

Blood samples (5 mL) were collected from an antecubital vein by an indwelling venous cannula using coded, sterile vacutainers containing ethylenediamine tetraacetic acid (EDTA) as an anticoagulant. Blood samples were obtained immediately prior to dosing (predose sampling, 0.00 h) and at 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 16.00, 18.00, and at 24.00 h after dosing. Blood samples were centrifuged at 4000 r/min (at 0-4℃) for 10 min, within 10 min of the sample collection, to separate the plasma. The plasma was separated and stored frozen at -20℃ ± 5℃ until assayed.

During the study periods, all the subjects were under medical supervision. Vital signs were examined at scheduled time as per the protocol.

Analytical procedure

A validated liquid chromatography/tandem mass spectrometry (LC/MS/MS) method was used for determination of lafutidine concentration in human plasma. Equipment used was a Perkin Elmer Series 200 pump fitted with Perkin Elmer series 200 autosampler and the software used was Analyst Software Integrator. Column type used was Cosmosil C_{18} (150 mm × 4.6 mm, i.d.) 5 μ and the mobile phase used was 0.01% Formic acid: Acetonitrile (20:80). Procedures of validation and acceptance criteria were based on "FDA Bio-analytical Method validation guidelines"[24].

Aliquots of 480.00 μL of drug free human plasma were taken in tubes and standard solutions were spiked to obtain concentrations of 5.00, 25.00, 50.00, 100.00, 200.00 and 400.00 ng/mL. The tubes were vortexed for 30 s. 100 μL of 0.2 mol/L sodium hydroxide was added and vortexed for 30 s. 5 mL of ethyl acetate was added to the tubes and the tubes were shaken for 10 min at 10 r/min in a shaker. The tubes were then centrifuged for 10 min at 3000 r/min. 4 mL of organic layer was collected and evaporated at 80℃ until dryness under a stream of nitrogen for 10 min in a low volume evaporator. The residue was then reconstituted in 200 μL of the mobile phase, and 10 μL of the reconstituted residue was injected onto the LC/MS/MS system.

Pharmacokinetic analysis

All pharmacokinetic parameters were determined by non-compartmental methods. Values below the quantification limit (< 5.00 ng/mL) were set to zero for calculation purposes.

The maximum plasma concentration (C_{max}) and the time to reach C_{max} (t_{max}) were taken directly from observed concentration *vs* time data. The elimination rate constant (K_{el}) was estimated by a non-linear least square regression

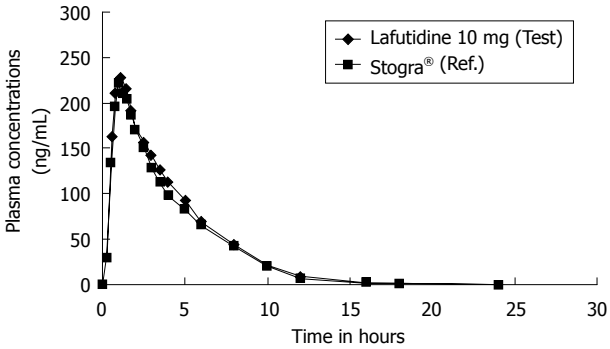

Figure 2 Mean plasma concentration *vs* time curves of the test and reference tablets, each containing 10 mg lafutidine.

analysis of the individual concentrations observed as a function of time during the elimination phase. The apparent elimination half life ($t_{½}$) was obtained by dividing 0.693 by K_{el}. The area under the curve (AUC) of lafutidine in plasma from time zero to last quantifiable time point (t), $AUC_{(0-t)}$, was calculated using the linear trapezoidal rule. The AUC from time zero to infinity, $AUC_{(0-\infty)}$, was calculated from the sum of $AUC_{(0-t)}$ and C_{last}/K_{el}, where C_{last} is the last measurable concentration of lafutidine in plasma.

The test and the reference formulations were considered to be bioequivalent if the calculated 90% confidence intervals (CI) for the log transformed ratios (test/reference) of the C_{max}, $AUC_{(0-t)}$, and $AUC_{(0-\infty)}$ were within the bioequivalence criteria range of 80.00-125.00 as established by the Central Drug Standard Control Organization (CDSCO), India; US Food and Drug Administration (US FDA) and European Medicines Evaluation Agency (EMEA).

Statistical analysis

Certified and validated WinNonlin version 3.0 (Pharsight Corp., USA) and Statistical Analysis System 9.1 (SAS 9.1) (SAS Institute Inc., USA) programs were used for statistical evaluations of the pharmacokinetic parameters.

The pharmacokinetic parameters were statistically analyzed by analysis of variance (ANOVA) test, and Schuermann's two one sided *t*-test. Standard descriptive analysis including mean, standard deviation (SD) and standard error (SE) were used for variables such as height, weight and age. These statistical parameters including coefficient of variance were used to describe plasma concentrations at each individual time point as well as pharmacokinetic parameters. $AUC_{(0-t)}$, $AUC_{(0-\infty)}$ and C_{max} were subjected to a four-way ANOVA accounting for sequence, subjects, period and treatment and the statistical significance was evaluated at 95% confidence level ($P \geqslant 0.05$). The statistical method for testing bioequivalence was based upon the 90% CI for the ratio of the calculated means (test/reference) for the parameter under consideration. The statistical analysis (e.g. ANOVA) took into account sources of variation that can be reasonably assumed to have an effective response.

RESULTS

Twenty eight healthy male subjects, including 4 subjects as standby to replace dropouts, were included in the study. Subject number 21 did not report for the second period of the study, and hence was considered as a dropout. Therefore subject number 26, carrying similar sequence of drug administration as that of subject number 21, was included for analysis. Thus twenty seven adult males completed the study. However twenty four subjects were considered for evaluation of pharmacokinetic parameters.

Table 1 Mean pharmacokinetic parameters of the test and the reference formulations, each containing 10 mg lafutidine

Parameters	Test (mean ± SD)	Reference (mean ± SD)
C_{max} (ng/mL)	265.15 ± 49.84	246.79 ± 29.30
$AUC_{(0-t)}$ (ng.h /mL)	1033.13 ± 298.74	952.94 ± 244.07
$AUC_{(0-\infty)}$ (ng.h/mL)	1047.61 ± 301.22	964.22 ± 246.45
t_{max} (h)	0.95 ± 0.24	1.01 ± 0.29
Kel (h^{-1})	0.44 ± 0.19	0.42 ± 0.22
t½ (h)	1.92 ± 0.94	2.05 ± 1.05

AUC: Area under the curve.

The two formulations were well tolerated by the subjects. No adverse event was observed during both the periods of the study in any of the subjects. Both clinical and laboratory parameters of all subjects showed no clinically significant changes.

The mean age, mean weight and mean height of twenty four subjects were (± SD) of 27.9 ± 5.22 years (range 19-37 years), 63.3 ± 7.74 kg (range 51-76 kg) and 167.2 ± 6.62 cm (range 157-184 cm) respectively. Figure 2 shows the plots of mean serum concentrations of lafutidine *vs* time. Both the formulations were rapidly absorbed and detected from 0.25 h in plasma.

Mean pharmacokinetic parameters of lafutidine for the test and the reference formulation, in 24 healthy Indian subjects are presented in Table 1.

The results of ANOVA revealed that sequence and period had no statistically significant effects on C_{max} ($P > 0.05$). However there was a significant effect of subject and treatment on C_{max} ($P < 0.05$). Similarly, there was a significant effect of sequence, subject and treatment on $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ ($P > 0.05$). The period had no significant effect on either $AUC_{(0-t)}$ or $AUC_{(0-\infty)}$ ($P < 0.05$). The intra-subject variation, calculated using mean square error obtained from the logarithmically transformed C_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ values, were 9.36%, 10.88% and 10.73% respectively. Additionally the 90% CI for the ratios of mean C_{max}, $AUC_{(0-t)}$, and $AUC_{(0-\infty)}$ were within the range of 80.00 to 125.00 (using log transformed data), meeting the regulatory criterion for bioequivalence as mentioned above. Table 2 represents the ratio (test/reference), 90% CI and the intra-subject variations of the C_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$.

For overall extent of absorption, both the formulations were equivalent, with test/reference formulation ratios of both $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ very close to 100%. Based on the plasma levels of the 24 completed subjects, the mean relative bioavailability of lafutidine was 107.90% as compared with the reference.

The pharmacokinetic data for each subject are illustrated in Table 3.

Table 2 Ratio (Test/Reference), 90% confidence interval and intra-subject variation following the administration of 10 mg lafutidine tablets

Parameters	Ratio (Test/Reference, %)	90% Confidence interval (Log transformed data)	Intrasubject variability (Log transformed data, %)
C_{max}	106.69	101.86-111.74	9.36
$AUC_{(0-t)}$	107.90	102.25-113.86	10.88
$AUC_{(0-\infty)}$	108.14	102.56-114.03	10.73

AUC: Area under the curve.

Table 3 Individual pharmacokinetic parameters of lafutidine 10 mg tablets

Subject	C_{max} (ng/mL)		t_{max} (h)		$AUC_{(0-\infty)}$ (ng.h/mL)		t½(h)	
No.	T	R	T	R	T	R	T	R
1	251.39	206.84	0.75	2.00	832.06	739.16	2.10	1.86
2	262.45	177.45	0.75	0.75	710.95	488.76	0.76	1.41
3	296.14	261.31	1.25	1.25	1194.42	1216.98	2.15	2.38
4	437.81	329.52	1.00	1.25	1794.64	1616.96	2.66	2.04
5	272.06	266.29	0.50	1.00	1514.49	1362.13	3.88	5.33
6	226.16	211.52	0.75	1.50	817.78	826.44	4.66	3.49
7	333.21	262.45	1.50	1.00	1670.60	919.94	2.74	2.38
8	367.03	284.66	1.00	0.75	1351.55	1029.20	2.83	2.60
9	217.84	264.98	0.75	0.75	915.96	946.37	1.47	1.17
10	239.84	263.04	0.75	1.00	908.95	955.31	1.68	2.17
11	261.86	230.72	1.00	0.75	934.60	1025.74	1.19	2.00
12	261.43	239.22	0.75	1.00	711.94	724.40	2.09	1.28
13	272.29	274.25	1.00	0.75	1079.63	1054.03	1.11	3.49
14	242.47	239.57	1.25	0.75	1338.51	1297.85	1.34	1.66
15	252.28	248.71	1.00	1.25	1132.98	1158.95	1.64	2.66
16	255.77	258.87	1.25	1.00	927.31	953.39	1.13	0.63
17	230.14	228.79	0.75	1.00	775.57	701.88	1.82	1.96
18	244.41	241.83	1.00	0.75	893.76	803.78	2.01	2.16
19	224.93	225.22	0.75	1.00	774.27	704.02	0.92	2.14
20	238.08	232.65	1.25	1.00	992.35	884.79	2.41	1.19
22	246.95	242.79	0.75	1.00	1109.70	1100.98	1.71	0.86
23	239.34	237.60	1.25	1.00	1163.50	984.41	0.95	1.31
24	240.45	245.86	0.75	1.00	727.85	825.47	1.32	1.01
26	249.33	248.84	1.00	0.75	869.33	820.28	1.42	1.97

T: Test formulation; R: Reference formulation; AUC: Area under the curve.

DISCUSSION

This is the first bioequivalence study of lafutidine conducted on an Indian population. This study assessed the bioequivalence of a 10 mg lafutidine tablet formulation with the Stogra® 10 mg tablet manufactured by Japanese company UCB Japan Co. Ltd. API and the test formulation (batch number FD/388/09; manufacturing date March' 2009) were indigenously manufactured by Emcure Pharmaceuticals Ltd., India.

Lafutidine has great potential for use in the treatment of gastric ulcers, duodenal ulcers, and gastric mucosal lesions associated with acute gastritis and acute exacerbation of chronic gastritis. Many published comparative clinical studies have established the superiority of lafutidine over proton pump inhibitors[2,6,16] and other H_2-receptor antagonists[1,25]. The normal dose of lafutidine is 10 mg once a day for gastric mucosal lesions associated with acute gastritis and acute exacerbation of chronic gastritis; 10 mg once a day as a preanesthetic medication; and 10 mg twice a day for gastric ulcers, duodenal ulcers and stomal ulcers[20]. As the dosage of 10 mg remains the mainstay, the pharmacokinetics of single dose of lafutidine 10 mg was evaluated in healthy male volunteers.

An internationally published pharmacokinetic study of lafutidine performed on healthy Japanese male volunteers showed a C_{max} of 133.90 ng/mL and t_{max} of 1.84 h[1]. A similar study performed on healthy Chinese volunteers showed a C_{max} of 151.55 ± 54.49 ng/mL and t_{max} of 1.60 ± 0.40 h[26]. This particular study performed on an Indian subpopulation showed a C_{max} of 265.15 ± 49.84 ng/mL and t_{max} of 0.95 ± 0.24 h. The estimated pharmacokinetic parameters of the test and the reference formulations in this study have higher levels than Japanese and Chinese clinical studies probably due to racial and genetic differences in the population studied.

The measured AUC and C_{max} values following oral administration of both formulations (test and reference) maintained 90% CI within 80.00-125.00 for the log transformed values, suggesting that the two formulations were bioequivalent.

Lafutidine was found to be well tolerated in the present study. No adverse effects were reported or observed in any of the subjects. This finding is consistent with previously

published clinical study by Ohya *et al*[27] where in no adverse event was observed in subjects given lafutidine.

In summary this study has demonstrated the bioequivalence of the 10 mg lafutidine tablet manufactured by Emcure Pharmaceuticals Ltd., India and the reference product, Stogra® manufactured by UCB Japan Co., Ltd., Japan. Hence it can be concluded that the two formulations can be used interchangeably in clinical settings.

COMMENTS

Background

Lafutidine is a newly developed second generation histamine H_2-receptor antagonist used in the treatment of gastric ulcers, duodenal ulcers, and gastric mucosal lesions associated with acute gastritis and acute exacerbation of chronic gastritis. The mechanism of lafutidine encompasses a multimodal action that not only reduces the gastric acid output but also exhibits mucosal anti-inflammatory and mucosal protective activity. That is why it is categorized as a second-generation H_2-receptor antagonist. The objective of the present study was to compare the pharmacokinetic profiles of a lafutidine formulation manufactured by Emcure Pharmaceuticals Ltd., India using indigenously developed active pharmaceutical ingredient and the commercially available formulation Stogra®, of UCB Japan Co., Ltd., Japan.

Research frontiers

The control of acid-peptic disease represents a major triumph for modern pharmacology. Second generation H_2-receptor antagonists, owing to their faster and multimodal mechanisms of action, can be used for on-demand treatment of mild to moderate acid-peptic disorders. Among the various second generation H_2-receptor antagonists available, Lafutidine seems to hold its own niche. Lafutidine boasts of a multi-modal and potent armamentarium of mechanisms of action thus giving it an edge over the other representative H_2-receptor antagonists. Lafutidine is presently approved in Japan as a tablet. This particular research aims at investigating the relative bioavailability and pharmacokinetic properties of two formulations of Lafutidine, and monitoring the safety and tolerability of a single dose of lafutidine 10 mg tablet in healthy adult Indian male volunteers.

Innovations and breakthroughs

Administration of the two lafutidine formulations (test and reference) to healthy adult Indian male volunteers did not significantly alter the pharmacokinetic profiles of either drug, demonstrating bioequivalence of the two formulations.

Applications

The study results suggest that lafutidine is safe and well tolerated. The formulations can be used interchangeably in clinical setting.

Terminology

Bioavailability: Bioavailability refers to the relative amount of drug from an administered dosage form which enters the systemic circulation and the rate at which the drug appears in the systemic circulation. Bioequivalence: Bioequivalence of a drug is achieved if its extent and rate of absorption are not statistically significantly different from those of the reference product when administered at the same molar dose. Pharmacokinetics: Pharmacokinetics describes the movement of the drug into, within, and out of the body, and its time-course. T_{max}: Time taken to achieve maximum plasma concentration. C_{max}: Maximum plasma concentration. AUC: Area under the plasma concentration time curve. K_{el}: Mean elimination rate constant. $t_{1/2}$: Mean elimination half-life. ANOVA: Analysis of variance.

Peer review

The study was well performed and the results are well discussed. The statistical tests are adequate.

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CASE REPORT

Henoch-Schönlein purpura complicating adalimumab therapy for Crohn's disease

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Abstract

Anti-tumour necrosis factor-α (TNF) therapy has revolutionised the management of chronic inflammatory conditions. With ever increasing numbers of patients being treated with these agents, uncommon adverse reactions will inevitably occur more frequently. Cutaneous manifestations are associated with many of these chronic conditions and can complicate anti-TNF therapy in about 20% of cases. Vasculitic complications are rarely associated with anti-TNF therapy. Henoch-Schönlein purpura (HSP), a small vessel vasculitis, has been described following infliximab and etanercept therapy but never with adalimumab, a fully humanized TNF antibody. The risk of such immune-mediated reactions is theoretically less with adalimumab compared to infliximab but can still occur. Here we report the first case in the literature of HSP that can be attributed to the use of adalimumab in a 19-year-old male with recalcitrant Crohn's disease.

Key words: Henoch-Schönlein purpura; Adalimumab; Anti-TNF therapy; Leukocytoclastic vasculitis; Crohn's disease

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INTRODUCTION

Anti-tumour necrosis factor-α (TNF) therapies have radically improved the management of chronic inflammatory conditions that are frequently associated with significant morbidity and a substantial burden to health service resources. These biologic agents are efficacious both for induction and long-term maintenance of remission in several conditions including; rheumatoid arthritis[1], ankylosing spondylitis[2], psoriasis and psoriatic arthritis[3], and the idiopathic inflammatory bowel diseases [Crohn's disease (CD)[4-8], ulcerative colitis[9]].

Anti-TNF agents can be broadly divided into neutralizing antibodies (infliximab, adalimumab, certolizumab) and recombinant receptors (etanercept). The neutralising antibodies are commonly used to treat patients with refractory, steroid-dependent, or fistulising CD. Adalimumab (HUMIRA®, Abbott), marketed in the UK for 6 years, is currently used primarily in those who have lost response to, or are unable to tolerate infliximab.

A number of cutaneous adverse events have been reported with anti-TNF therapy including; infusion and

injection site reactions, psoriatic eruptions, lupus-like disorders, vasculitis, granulomatous reactions, and cutaneous infections/ neoplasms[10,11]. Whilst infusion and injection site reactions are unquestionably related to administration of anti-TNF agents, all other events have a varying strength of association and severity, not necessarily requiring drug discontinuation. Eczematous/ psoriatic eruptions and infections are the commonest cutaneous complications with vasculitic manifestations occurring less frequently[12,13]. Henoch-Schönlein purpura (HSP), a small vessel vasculitis, is an extremely rare complication of anti-TNF therapy but has been described following infliximab[14] and etanercept[15,16] therapy. Although adalimumab is associated with various features of HSP there are no formal reports of adalimumab-induced HSP in the literature. The risk of such immune-mediated reactions is theoretically less with adalimumab, a fully humanized TNF antibody, compared to infliximab, a chimeric monoclonal antibody, but can still occur.

CASE REPORT

A 19-year-old male non-smoker with quiescent ileo-colonic and perianal CD on combination therapy with azathioprine (AZA) and fortnightly adalimumab, presented with a 10 d history of a purpuric rash on both legs and diffuse joint pain and swelling. These symptoms were preceded by a short-lived coryzal illness from which he had made a full recovery. There were no gastrointestinal symptoms of note. He had taken his most recent dose of adalimumab 2 d prior to developing the rash.

His CD, initially diagnosed in 1999, was not associated with any extra-gastrointestinal manifestations. Over the decade since diagnosis his CD had failed to respond to various established (5-aminosalicylates, AZA, methotrexate, infliximab) and experimental (natalizumab and leukocytapheresis (adacolumn)) medical therapies, and surgical interventions including a subtotal colectomy, ileal resection and ileostomy formation had been required. Adalimumab therapy was initiated 7 mo previously due to grumbling active disease on AZA monotherapy. The patient received a routine induction regimen with 160 mg at week 0 and 80 mg at week 2, and continued on a fortnightly maintenance dose of 40 mg. This was well tolerated achieving a prolonged remission.

On examination he was haemodynamically stable with a non-blanching purpuric rash on his lower limbs (Figure 1), marked synovitis and swelling of both hands and a tender swollen right elbow. There were no signs of meningism. The full blood count was normal apart from a mildly elevated eosinophil count of 0.44×10^9/L (normal range 0-0.4 $\times 10^9$/L). The C-reactive protein (CRP) was 15.1 mg/L (normal range 0-5 mg/L) and erythrocyte sedimentation rate (ESR) 13 mm/h (normal range 1-5 mm/h). Urea and electrolytes and liver function tests were within normal limits and a chest x-ray and x-rays of the swollen joints were unremarkable. The total IgA was elevated at 4.38 g/L (normal range 0.5-3.5 g/L) with normal IgG and IgM levels. The results of other blood tests that were performed are shown in Table 1. Urinalysis showed proteinuria and numerous amorphous deposits but no casts, red or white cells. The total urine protein was 0.2. An ultrasound scan of the renal tract was normal. A skin biopsy was also taken under local anesthetic.

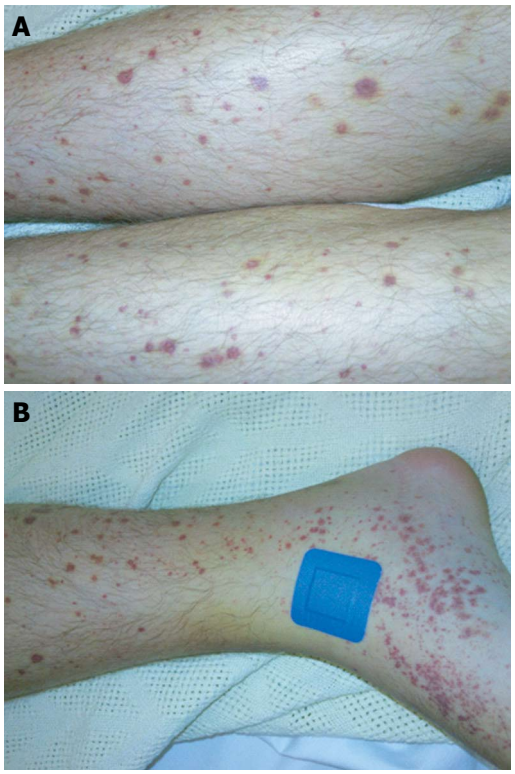

Figure 1 Vasculitic skin rash of Henoch-Schönlein purpura. A: Typical palpable purpuric lesions on the legs; B: Site of skin punch biopsy.

The patient remained systemically well and was managed conservatively in conjunction with the rheumatologists and nephrologists. After a few days the pain and joint swelling improved dramatically and the rash began to fade. The skin biopsy confirmed a leukocytoclastic vasculitis (Figure 2) consistent with the clinical diagnosis of HSP, although the precise aetiology was unclear (recent viral illness *vs* adalimumab). A multi-disciplinary decision was taken to commence a further trial of adalimumab, given the favorable response of his previously recalcitrant CD to treatment and a paucity of evidence linking it to HSP. After a repeat dose of adalimumab on the ward, which was well tolerated, he was discharged with outpatient follow-up.

He re-presented 3 d later with polyarthropathy and a florid vasculitic rash on his legs. Although he remained systemically well he was finding it difficult to weight bear due to pain in his knees and right ankle. Baseline investigations were again unremarkable apart from raised inflammatory markers (CRP 22.2 mg/L, ESR 8 mm/h) and serum IgA level. Urinalysis revealed proteinuria and microscopic haematuria. The right ankle was aspirated excluding septic and crystal arthropathy. He was treated with intra-articular (right ankle) and systemic steroids. His

Table 1 Blood tests

Blood cultures: No growth
ASO titre: Negative
HSV 1/2, CMV antibodies: Not detected
EBV IgM: Not detected
EBV IgG: Positive consistent with past infection
Parvovirus IgM: Not detected
Parvovirus IgG positive consistent with past infection
HBV/HCV serology negative
ANA, ANCA, Rheumatoid factor: negative
C3, C4 levels: Normal
Cryoglobulins: Not detected
Serum protein electrophoresis: Normal

symptoms rapidly improved and he was discharged home on a reducing dose of prednisolone. Adalimumab therapy was discontinued. His HSP-related symptoms completely resolved over the next few weeks and did not recur on completion of the course of steroids. Seven months later his CD is mildly active again on AZA monotherapy and he is being considered for a trial of a novel biological agent (anti-IL-12/23, ustekinumab) in CD.

DISCUSSION

HSP is a multi-system small vessel vasculitis, usually affecting children, which can be defined according to multiple classifications[17]. It commonly manifests with palpable purpura along with acute arthritis (typically involving the ankles, knees and elbows), enteritis and nephritis (causing haematuria and/or proteinuria). Neurological, pulmonary, cardiac and genitourinary complications occur rarely. The prognosis is generally favorable in children, where symptoms tend to last about 4 wk, resolving spontaneously. Adult onset is rare but associated with more severe manifestations and a poor prognosis.

Although the cause is unknown, HSP can develop after various viral and bacteria infections and as an idiosyncratic reaction to several drugs. IgA is thought to play central role in the immunopathogenesis. The rare association between HSP and CD is recognized but poorly understood[18,19].

The diagnosis is based on the combination of symptoms, as very few other diseases cause the same symptoms together. There are no diagnostic laboratory investigations although platelet count, urea, creatinine, IgA (50%), CRP and ESR may be elevated. Histology typically shows a hypersensitivity vasculitis and immunofluorescence demonstrates IgA and C3 (complement system protein) in the blood vessel wall. Treatment is usually supportive and directed against the precipitating cause. Immunosuppressants and immunoglobulin infusions are occasionally required in serious cases.

There have been several prior reports of localised cutaneous adverse events, including necrotizing and leukocytoclastic vasculitis, with infliximab and etanercept[20]. Most cases of cutaneous vasculitis develop within 3 mo of initiating anti-TNF therapy. HSP complicating anti-TNF therapy appears to be rare. The Medicines and Healthcare products Regulatory Agency (MHRA) has received one report each for etanercept and infliximab since 1999. In the literature there are two reports of HSP following etanercept[15,16] and one following infliximab[14]. It has not yet been associated with adalimumab although features of it such as vasculitic rash, arthralgia and glomerulonephritis have. Since January 2003 the MHRA has received 7 reports of vasculitic rash, 27 of arthralgia and 4 of glomerulonephritis for adalimumab. There is one case report of a cutaneous small vessel vasculitis and necrotizing crescentic glomerulonephritis in an anti-neutrophil cytoplasmic antibody positive patient that resolved on withdrawal of adalimumab and immunosuppressive therapy[21]. It is possible that some of these reactions may have been manifestations of undiagnosed HSP.

Here we describe the first case of adalimumab-associated HSP occurring after 7 mo of treatment for CD.

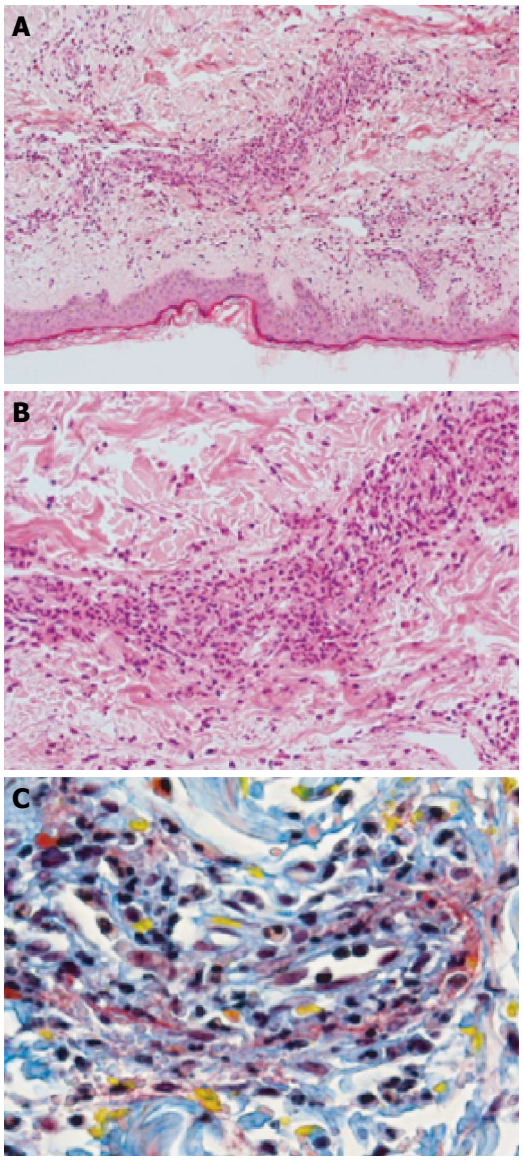

Figure 2 Punch biopsy of skin showing leukocytoclastic vasculitis. A: Low (× 20); B: High (× 100) power views (H&E); C: MSB stain highlighting fibrinoid necrosis (red) and extravasation of red blood cells (yellow) in leukocytoclastic vasculitis.

Discontinuation of the drug and treatment with systemic steroids led to the complete resolution of the vasculitis and polyarthropathy. HSP has now been described with all three commonly used anti-TNF agents- and occurred after several months of anti-TNF therapy in all cases. Thus HSP or features of it, occurring during chronic use of these biologics must be considered as possibly related to the therapy. HSP manifests more severely and is associated with a worse prognosis in adults. Renal involvement, a key discriminator of long-term outcome, is more common in this group. It has been reported that up to one third of HSP patients with renal involvement eventually progress to end stage renal failure[22]. Given that the use of anti-TNF therapies is likely to increase further in the future, prescribers need to be aware of this potentially serious complication.

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Meetings

Events Calendar 2010

January 15-16, 2010
AGA Clinical Congress of Gastroenterology and Hepatology
Las Vegas, United States
http://www.gilearn.org/clinicalcongress

February 4, 2010
New Developments in Pain Therapy sponsored by the Swiss Society of Pharmacology and Toxicology
Bern, Switzerland
http://pharmacology.unibe.ch/SSPT2010

February 5-9, 2010
Cancer Genomics, Epigenomics & the Development of Novel Therapeutics
Waikoloa, United States

February 7-10, 2010
53rd Annual Meeting of the Western Pharmacology Society
San Diego, United States
http://www.medicine.nevada.edu/wps/annualmeeting.html

February 25, 2010
Multidisciplinary management of acute pancreatitis symptoms
London, United Kingdom
http://www.rsm.ac.uk/academ/pancreatitis10.php

March 16-18, 2010
83rd Annual Meeting of the Japanese Pharmacological Society
Osaka, Japan
http://www2.convention.co.jp/83jps/english/english_top.html

March 17-20, 2010
Annual Meeting of the American Society for Clinical Pharmacology and Therapeutics
Atlanta, United States
http://www.ascpt.org/annualmeeting2010/index.cfm

March 23-25, 2010
51st Annual Meeting of the German Society for Experimental and Clinical Pharmacology and Toxicology
Mainz, Germany
http://www.pharmakologie.uni-mainz.de/JTG/JTG.html

March 25-28, 2010
20th Conference of the Asian Pacific Association for the Study of the Liver
Beijing, China
http://www.apasl2010beijing.org/en/index.aspx

May 15, 2010
Digestive Disease Week 2010
New Orleans, United States
http://www.ddw.org/

June 2-4, 2010
Annual meeting of the Canadian Society of Pharmacology and Therapeutics
Toronto, Canada
http://www.pharmacologycanada.org

July 16-17, 2010
WorldPharma2010 Satellite Meeting: The role of clinical pharmacology in therapeutic drug monitoring and clinical pharmacogenetics
Copenhagen, Denmark

July 17-23, 2010
16th World Congress on Basic and Clinical Pharmacology (WorldPharma2010)
Copenhagen, Denmark
http://www.WorldPharma2010.org

September 12-14, 2010
39th Annual Meeting of the American College of Clinical Pharmacology
Baltimore, United States
http://www.accp1.org

September 23-26, 2010
The 1st World Congress on Controversies in Gastroenterology & Liver Diseases
Prague, Czech

October 15-20, 2010
ACG 2010: American College of Gastroenterology Annual Scienitfic Meeting
San Antonio, United States

October 20-23, 2010
Australian Gastroenterology Week
Melbourne, Australia
http://www.gesa.org.au/agw.cfm

November 11-12, 2010
20th Neuropharmacology Conference co-organized by the Nomenclature Committee of IUPHAR (NC-IUPHAR): Receptor Structure and Drug Design
San Diego, United States
http://www.neuropharmacology-conference.elsevier.com

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Case-Based Approach to the Management of Inflammatory Bowel Disease
San Francisco, United States

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Patent (list all authors)

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