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EDITORS FOR THIS ISSUE

Pseudopemphigoid as caused by topical drugs and pemphigus disease

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Abstract

Pseudopemphigoid can cause a chronic cicatricial conjunctivitis that is clinically identical to the manifestations seen in mucous membrane pemphigoid, a disorder with a common clinical phenotype and multiple autoimmune links. For the purpose of this review, we will describe pseudopemphigoid as caused by topical drugs, the most common etiology with ocular manifestations, and as caused by the pemphigus disease, a more rare etiology. Specifically, we will discuss the ophthalmological features of drug-induced cicatricial conjunctivitis, pemphigus vulgaris, and paraneoplastic pemphigus. Other etiologies of pseudopemphigoid exist that will not be described in this review including autoimmune or inflammatory conditions such as lichen planus, sarcoidosis, granulomatosis with polyangiitis (Wegener's granulomatosis), erythema multiforme (minor, major, and Stevens-Johnson syndrome), bullous pemphigoid, skin-dominated linear IgA bullous dermatosis, and skin-dominated epidermolysis bullosa acquisita. Prompt diagnosis of the underlying etiology in pseudopemphigoid is paramount to the patient's outcome as certain diseases are associated with a more severe clinical course, increased ocular involvement, and differential response to treatment. A complete

history and ocular examination may find early cicatricial changes in the conjunctiva that are important to note and evaluate to avoid progression to more severe disease manifestations. When such cicatricial changes are noted, proper diagnostic techniques are needed to help elucidate a diagnosis. Lastly, collaboration between ophthalmologists and subspecialists such as dermatologists, pathologists, immunologists, and others involved in the care of the patient is needed to ensure optimal management of disease.

Key words: Pseudopemphigoid; Mucous membrane pemphigoid; Cicatricial conjunctivitis; Pemphigus vulgaris; Paraneoplastic pemphigus; Drug-induced conjunctival cicatrization

Core tip: Pseudopemphigoid in the context of chronic cicatricial conjunctivitis mimicking mucous membrane pemphigoid is a disease with terminology that has continuously evolved since its inception. Recent understanding of the ophthalmological and systemic manifestations of pseudopemphigoid as caused by topical drugs and the pemphigus disease demonstrates that significantly decreased vision and/or increased mortality due to paraneoplastic associations may result. Proper diagnosis and treatment of the underlying disease is therefore critical in order to provide maximal care to the patient.

INTRODUCTION

The first use of the term pseudopemphigoid referred to a non-progressive, unilateral cicatricial conjunctivitis that developed in response to certain aggravating topical medications[1]. Pseudopemphigoid was originally named due to its clinical similarity to mucous membrane pemphigoid (MMP) - an autoimmune blistering disease characterized by subepithelial deposition of antigen-antibody complexes at the basement membrane zone. Subepithelial deposition of autoantibody complexes seen in pemphigoid disease differentiates it from pemphigus that is characterized by intraepithelial deposition of autoantibody complexes. If clinical manifestations of pemphigus produce conjunctival cicatrization identical to MMP, then pemphigus may therefore be characterized as "pseudopemphigoid" and the modern terminology of pseudopemphigoid now includes any etiology that mimics MMP in clinical presentation.

The purpose of this review is to elaborate on the epidemiology, clinical features, diagnosis, and treatment

options available for patients with pseudopemphigoid. This paper will review the ocular manifestations associated with three etiologies of pseudopemphigoid including the most common cause, drug-induced cicatricial conjunctivitis[2], and two rare causes from the pemphigus family, pemphigus vulgaris and paraneoplastic pemphigus. Pemphigus foliaceus, a third subset of the pemphigus family, does not involve the conjunctiva and will not be discussed in this review.

Pseudopemphigoid may be caused by a variety of other conditions not included in this review article such as sarcoidosis, granulomatosis with polyangiitis (Wegener's granulomatosis), bullous pemphigoid, skin-dominated linear IgA bullous dermatosis, and skin-dominated epidermolysis bullosa acquisita[3]. Additionally, inflammatory and/or autoimmune disease associated cicatricial conjunctivitis characterized by an interface/lichenoid lymphocytic infiltrate such as lichen planus[4], graft *vs* host disease, erythema multiforme spectrum, and discoid lupus erythematosis[5] are not included in this review article.

PSEUDOPEMPHIGOID

How pseudopemphigoid differs from mucous membrane pemphigoid

Historically, pseudopemphigoid referred to a unilateral drug-induced cicatricial reaction identical to MMP that did not progress upon removal of the inciting drug. The term has since evolved and for the purposes of this review, pseudopemphigoid will be characterized according to the criteria proposed by Thorne *et al*[2]: (1) Chronic cicatricial conjunctivitis; (2) A biopsy that rules out MMP; and (3) The existence of an alternate cause for cicatrization.

MMP refers to a group of autoimmune, subepithelial, blistering diseases that predominantly affect the mucous membranes and are notable for linear deposition of autoantibodies (IgG, IgA, or C3) along the epithelial basement membrane zone on biopsy. The primary distinction between MMP and pseudopemphigoid is that MMP consists exclusively of autoimmune blistering diseases with subepidermal deposition of autoantibodies as opposed to pseudopemphigoid that simply mimics MMP in clinical presentation but does not involve subepidermal deposition of autoantibodies.

How pseudopemphigoid mimics mucous membrane pemphigoid

Clinical features: Pseudopemphigoid, similarly to MMP, may produce a chronic cicatricial conjunctivitis in patients characterized by the presence of scarring. The clinical presentation of a patient with cicatricial conjunctivitis includes irritation, burning, a foreign body sensation, photophobia, tearing, dryness, redness or blurry vision and hyperemic conjunctiva, misalignment of eyelashes, cicatricial entropion, and trichiasis[6].

The Foster[7] staging system developed for MMP may be utilized to characterize the severity of chronic cicatricial conjunctivitis secondary to pseudopemphigoid

Table 1 Staging for severity of cicatricial conjunctivitis

Staging for severity of cicatricial conjunctivitis

Foster Staging[7]

I	Subepithelial fibrosis, positive rose-bengal staining in conjunctiva, conjunctival "shrinkage" from abnormal connective tissue due to small white striae that form around the superficial vessels in substantia propria
II	Marked foreshortening of inferior conjunctiva described by (1) 0%-25%; (2) 25%-50%; (3) 50%-75%; and (4) 75%-100%
III	Corneal neovascularization, trichiasis, dystichiasis, keratopathy, subepithelial bands of connective tissue resulting in symblepharon (conjunctival adhesions) formation that is described by (1) 0%-25%; (2) 25%-50%; (3) 50%-75%; and (4) 75%-100%
IV	Severe sicca syndrome, keratinization, ankyloblepharon

Mondino Staging[134]

I	0%-25% loss of inferior conjunctival fornix depth
II	25%-50% loss of inferior conjunctival fornix depth
III	50%-75% loss of inferior conjunctival fornix depth
IV	75%-100% loss of inferior conjunctival fornix depth

Table 2 Conditions associated with cicatricial conjunctivitis

Trauma
  Physical trauma
  Chemical burn
  Thermal burn
  Radiation burn
Infection
  Trachoma
  Membranous conjunctivitis
Allergic
  Chronic atopic keratoconjunctivitis
Mucocutaneous disease
  Erythema multiforme
  Stevens-Johnson Syndrome
  Toxic epidermal necrolysis
Immunobullous disorders
  Mucous membrane pemphigoid
  Bullous pemphigoid
  Pemphigus vulgaris
  Paraneoplastic pemphigus
  Lichen planus
  Dermatitis herpetiformis
  Systemic lupus erythematosus
Systemic disorders
  Rosacea
  Sjogren's syndrome
  Graft-*vs*-host disease
  Sarcoidosis
  Ectodermal dysplasia
  Erythroderma ichthyosiform congenital
Drug-induced
  Systemic
  Topical

as well. In Stage I, conjunctival inflammation develops with mucous discharge, subepithelial fibrosis, and areas of degenerated cells in the conjunctival epithelium. Abnormal connective tissue and small white striae develop around the superficial vessels of the substantia propria, producing conjunctival "shrinkage." In Stage II, inferior conjunctival foreshortening occurs. In Stage III, subepithelial bands of connective tissue create symblepharon (conjunctival adhesions), corneal neovascularization, trichiasis (misdirected eyelash growth), dystichiasis (eyelash growth arising from meibomian glands), and keratopathy due to scarring of the conjunctival goblet cells, lacrimal gland ducts, and meibomian gland orifices. In Stage IV, severe sicca syndrome, keratinization, and ankyloblepharon (lid adhesions) develop (Table 1).

Despite a severe end-stage presentation, early cicatrization secondary to pseudopemphigoid and MMP is often nonspecific and subtle which causes patients to present with disease that is already erosive and scarring[8]. Additionally, more than 65% of patients may have cicatricial conjunctivitis develop without any symptoms[9]. In a prospective study of 163 eyes with cicatricial change, a diagnostic delay of a median 225 d after symptom onset was noted causing 59% of patients to present as Stage III at diagnosis[10]. Therefore, it is paramount to have etiologies such as MMP and pseudopemphigoid on the differential diagnosis for any patient who presents with cicatrization to best optimize management.

Diagnosis: The first step to discovering the etiology of chronic cicatricial conjunctivitis involves the history. The patient should be asked about any past medical history of chemical or thermal burns; membranous conjunctivitis caused by infectious organisms such as adenovirus; mucocutaneous disorders such as erythema multiforme (minor, major, and Stevens-Johnson syndrome), Sjogren's syndrome; systemic allergic disease such as chronic atopic conjunctivitis or rosacea; chronic graft-*vs*-host disease following organ transplantation; history of trachoma if from endemic areas;

previous eyelid surgery; or previous use of any aggravating topical or systemic medications (Table 2). Additionally, because many etiologies of pseudopemphigoid include systemic autoimmune bullous disease, the physician should inquire about new onset cutaneous or oral mucosal lesions. Most patients presenting with cicatricial conjunctivitis will have a history that makes the diagnosis simple. If inconclusive evidence is found from the history, then other methods of diagnosis are needed including a conjunctival biopsy.

ETIOLOGIES OF PSEUDOPEMPHIGOID

Drug-induced cicatrization

Epidemiology: Drug-induced conjunctival cicatrization (DICC), also known as drug-induced ocular pseudopemphigoid, produces clinical findings identical to MMP in response to varying offending topical and systemic drugs[11]. The incidence of DICC remains unknown, but has been documented to occur most often in patients who require long-term use of glaucoma medications[11]. In a retrospective cohort study of 145 pseudopemphigoid patients, DICC was the most common cause of pseudopemphigoid in this population occurring in 28.3% of patients[2].

Pathophysiology: DICC can develop as a non-progressive, self-limiting "toxic" reaction to an offending topical drug or as a progressive, immunological process that continues

despite cessation of the offending drug[12-14]. Although increased activity of fibroblasts has been implicated as a possible effect on the local immune system, the exact mechanism by which offending topical drugs directly induce cicatricial conjunctivitis remains unknown[15].

When IgG localized to the ocular epithelial basement membrane zone are found, then autoimmune phenomenon are suggested[15,16]. Practolol, an oral beta-blocker, and its derivative metipranolol, a topical beta-blocker that treats glaucoma, have been implicated to induce immunologically mediated DICC[17-20]. This is related to the chemical structure and pharmacologic metabolism in the body - both compounds require deacetylation for metabolic activation, which produces a toxic aniline derivative in practolol and a slightly less toxic phenol derivative in metipranolol[17]. When oxidized, these derivatives become highly reactive and are normally neutralized in the body by the addition of glucuronic acid or sulfate. However, this mechanism is insufficient in patients that have a lower capacity for enzymatic detoxification[17]. When this occurs, proteins can bind these reactive oxidative products to create antigens[17]. Therefore, the toxicity potential of practolol and metipranolol to produce immunologically mediated cicatricial conjunctivitis occurs in patients who are susceptible to these reactions required for metabolic activation of the drug due to its pharmacologic structure. Drug chemical structure has not been implicated in the mechanism of cicatricial conjunctivitis induced by other offending topical drugs and in many cases of DICC, a toxic or immune-mediated reaction cannot be further defined.

Epitope spreading is one possible theory that may elucidate the mechanism behind autoimmune phenomenon as induced by topical drugs. Epitope spreading[21,22] refers to the phenomenon of autoimmune reactivity not only against one protein, but also against other epitopes on the same protein or other proteins in the same tissue. Intramolecular epitope spreading that occurs between different epitopes on the same protein is often used to explain the molecular pathogenesis and severity of disease in bullous pemphigoid[23]. Additionally, epitope spreading may occur due to tissue damage that causes certain antigens to become newly exposed to autoreactive T or B cells, thus producing an autoimmune disease in predisposed individuals[21,24]. This mechanism of epitope spreading can be promoted by injury that exposes previously sequestered antigens, causing activation of antigen presenting cells that attract autoreactive lymphocytes in these individuals[22]. Intermolecular epitope spreading that occurs between two different proteins has been cited to explain the conversion of one autoimmune disease into another. Pemphigus autoimmune disease converting into pemphigoid disease, or conversions between other autoimmune blistering diseases either simultaneously or separated by a few years, is hypothesized to occur when tissue damage exposes protein parts that are normally undetected by the immune system[25,26]. In a similar manner, ocular mucosal injury due to Stevens-Johnson syndrome, Lyell Syndrome, or direct chemical injury from drugs may be implicated to expose normally hidden antigens to processing and presentation by activated T-cells, resulting in the formation of MMP[12,21,24].

Incidences of MMP developing in uninvolved eyes of patients that did not receive the inciting drug may indicate an immunological etiology[12]. On the other hand, instances of unilateral changes histologically and immunologically identical to MMP that occur in only the eye that received an offending drug is considered to be drug-induced[16]. The absence of bilateral ocular involvement, other mucosal or cutaneous manifestations, and disease that is non-progressive after cessation of the offending drug suggests a drug-induced reaction. Therefore, DICC may involve either a toxic mechanism of damage or an autoimmune etiology where inciting topical medications sensitize predisposed individuals to developing a more rapid onset of ocular MMP.

Clinical findings: DICC produces symptoms of cicatrization clinically identical to MMP. Two distinguishing factors that differentiate DICC from MMP include unilaterality of symptoms localized to the eye that received the topical therapy as well as non-progression of disease after cessation of the drug. However, reports of progressive DICC have occurred in the literature[13,14].

A total of 7 studies comprising 63 cases of drug-induced conjunctival cicatrization were found in the literature[1,2,14,16,27-29]. The most commonly used inciting topical drugs and the average duration of utilization before onset of DICC symptoms consisted of: timolol (73% or 46/63) for an average 10.5 years, pilocarpine (51% or 32/63) for an average 9 years, dipivefrin (49% or 31/63) for an average 7 years, latanoprost (13% or 8/63) for an average 8 years, echothiophate iodide (11% or 7/63) for an average 8 years, epinephrine (10% or 6/63) for an average 2 years, acetazolamide (6% or 4/63) for an average 9 years, betaxolol (3% or 2/63) for an average 6 years, idoxuridine (6% or 4/63) for an average 2 years, dichlorphenamide (5% or 3/63) for an average 3 years, and bromonidine (8% or 5/63) and other beta blocker antiglaucomatous medications for an unknown duration of time (48% or 30/63).

Of 7 studies comprising 23 patients with drug-induced conjunctival cicatrization found in the literature, the most common clinical findings included: forniceal foreshortening (57% or 13/23), symblepharon formation (48% or 11/23), trichiasis (48% or 11/23), corneal epithelial defects (35% or 8/23), entropion (30% or 7/23), corneal pannus (30% or 7/23), pseudopterygium formation (4% or 1/23), and corneal perforation (4% or 1/23)[1,14,16,27-30].

Diagnostic studies: There are no specific changes associated with medications that induce cicatrization nor is there a favored location of conjunctival involvement to distinguish DICC from idiopathic MMP[11]. Histopathological features seen on conjunctival biopsy can vary according to whether cicatrization is mild or severe[31]. When histopathological changes are seen, biopsy specimens can be identical to that of MMP and include subepithelial fibrosis, subepithelial infiltration with inflammatory cells,

reduction or loss of goblet cells, and basement membrane thickening[1,12,19,28]. Conjunctival biopsy with the use of direct immunofluorescence (DIF) is often not helpful as the findings are usually absent or nonspecific. However, IgG and complement staining to the epithelial basement membrane zone have been reported[1,15]. Although it is more common for immunofluorescent testing to lack positive findings, patients who present with both DICC and positive basement membrane zone autoantibody deposition should be considered to have MMP[3]. Otherwise, if the patient presents with a unilateral, non-progressive cicatrization of the conjunctiva, lacks other cutaneous or oral mucosal lesions, has a history of topical medication use for a prolonged amount of time, and other causes of conjunctival shrinkage have been excluded, then DICC should be considered.

Treatment: Management of DICC involves withdrawing the causative drug as early as possible and monitoring the patient carefully for the progressive type of disease. As topical intraocular lowering pressure therapies are commonly implicated in the pathogenesis of DICC, therapy involves a dual approach that includes controlling intraocular pressure and treating the signs of cicatrization. The primary management to resolve or inhibit the progression of fibrosis is cessation of intraocular lowering pressure medication[12]. The treatment to control intraocular pressure includes systemic carbonic anhydrase inhibitors followed by early surgical trabeculectomy[14,16]. If there are no other treatment options for the topical preparation suspected to be the offending drug, then re-introducing the medication in an unpreserved preparation may help. The patient should be followed closely for progressive disease and if progression occurs, then one must consider that the patient has developed ocular MMP and begin the patient on therapy.

A total of 7 studies comprising 63 cases of DICC or drug-induced pseudopemphigoid were found in the literature[1,2,14,16,27-29]. Aside from cessation of the inciting drug, management to control signs of cicatrization included medical treatment involving dapsone (10% or 2/21) or steroids (10% or 2/21). Procedural treatments to control sequelae of cicatrization include electrolysis and cryotherapy. Surgical treatments to control sequelae of cicatrization included anterior lamellar repositioning, tarsectomy, mucous membrane grafting, lower lid retractor tightening, lamellar keratoplasty, conjunctival transplant, terminal tarsal rotation procedure, and everting sutures. When switching to a different anti-glaucomatous medication, acetazolamide (14% or 3/21) or methazolamide (14% or 3/21) were utilized. Surgical treatment to manage uncontrolled intraocular pressure included trabeculectomy.

Prognosis: Clinical outcomes after procedural and/or medical treatment included persistence of ocular lesions without progression (48% or 10/21), remission of ocular lesions defined as regression (33% or 7/21), progression of ocular lesions (10% or 2/21), and recurrence of ocular lesions (5% or 1/21). Overall, average follow up time was 25 mo. If cicatrization is non-progressive upon withdrawal of inciting drug, then prognosis is favorable and management should treat the signs of scarring. If cicatrization is progressive upon withdrawal of inciting drug, then management and prognosis should be according to that of MMP.

Pemphigus vulgaris

Epidemiology: Pemphigus vulgaris (PV) is an intraepithelial blistering disease with a reported incidence of 4 to 4.7 cases per one million individuals[32,33]. PV most often affects patients in the fourth to fifth decade of life with equal occurrence in both sexes[34,35]. This differs from MMP that occurs less commonly at an incidence of 1.13 cases per one million individuals and presents in older individuals with a female predominance[8,36-41]. PV affects all races, but a higher predilection is associated with certain HLA subtypes such as the HLA-DRB1*0402 in Ashkenazi Jews and DRB1*1401/04 and DQB1*0503 in patients of European or Asian origin[42-44].

Pathophysiology: PV is an autoimmune disease characterized by suprabasal acantholysis (loss of cell-to-cell adhesions in epidermal cells that occurs just above the basal layer) induced by IgG binding to target antigens desmoglein 1 and 3 of the cadherin family[45-47]. Acantholysis leads to formation of a cleft which subsequently develops into an intraepithelial bulla[48]. This differs from MMP that is characterized by subepithelial lesions due to autoantibodies directed against various target antigens identified in the basement membrane zone.

PV antigens, desmoglein 1 and 3, are part of desmosome complexes that anchor intermediate filaments for adhesion between adjacent cells. These complexes consist of plakoglobin, plakophilin, desmoplakin, and desmosomal cadherins[45]. Desmoglein 1 (160 kDa) is located more superficially just below the stratum corneum whereas desmoglein 3 (130 kDa) is confined to the lower levels just above the basal cell layer[45,48].

Ocular involvement in PV is rare and its low incidence in the literature may be related to the course of disease or due to underreporting. Desmoglein 3 is heavily expressed in the basal layer of conjunctival epithelium along with strong expression of desmocollin 3, and desmoplakin 1 and 2, throughout the conjunctiva[49,50]. The mechanism on why ocular involvement in PV is rare despite the presence of anti-desmoglein 3 autoantibodies in disease is unclear. Suggestions include that the ocular surface is less exposed to trauma than other tissues normally affected by PV[51]; that there is inactivation of desmoglein 3 in ocular epithelium that is readily compensated by other desmosomal proteins thereby leaving only a minority of patients susceptible to disease if compensation cannot be attained[49]; or that conjunctival involvement in PV is simply underreported.

Clinical findings: PV is characterized by the development of large, flaccid cutaneous blisters and mucosal surface

Figure 1 Clinical manifestations of ocular pemphigus vulgaris. A: Lid margin erosions of the medial aspect on the lower lid and blisters of the upper lid; B: Higher magnification of eyelid demonstrating erosions and crusting of the upper eyelid with superficial blisters; C: Conjunctival hyperemia, crusting from lid margin erosions, and mucoid discharge.

involvement including the oral mucosa, conjunctiva, esophagus, larynx, and genitalia. Cutaneous lesions are fragile blisters that bleed easily and are characteristic for demonstrating Nikolsky's sign (rubbing of the perilesional skin with slight pressure produces exfoliation of the outer layer) and the indirect Nikolsky sign (moving an intact blister laterally and enlarging it with pressure)[52].

Mucosal involvement is the most common manifestation of PV and painful, chronic erosions of mucus membranes are often the initial presentation[39,53,54]. Ocular involvement in PV is rare and typically benign - ocular lesions do not usually progress to scarring and patients often fully recover without sequelae[51,55]. If ocular involvement occurs, it typically induces bilateral conjunctivitis without fibrosis (Figure 1)[56]. Lid margin erosions in the medial aspect of the lower eyelid can be characteristic of ocular pemphigus vulgaris (OPV)[57]. This differs from MMP that most commonly presents ophthalmologically with signs of overt cicatrization including symblepharon, trichiasis, punctate keratitis, and entropion[10].

A total of 10 studies comprising 36 OPV patients were found in the literature[34,49,51,55,58-63]. The most common ocular symptoms included the following: conjunctival hyperemia (49% or 17/35), conjunctivitis (46% or 16/35), conjunctival ulceration (14% or 5/35), lid margin erosions (14% or 5/35), corneal erosions (6% or 2/35), erosions of the medial canthus (3% or 1/35), and pseudomembrane formation (3% or 1/35). Concomitant systemic manifestations most commonly included oral involvement (80% or 28/35) followed by cutaneous lesions (54% or 19/35). The initial presentation of disease included ocular involvement in 41% (14/34) of cases.

Despite the seemingly benign nature of OPV, other studies suggest that ocular involvement in PV may be a sign of severe or recurrent disease that can occur in conjunction with exacerbation of systemic disease or in patients who have previously failed conventional immunosuppressive therapy[34,51,58]. Although fibrosis is very uncommon in PV, a subset of patients characterized by ocular involvement as the first manifestation of disease can produce a progressive cicatricial conjunctivitis in a similar manner to MMP[35].

In the largest series in the literature regarding OPV

patients, Chirinos-Saldaña *et al*[35] described 15 patients whose presentation included the following: conjunctival hyperemia (100% or 15/15), cicatrization (100% or 15/15), subconjunctival scarring (100% or 15/15), conjunctival cul-de-sac shortening (73% or 11/15), symblepharon formation (40% or 6/15), eyelid involvement including trichiasis or entropion (33% or 5/15), corneal perforation (27% or 4/15), and ankyloblepharon formation (7% or 1/15). Concomitant systemic manifestations included oral (20% or 3/15) and cutaneous involvement (7% or 1/15). The initial presentation of disease included ocular involvement in 100% (15/15) of cases. These results, alongside other reports in the literature involving progressive keratolysis with secondary corneal perforation[64,65], have led authors to conclude that a subset of patients exist with atypical pemphigus characterized by severe ocular involvement as the primary manifestation of disease[35]. Although all patients in this series had immunopathological diagnoses of PV, additional serology studies and/or secondary confirmatory biopsies were not performed to determine the coexistence of MMP. Dual diagnoses of MMP and PV have been previously reported in the literature[66,67] and therefore remain a possibility in this series.

Diagnostic studies: Histopathological studies utilizing hematoxylineosin staining of conjunctival biopsies in OPV demonstrate suprabasal and intraepithelial acantholysis that is characteristic of the pemphigus disease with splitting that occurs above the basal layer[51,61]. This differentiates OPV from MMP where most changes occur at the basement membrane zone with subepithelial conjunctival shrinkage; inflammatory infiltrate involving lymphocytes, macrophages, and plasma cells; and squamous metaplasia progressing to parakeratosis and keratinization of conjunctival epithelium[56,68].

The initial laboratory method to diagnose PV includes a conjunctival biopsy with subsequent DIF to IgG deposits in the intercellular space (Figure 2). This demonstrates antibodies directed against pemphigus antigens including desmosomal proteins desmoglein 3 and 1. This differs from MMP that demonstrates IgG deposits in the subepithelial space with antibodies directed against a variety of antigens not including desmoglein 3 and 1.

Figure 2 Direct immunofluorescence studies of conjunctival biopsies. A: Conjunctival mucous membrane pemphigoid showing thick linear IgG along the lamina propria in a background of squamous metaplasia; B: Conjunctival pemphigus vulgaris showing linear IgG deposition on desmosomal areas of epithelial cell surfaces displaying a classic "chicken-wire" pattern; C: Conjunctival paraneoplastic pemphigus (PNP) showing linear IgG along the lamina propria with a hemidesmosomal pemphigoid-like in conjunction with a desmosomal pemphigus vulgaris-type epithelial cell surface "chicken-wire" type pattern. The pattern in PNP is due to the presence of IgG autoantibodies against hemidesmosomal antigens (plakin proteins: BP230/BPAG1 and plectin) as well as desmosomal antigens (plakin proteins: desmoplakin, envoplakin, periplakin, and desmogleins 3 and 1); D: Conjunctival pseudopemphigoid (most likely drug-induced) showing negative IgG deposition along the lamina propria in a background of subepithelial clefting, mild submucosal fibrosis, and incipient epithelial metaplasia.

Indirect immunofluorescence (IIF) is utilized to detect a titer of circulating autoantibodies through serologic assays. IIF in PV will have serum positive for anti-intercellular substance antibodies in greater than 80%-90% of untreated cases which can be correlated with disease activity[68,69]. This differs from MMP where circulating antibodies are found less commonly than in PV, but may also be utilized to monitor disease activity[3,15,70].

Direct immunoelectron microscopy (IEM) detects peroxidase-labeled antibodies attached to autoantigens in tissue that react with various agents to form electron-dense material[71]. Indirect IEM localizes pemphigus-associated antigens to the extracellular hemidesmosomes in the upper portion of the lamina lucida. This differs from MMP where IEM localizes immune deposits to the lower lamina lucida and lamina densa[56].

The detection of target antigen in OPV can be accomplished through immunoblotting and immunoprecipitation techniques, which identify unknown target antigens bound to autoantibodies. Although immunoprecipitation is less available and more difficult to perform, it is more sensitive than immunoblotting because it utilizes native protein as opposed to denatured protein substrates[52]. A standardized enzyme-linked immunosorbent assay can be utilized to measure autoantibody titers to both desmoglein 3 and desmoglein 1 with higher sensitivity compared to IIF[72]. This differs from MMP that demonstrates immunoprecipitation of various target antigens not including desmoglein 3 and 1.

Treatment: Before the availability of immunosuppressive therapy, mortality for PV reached up to 90% but has now dropped to 3.3% with the use of corticosteroids, cytotoxic drugs, and other biologic agents with immunomodulatory effects[68,73]. Ocular lesions appear to be more responsive to treatment compared to other sites of mucosal involvement[51].

First line therapy for PV includes corticosteroids. Corticosteroids may be used alone or in conjunction with corticosteroid-sparing immunosuppressive agents to allow gradual weaning of steroids to decreased doses or alternate-day therapeutic regimens[60,74,75]. Side effects of corticosteroid treatment most commonly seen include weight gain, cushingoid features, infection, gastrointestinal bleeding, hypertension, hyperglycemia, osteoporosis, and acne[73]. To avoid the occurrence of these side effects, corticosteroids may be used concurrently with sulfone derivatives, immunosuppressive agents, antimetabolites, alkylating agents, and biologic agents.

A total of 10 studies encompassing 39 OPV patients and treatments with multi-drug systemic regimens were found in the literature[34,35,49,55,58-63]. These regimens most commonly consisted of: systemic steroids (82% or 32/39), dapsone (18% or 7/39), azathioprine (13% or 5/39), cyclophosphamide (13% or 5/39), mycophenolate mofetil (10% or 4/39), methotrexate (10% or 4/39), and

rituximab (5% or 2/39). Additionally, adjunctive topical drops were utilized in 40% (4/10) studies, of which the most commonly used were topical steroids (8% or 3/39) followed by topical diclofenac, naphazolin, zinc sulphate, chloramphenicol, cyclosporine, and tacrolimus (each 3% or 1/39). Surgical procedures for treatment consisted of penetrating keratoplasty (8% or 3/39) and manual removal of pseudomembranes (3% or 1/39). Overall, the average duration of treatment was 42.1 d.

Prognosis: Factors associated with worse prognosis in PV include ethnicities such as Indo-Asian and Jewish origin, younger age of onset, higher initial intercellular antibody titer, and higher initial desmoglein 3 titer[76,77]. If left untreated, the spread of erosions and bullae leads to severe infection and eventually death with 50% mortality at 2 years and almost 100% mortality at 5 years[78]. If treated, cutaneous lesions heal with re-epithelialization leaving residual hyperpigmentation without scarring.

Of 10 studies encompassing 39 OPV patients treated with multi-drug systemic regimens, outcomes of treatment included remission defined as regression of ocular lesions (54% or 21/39), remission of ocular lesions with persistence of other systemic manifestation of disease (13% or 5/39), persistence of ocular lesions without progression (8% or 3/39), progression of ocular lesions (18% or 7/39), and recurrence of ocular lesions (8% or 3/39). Overall, average follow-up time was 26.6 mo[34,35,49,55,58-63].

Paraneoplastic pemphigus

Epidemiology: Paraneoplastic pemphigus (PNP), also known as paraneoplastic autoimmune multiorgan syndrome, is a rare intraepithelial blistering disease that occurs less commonly than MMP. PNP occurs at an unknown incidence although approximately 250 cases have been reported in the literature[79]. PNP typically affects patients aged 45-70 years old although cases have occurred in children[80] and males appear to be more commonly affected compared to females[81]. The disease affects all races, but a higher predilection is associated with certain HLA subtypes such as the DRB1*03 allele in Caucasian patients and HLA Cw*14 in Chinese patients[82,83]. Additionally, PNP is strongly associated with underlying malignancy, more often lymphoproliferative neoplasms (chronic lymphocytic leukemia and non-Hodgkin Lymphoma), and the type of malignancy may be related to the ethnic background of the patient. A high prevalence of PNP associated with Castleman's disease and follicular dendritic cell sarcomas in Chinese and Korean patients has been documented[84].

Pathophysiology: PNP is an intraepithelial blistering disease characterized by autoantibodies that bind desmoglein 3, similarly to the pathogenic mechanism seen in PV. However, in PNP, the autoantibodies bind to epitopes distributed throughout the extracellular domain of desmoglein 3 as opposed to solely the N-terminal extracellular domain in PV[85]. Additionally, PNP has multiple other target antigens including the plakin protein family that connects cytoskeletal networks. These target antigens include desmoplakin I (250 kDa), desmoplakin II (210 kDa), bullous pemphigoid antigen 1 (BPAG1, 230 kDa), envoplakin (210 kDa), periplakin (190 kDa), plectin (500 kDa), desmocollin 2 (105 kDa), desmocollin 3, α2-macroglobulin-like-1 (A2LM1, 170 kDa), desmoglein 1 (160 kDa), and desmoglein 3 (130 kDa)[85-93]. This differs from MMP that is characterized by subepithelial lesions due to autoantibodies directed against various target antigens not including the plakin protein family.

Clinical features: PNP is a systemic autoimmune disease that occurs mostly in the setting of lymphoproliferative malignancies. PNP manifests as persistent painful erosions of mucous membranes and chronic cicatricial conjunctivitis clinically identical to MMP. Anhalt *et al*[94] termed paraneoplastic pemphigus to refer to a distinct clinical, histopathologic, and immunopathologic condition that included 5 criteria, of which Camisa *et al*[95] later revised these into major and minor criteria (Table 3)[69,94,95].

The distinguishing clinical manifestations that differentiate PNP include a painful and intractable ulcerating stomatitis that extends to the vermillion surface of the lips[45,96,97] and tense bullae that develop on the palms and/or soles[98]. Cutaneous manifestations of PNP are widely variable and can include superficial vesicles and flaccid blisters (pemphigus-like); scaly erythematous papules with or without tense blisters (bullous pemphigoid-like); polymorphic lesions (erythema multiforme-like); disseminated red scaly papules (graft *vs* host disease-like); or small violaceous papules with predominant mucosal membrane involvement (lichen planus-like)[99].

A total of 12 studies comprising 23 PNP patients with ocular involvement were found in the literature[84,94,100-109]. The most common ocular symptoms included the following: conjunctival erosions (68% or 15/22), conjunctivitis (45% or 10/22), pseudomembrane formation (27% or 6/22), conjunctival scarring (23% or 5/22), symblepharon formation (18% or 4/22), conjunctival shrinkage (14% or 3/22), forniceal foreshortening (9% or 2/22), corneal epithelial defect (5% or 1/22), and corneal perforation (5% or 1/22). Concomitant systemic manifestations included oral involvement (100% or 23/23) and cutaneous involvement (96% or 22/23). The most common initial presentation of disease was oral involvement (94% or 17/18) followed by ocular (17% or 3/18) and cutaneous (11% or 2/18) lesions.

Additionally, PNP is associated with malignant neoplasms. PNP may be the initial manifestation of a previously undetected malignancy in up to 33% of cases[81,102,110] or PNP may arise years after a patient has already undergone treatment for a previously known malignancy[111]. Of 12 studies comprising 23 PNP patients with ocular involvement, associated malignancies included the following: non-Hodgkin lymphoma (43% or 10/23), Castleman's disease (22% or 5/23), follicular dendritic cell sarcoma (22% or 5/23), peripheral T cell lymphoma (4% or 1/23), thymoma (4% or 1/23), and squamous cell lung carcinoma (4% or 1/23)[84,94,100-109]. Others have reported

Table 3 Diagnostic criteria for paraneoplastic pemphigus

Diagnostic criteria for paraneoplastic pemphigus	
Anhalt *et al*[94]	Camisa *et al*[95]
Painful mucosal and polymorphous skin erosions that involves the trunk, extremities, palms, and soles of a patient with a neoplasm	Major criteria
	Polymorphous mucocutaneous eruption
Histological changes including intraepidermal acantholysis, keratinocyte necrosis, and vacuolar interface dermatitis)	Concurrent internal neoplasia
	Specific serum immunoprecipitation pattern
Direct immunofluorescence findings of IgG and complement localized to the intercellular regions of the epithelium in a linear or granular fashion at the basement membrane zone	Minor criteria
	Histology demonstrating acantholysis
	Direct immunofluorescence demonstrating intercellular and basement membrane staining
Circulating autoantibodies that bind to stratified squamous epithelium as well as simple, columnar, and transitional epithelium	Indirect immunofluorescence staining with rat murine epithelium
Immunoprecipitation studies that demonstrate the presence of autoantibodies directed against a complex of five proteins of 250, 230, 210, 190, and 170 kDa	Diagnosis: All three major or two major and two minor required to diagnosis paraneoplastic pemphigus

the occurrence of chronic lymphocytic leukemia in up to 18%-29% of cases[97,108] as well as adenocarcinoma of various solid organs including the pancreas, colon, breast, prostate, and liver and squamous cell carcinoma of the tongue, cervix, and kidney[107,108,112-116].

Diagnostic studies: Histopathological studies utilizing hematoxylin-eosin staining in conjunctival biopsies from patients with PNP include the characteristic feature of pemphigus - vacuolization of basal cells and suprabasilar intraepithelial acantholysis[109]. Specimens taken from cutaneous biopsy may be widely variable and reflect the clinical polymorphisms present in this disease. Histopathologic features that are unique to PNP and are not found in other pemphigus subsets include vacuolar degeneration of basal keratinocytes with lichenoid or lymphohistiocytic infiltration as well as apoptotic keratinocytes located throughout the epidermis[84,94]. These changes differentiate PNP from MMP where changes occur exclusively at the basement membrane zone with subepithelial conjunctival shrinkage; inflammatory infiltrate involving lymphocytes, macrophages, and plasma cells; and squamous metaplasia progressing to parakeratosis and keratinization of conjunctival epithelium[56,68].

DIF studies show IgG and complement (C3) distributed both intercellularly and at the basement membrane zone in a linear or granular distribution[98]. This differentiates PNP from other types of pemphigus where only intercellular deposits are found and from MMP where only subepithelial deposits are found. Histological examination of biopsy parallels the clinical phenotype categorization and may demonstrate suprabasal acantholysis; keratinocytic dyskeratosis, apoptosis, and necrosis; vacuolization of the basal layer; or a lichenoid appearance seen along the dermal-epidermal junction[117,118].

IIF in PNP demonstrates autoantibodies binding to a variety of epithelium including simple columnar, transitional, respiratory, gastrointestinal, and myocardium[88]. This differentiates PNP from PV where autoantibodies against desmoglein 3 are restricted to stratified squamous epithelial tissues and from MMP where autoantibodies are found only in the epidermal basement membrane zone.

Additionally, IIF using murine bladder and tongue or monkey esophagus distinguishes PNP because these tissues express desmoplakin 1 without desmoglein 3[81,119]. When IIF results are indeterminate, western blotting and immunoprecipitation may provide more sensitive techniques. Detection of autoantibodies to envoplakin and periplakin is most specific followed by desmoplakin I and II[79].

Utilization of IEM enables visualization of autoantibodies binding to desmosomes, hemidesmosomes, and spreading along the keratinocyte cell surface including the lamina lucida[120,121].

Immunoprecipitation demonstrating polyclonal auto-antibodies that target a complex of plakin proteins is best for diagnosis and is a major criterion for diagnosis of PNP[95,122]. The combination of rat bladder IIF and immunoblotting has equally sensitive results that are highly specific; this can be utilized as an alternative approach to immunoprecipitation for serologic diagnosis[122].

Treatment: Treatment for PNP is directed towards relieving symptoms of PNP as well as treating the underlying neoplasm. Resection of benign neoplasms may lead to improvement or remission of cutaneous lesions in 6-11 wk[86,105,118]. However, the disease often progresses despite surgical excision and chemotherapy[118]. Aside from treating the associated neoplasm, management of PNP includes corticosteroids and adjunctive corticosteroid-sparing agents to decrease the incidence of side effects. Concurrent use of corticosteroids with sulfone derivatives, immunosuppressive agents, antimetabolites, alkylating agents, and biologic agents may occur although PNP is much less responsive to therapy compared to other forms of pemphigus[123]. In general, skin lesions are usually more responsive whereas mucosal lesions are highly refractory to treatment and recover more slowly[57,86,124].

A total of 12 studies encompassing 23 PNP patients with ocular involvement were found in the literature[84,94,100-109]. Treatment regimens most commonly consisted of: systemic steroids (81% or 13/16), rituximab (31% or 5/16), cyclophosphamide (31% or 5/16), cyclosporine (25% or 4/16), azathioprine (25% or 4/16), IVIG (25% or 4/16), vincristine (25% or 4/16), chlorambucil (19% or 3/16), and fludarabine, doxorubicin, bleomycin, double filtration

Table 4 Overview of mucous membrane pemphigoid and pseudopemphigoid as caused by the pemphigus disease

	MMP	Pseudopemphigoid	
		PNP	OPV
Location	Subepidermal	Intraepidermal	Intraepidermal
DIF	IgG/IgA/IgM/C3	IgG/C3	IgG/C3
IIF on salt-split skin	Dermal, epidermal, or combined depending on antigen	Not applicable	Not applicable
IEM: ultrastructural location of antigen	Lamina lucida	Hemidesmosomes	Desmosomes
	Lamina densa	Desmosomal plaques	
	Sublamina densa (anchoring fibrils)	Lamina lucida	
Immunoblot: determination of antigen	Bullous pemphigoid antigen 1 (Bullous Pemphigoid 230)	Plakin protein family:	Desmoglein 1 (160 kDa)
	Bullous pemphigoid antigen 2 (Bullous Pemphigoid 180, type XVII collagen)	Desmoplakin I (250 kDa)	Desmoglein 3 (130 kDa)
	Type VII collagen (290 kDa)	Bullous pemphigoid antigen 1 (230 kDa)	
	Laminin332, epiligrin, or laminin 5 $\alpha3\beta3\gamma2$ (165, 145, 140, 105 kDa)	Desmoplakin II and envoplakin (210 kDa)	
	Laminin 6 (α3)	Periplakin (190 kDa)	
	Integrin beta 4	Plectin (500 kDa)	
	45 kDa epithelial protein	Desmocollin 2 (105 kDa)	
	130 kDa epithelial protein	Desmocollin 3	
	140 kDa epithelial protein	α2-macroglobulin-like-1 (A2LM1, 170 kDa)	
	205 kDa epithelial protein	Desmoglein 1 (160 kDa)	
	168 kDa epithelial protein	Desmoglein 3 (130 kDa)	
	Uncein		
	LAD-1 (97/120 kDa)		
Increased malignancy	Yes - solid malignancies (laminin 332 subtype)	Yes - lymphoproliferative malignancies	--

MMP: Mucous membrane pemphigoid; PNP: Paraneoplastic pemphigus; OPV: Ocular pemphigus vulgaris; DIF: Direct immunofluorescence; IIF: Indirect immunofluorescence; IEM: Immunoelectron microscopy; LAD-1: Linear IgA bullous dermatosis autoantigen 1.

membrane plasmapheresis, methotrexate, and daclizumab (each 6% or 1/16). Additionally, adjunctive topical drops were utilized in 27% (3/11) of studies, of which the most commonly used were topical steroids (13% or 2/16) followed by topical tacrolimus (6% or 1/16). Surgical procedures consisting of resection of primary tumor occurred in 48% (11/23) of cases. Discontinuation of treatment due to side effects occurred in 13% of cases (3/23) and included plasmapheresis secondary to hypogammaglobulinemia and hypoalbuminemia; cyclosporine secondary to renal dysfunction; and cyclophosphamide, vincristine, prednisone, and rituximab regimen secondary to chemotherapy related side effects.

Prognosis: Of 12 studies encompassing 23 PNP patients with ocular involvement treated medically and/or surgically, outcomes included remission defined as regression of ocular lesions (30% or 7/23), remission of ocular lesions but persistence of other systemic manifestations (9% or 2/23), persistence of ocular lesions without progression (22% or 5/23), progression of ocular lesions (22%, 5/23), and recurrence of ocular lesions (4% or 1/23). Prognosis including death as final outcome occurred in 61% of cases (14/23) at an average 26 mo after onset of symptoms due to PNP[84,94,100-109]. Although reports of long-term survival have been described in the literature[104,125-128], others indicate that mortality rates may reach up to 90% with a mean survival of less than 1 year[128-130].

Death most commonly occurs secondary to malignancy, sepsis, or respiratory failure due to pulmonary involvement producing bronchiolitis obliterans[131,132]. Pulmonary involvement may occur and can continue to progress despite treatment with immunosuppressants, resection of malignancy, and improvement of other mucocutaneous symptoms[133]. Factors associated with worse prognosis in PNP include presence of erythema multiforme-like lesions, keratinocyte necrosis on biopsy specimens, and non-Hodgkin lymphoma patients with an increased risk of infection due to systemic chemotherapy and corticosteroids[128].

CONCLUSION

Pseudopemphigoid as caused by topical drugs and pemphigus disease may produce a chronic cicatricial conjunctivitis that can present clinically identical to MMP. In these cases, a vigilant history and examination combined with thorough diagnostic methods are needed to differentiate these diseases (Table 4). Distinguishing between the different causes of pseudopemphigoid that includes but is not limited to drug-induced cicatricial conjunctivitis, pemphigus vulgaris, and paraneoplastic pemphigus is paramount as there may be a poorer prognosis or a more severe clinical course unresponsive to medical management. Collaboration of the ophthalmologist with subspecialists such as the dermatologist, immunologist, and others involved in care of the patient is critical to prevent progression of the disease.

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MINIREVIEWS

Cranial neuropathies in sarcoidosis

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Abstract

Sarcoidosis is a multisystem, chronic inflammatory disease that is characterized by the development of non-caseating granulomas in multiple body tissues and organ systems. Neurological complications of systemic sarcoidosis include peripheral and cranial neuropathies, myopathies, seizures, gait dysfunction, and cognitive decline. Because sarcoidosis has a predilection to involve the basilar meninges, cranial neuropathy is the most prevalent neurological deficit seen when the nervous system is involved. Sarcoidosis cranial neuropathy may occur at different stages of the disease and even as the initial clinical manifestation of central nervous system involvement. Attributing a cranial neuropathy to sarcoidosis can be challenging, particularly in the setting of normal imaging studies. In this review, cranial neuropathies in sarcoidosis are discussed in detail.

Key words: Sarcoidosis; Neurosarcoidosis; Cranial neuropathy; Central nervous system

Core tip: Sarcoidosis is a multisystem, chronic inflammatory disease that is characterized by the development of non-caseating granulomas in multiple body tissues and organ systems. Neurological complications occur in 5%-15% of the cases. Because sarcoidosis has a predilection to involve the basilar meninges, cranial neuropathy is the most prevalent neurological deficit seen when the nervous system is involved. Several review papers on neurosarcoidosis have been published, but none has elaborated on cranial neuropathies. In this review, cranial neuropathies in sarcoidosis are discussed in detail, with elaboration on each cranial nerve individually and a representation of case reports from the literature.

INTRODUCTION

Sarcoidosis is a multisystem, chronic inflammatory disease that is characterized by development of non-caseating granulomas in multiple body tissues and organ systems. Sarcoidosis affects more women than men and more adults than children. In the United States, the disease affects more African Americans than Caucasians. Neurological complications occur in 5%-15% of individuals diagnosed with systemic sarcoidosis[1-4], imaging studies reveal neurological disease in 10% of all patients[5], and postmortem

Table 1 Frequencies of clinical signs and symptoms associated with neurosarcoidosis	
Symptoms	**%**
Cranial nerve palsies	50-75
Overall parenchymal disease	50
Headache	30
Meningeal signs	10-20
Endocrinopathies	10-15
Hydrocephalus	10
Mass lesion(s)	5-10
Seizures	5-10
Encephalopathy/vasculopathy	5-10

Source: Stern *et al*[39].

Table 2 Frequencies of occurrence of cranial neuropathies in sarcoidosis	
Cranial nerve	**Frequency of occurrence**
CN-I	Rare
CN-II	5% of all patients with sarcoidosis
CN-III, -IV, -VI	Rare
CN-V	Rare
CN-VII	25%-50% of all patients with sarcoidosis
CN-VIII	1%-7% of all patients with sarcoidosis
CN-IX, -X, -XI	Common
CN-XI	Rare
CN-XII	Rare

CN: Cranial nerve.

studies report that ante-mortem diagnosis is made in only half of the cases with nervous system involvement[6]. The exact site of involvement and pathogenesis are difficult to establish, as biopsy and autopsy material is not commonly obtained. Neurological manifestations of sarcoidosis include peripheral and cranial neuropathies, myopathies, seizures, gait dysfunction, and cognitive decline. The presenting symptoms of intracranial sarcoidosis are typically related to meningeal, cranial nerve, hypothalamus, and pituitary involvement[7-9]. Common imaging findings include hydrocephalus, mass lesion(s), and leptomeningeal enhancement.

Because sarcoidosis has a predilection to involve the basilar meninges, cranial neuropathy is the most prevalent neurological deficit seen when the nervous system is involved[10], and has been reported in as many as 50%-75% of patients with neurosarcoidosis[7]. Table 1 outlines the frequency of some of the most common neurological signs and symptoms associated with neurosarcoidosis. Granulomatous basal meningitis, direct infiltration of cranial nerve(s), and increased intracranial pressure are all potential mechanisms causing cranial neuropathies. Attributing a cranial neuropathy to sarcoidosis can be challenging, especially in the event of normal brain imaging and the often poor correlation between abnormal imaging and clinical findings. For example, in 13 patients with central nervous system (CNS) sarcoidosis and cranial neuropathies, only 9 had correlating brain imaging findings[11]. Several explanations of negative brain imaging in patients with cranial neuropathies related to sarcoidosis have been proposed, including extra-cranial nerve involvement, minimal infiltration of the involved cranial nerve by the disease, and small size granulomas.

Cranial neuropathy of neurosarcoidosis can involve one or multiple cranial nerves simultaneously. Table 2 shows the frequency of occurrence of cranial nerve involvement. Cranial nerves can be affected by direct infiltration of the nerve at any anatomical location, extra- or intra-cranially, or by other processes such as increased intracranial pressure and mass lesions.

THE PATHOGENESIS OF NEUROSARCOIDOSIS

Several mechanisms have been proposed to explain the pathogenesis of sarcoidosis, but none are conclusive. Several studies suggest a particular role of T-lymphocytes, triggered by an antigen of an unknown origin, in amplifying a local cellular immune response that is crucial for the development of sarcoidosis[12,13]. Non-necrotizing granulomas of sarcoidosis are composed of epithelioid macrophages, lymphocytes, and monocytes, and the consequential inflammation is often perivascular. Thickening of the vascular intima and media, along with fibrosis, may lead to ischemic injury.

CNS sarcoidosis has a predilection to involve the leptomeninges, with a granulomatous inflammatory exudate that infiltrates brain parenchyma through the Virchow-Robin spaces[14,15]. This pattern of infiltration may explain the predilection of neurosarcoidosis to the base of the brain where the Virchow-Robin spaces are particularly large and, consequently, the high incidence of cranial neuropathies[16-18].

OLFACTORY NERVE, OR CRANIAL NERVE-I

Involvement of the olfactory nerve, cranial nerve-I (CN-I), in sarcoidosis is considered rare[19]. Clinical signs and symptoms include anosmia and hyposmia. Isolated involvement of CN-I in patients with neurosarcoidosis is rare, and anosmia is an extremely infrequent isolated clinical presentation[20]. In a series published by Delaney[2], 17% of patients with neurosarcoidosis had anosmia, whereas Colover *et al*[20] reported this symptom in only 2 of 118 cases (< 0.2%). CN-I can be affected by direct infiltration of the nasal mucosa, intracranial disease, basal granulomatous meningitis, or a combination of these mechanisms. Kieff *et al*[21] reported a case of a 51-year-old man who presented with a 6-wk history of anosmia and visual difficulty. Magnetic resonance imaging (MRI) of the brain showed an enhancing subfrontal, extra-axial mass with accompanying edema. Tissue biopsy demonstrated non-caseating granulomas, consistent with the diagnosis of neurosarcoidosis.

OPTIC NERVE, OR CRANIAL NERVE-II

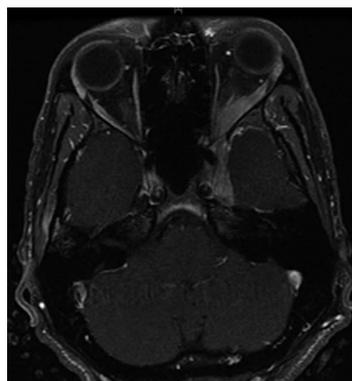Following the facial nerve, the optic nerve, or cranial nerve-II (CN-II), is the second most commonly involved

nerve in patients with neurosarcoidosis[7]. Approximately 5% of patients with sarcoidosis experience some type of optic neuropathy during the course of the disease, and about 30% of those will have other signs of neurosarcoidosis. Granulomatous infiltration of the optic nerves, chiasm, or tracts has been reported in autopsy studies[22].

Clinical signs of optic neuropathy occur as a result of increased intracranial pressure and papilledema, intracranial compression leading to optic atrophy, and/or direct invasion of the nerve by the forming granulomas. Optic nerve involvement is associated with papilledema, disc edema, or optic nerve head granulomas. Disc edema is the most common optic nerve abnormality in patients with neurosarcoidosis, with optic atrophy and neuritis being much less frequent[23]. Retrobulbar involvement of the optic nerve may mimic the clinical picture of optic neuritis, with acute loss of vision, with or without optic disc edema[24,25]. Pituitary granulomatous disease may also extend to affect the optic chiasm, with a correlating clinical picture of bi-temporal visual field loss and pituitary dysfunction[26,27]. Infiltration of the optic tract or the visual cortex is much less common.

OCULOMOTOR, TROCHLEAR, AND ABDUCENS NERVES (CN-III, -IV, AND -VI)

External ophthalmoplegia is an infrequent manifestation of CNS sarcoidosis[2]. Involvement of CN-III, -IV and -VI is rare[20]. Potential pathological mechanisms leading to ophthalmoplegia include direct invasion of a cranial nerve or extraocular muscles by granuloma, increased intracranial pressure, leptomeningeal disease, or orbital mass effect. Ischemia to the involved cranial nerve as a result of perivasculitis has also been suggested as a mechanism contributing to ophthalmoplegia in a patient with neurosarcoidosis[28]. Overall, the frequency of extraocular muscles and/or innervating cranial nerve involvement in neurosarcoidosis is felt to be under-reported, as biopsy of these structures is rarely performed. Clinical signs and symptoms include double vision, ptosis, pupillary involvement, and ophthalmoplegia.

There are several reports of CN-III palsy as a manifestation of CNS sarcoidosis, typically as a result of aseptic meningitis causing multiple cranial neuropathies[29-31]. Ueyama *et al*[30] reported a patient with isolated CN-III palsy as an initial manifestation of sarcoidosis. The case was of a 28-year-old man who presented with sudden onset of complete CN-III palsy. A conventional cerebral angiogram was unremarkable. Cerebrospinal fluid analysis revealed elevated lymphocytes and protein, but negative cytologic analysis. Brain MRI showed enhanced thickening of CN-II at the level of the ponto-midbrain junction. A chest radiograph revealed bilateral hilar lymphadenopathy, and lymph node biopsy showed non-caseating granulomas confirming sarcoidosis[30]. Velazquez *et al*[31] reported a case of a 53-year-old woman who presented with bilateral CN-III palsy and was subsequently found

Figure 1 Brain magnetic resonance imaging with gadolinium showing diffuse thickening and enhancement of the dura involving the left cavernous sinus, with mild mass effect on the left temporal lobe, and soft tissue enhancement extending anteriorly through the foramen rotundum and left orbital apex.

to have biopsy-proven sarcoidosis. The majority of other cases reported on CN-III palsy related to sarcoidosis were associated with multiple cranial neuropathies[11,28].

As stated previously, involvement of CN-IV or -VI is rare[2,20]. In the series published by Wiederholt *et al*[19], 18 of 807 patients with sarcoidosis had cranial nerve lesions. No trochlear or abducens nerve involvement was reported, whether in isolation or in combination with other cranial neuropathies.

We evaluated a 23-year-old African American man who presented to our institution with painless bulging of the left eye of three months duration, associated with diplopia. On the day of admission, he had a first-event witnessed generalized tonic-clonic seizure. The patient had a normal neurological examination except for left CN-VI palsy. Brain MRI revealed diffuse thickening and enhancement of the dura involving the left cavernous sinus (Figure 1). A computed tomography of the chest, abdomen, and pelvis with and without contrast was unremarkable for any sarcoidosis lymphadenopathy or malignancy. A left cavernous sinus dural biopsy revealed extensive chronic inflammation containing non-necrotizing granulomas, consistent with sarcoidosis.

TRIGEMINAL NERVE, OR CRANIAL NERVE-V

Involvement of the trigeminal nerve (CN-V) is exceedingly uncommon in patients with sarcoidosis[7]. Sarcoidosis can infiltrate any of the three divisions of CN-V, with or without eye involvement. Involvement of CN-V is usually sensory and unilateral, and commonly accompanied by other cranial neuropathies[20]. Clinical signs and symptoms include facial numbness, hypesthesia, and/or corneal ulcers. Biopsy of CN-V is not a common practice, and the physician must thus rely on the clinical presentation and neurological examination.

Three cases of isolated unilateral trigeminal nerve involvement in patients with sarcoidosis have been reported[32]. The first was of a patient with pulmonary

sarcoidosis who presented with complete unilateral ophthalmoplegia and cavernous sinus syndrome involving CN-V[33]. A case of another patient with mediastinal and parotid sarcoidosis and bilateral Gasser's ganglion cistern involvement has been reported with no ocular findings[34].

Absence of corneal sensation can result from impairment of trigeminal corneal innervation, a condition known as neurotrophic keratopathy. Gupta *et al*[35] reported a particularly rare case of isolated bilateral CN-V neuropathy in a patient with sarcoidosis who presented with neurotrophic corneal ulcers and was diagnosed with biopsy-proven cutaneous sarcoidosis. The patient also had decreased sensation to light touch involving all divisions of the trigeminal nerve bilaterally, with no other cranial neuropathies. After all potential causes of CN-V neuropathy were ruled out, isolated bilateral trigeminal neuropathy as a result of sarcoidosis was the confirmed diagnosis[35].

FACIAL NERVE, OR CRANIAL NERVE-VII

Of all the cranial nerve syndromes associated with sarcoidosis, peripheral cranial nerve-VII (CN-VII) palsy is the most common and is the single most frequent neurologic manifestation[36,37]. Facial neuropathy makes up 25%-50% of neurological manifestations of sarcoidosis[7,28]. Although usually unilateral, bilateral CN-VII involvement can occur, presenting with either simultaneous or sequential paralysis[37,38]. CN-VII can therefore be affected unilaterally, bilaterally, or simultaneously with other cranial nerves[39]. Sarcoidosis affects CN-VII either secondary to meningitic reaction or parotid gland inflammation, and may precede or follow parotitis. Clinical signs and symptoms include facial diplegia, peripheral facial palsy, and/or hemiageusia. Other potential etiologies including Lyme disease, human immunodeficiency virus, syphilis, brain stem lesions, leukemia, meningitis, Guillain-Barré syndrome, and diabetes mellitus need to be considered and investigated[40-42].

Facial nerve infiltration can occur at different anatomical locations. Rarely is the facial palsy caused by parotid inflammation[43] or part of uveoparotid fever (Heerfordt's syndrome), which includes fever, enlarged parotid glands, uveitis, and unilateral or bilateral facial neuropathy. In patients with sarcoidosis, CN-VII is more commonly affected as it traverses the meninges and subarachnoid space. Facial nerve paresis could also be due to intra-axial sarcoidosis-induced inflammation[43]. Necrotizing nerve ischemia and granulomatous infiltration of the epineurium are suggested mechanisms of facial neuropathy[44]. CN-VII involvement can be part of multiple cranial neuropathies, especially with meningeal infiltration[36,45]. In general, the prognosis for CN-VII is good, with over 80% of patients having a favorable outcome if treated early[46].

VESTIBULOCOCHLEAR NERVE, OR CRANIAL NERVE-VIII

Involvement of cranial nerve-VIII (CN-VIII) has been reported as a neurological manifestation in 1%-7% of patients with sarcoidosis[7,45,47-50]. Clinical signs and symptoms include vertigo, tinnitus, deafness, and sensorineural hearing loss. Neurosarcoidosis should be entertained as a diagnosis in a patient with sensorineural hearing loss of an unknown source, especially if a diagnosis of systemic sarcoidosis is known. Several cases of sensorineural hearing loss have been reported in patients with sarcoidosis[51-54]. In a report by Babin *et al*[55], autopsy findings in a patient with a known diagnosis of sarcoidosis and deafness included perivascular granulomatous inflammation within the internal auditory meatus. The authors attributed the vestibulocochlear impairment to vascular occlusion, as the severity of cochlear destruction did not correlate with the degree of cochleae infiltration[55].

Cama *et al*[56] reported two patients with sudden hearing loss that was attributed to sarcoidosis, with different findings on brain imaging studies. The first reported case was of a 29-year-old man who presented with left-sided hearing loss and facial nerve paralysis. Initial evaluation revealed bilateral sensorineural hearing loss and right anterior uveitis. Brain MRI with gadolinium was normal. Further imaging studies revealed multiple small pulmonary cavities and abdominal lymphadenopathy. Percutaneous hepatic biopsy revealed giant-cell granulomas. The initial presenting symptom of hearing loss was attributed to systemic sarcoidosis with CNS involvement[56].

The second case was of a 44-year-old man with a known diagnosis of systemic sarcoidosis who presented with diplopia and unsteadiness, followed by sudden right-sided hearing loss a few weeks later. Initial evaluation revealed sensorineural hearing loss of a cochlear origin. Contrast-enhanced brain MRI was negative. One month later he had worsening of the right-sided and new left-sided hearing loss. Brain MRI with gadolinium showed bilateral enhancement of the internal auditory meatus. A follow-up MRI two months later showed diffuse enhancement of basal leptomeninges, myelinic sheath of both optic nerves, trigeminal nerves, and pial surfaces of the cerebellar folia. The patient's hearing impairment, secondary to CNS involvement of systemic sarcoidosis, remained stable on oral corticosteroids[56].

GLOSSOPHARYNGEAL NERVE, OR CRANIAL NERVE-IX

Isolated glossopharyngeal neuropathy associated with sarcoidosis is extremely rare[57]. Combined involvement of cranial nerves IX, X, and XI is the third most common cranial neuropathy after facial and optic nerves involvement[58]. The most common site of involvement is in the lateral medulla or subarachnoid space[57]. The main presenting symptoms are dysphagia and hoarseness of voice[57,58].

VAGUS NERVE, OR CRANIAL NERVE-X

Cranial nerve-X (CN-X) involvement in neurosarcoidosis

is rare, with only a few cases reported in the literature[59]. Vagal neuropathy can occur in isolation as a manifestation of neurosarcoidosis or in combination with other cranial neuropathies. Neurosarcoidosis should be considered in a patient with vocal fold paresis of no apparent etiology. Two cases of CN-X involvement were reported in a retrospective review of 35 cases of confirmed neurosarcoidosis[3]. Additionally, Alon *et al*[60] conducted a retrospective study of a small cohort of 53 patients who presented with neurosarcoidosis and found only four with clinical or radiological findings suggestive of CN-X involvement. None of the four patients had a known diagnosis of systemic sarcoidosis. All four patients had vocal fold motion impairment. In one patient, a retropharyngeal mass was identified with biopsy-proven noncaseating granulomas, which extended to the jugular foramen several months later. The patient was found to have unilateral vocal fold paralysis and palatal weakness. Another patient with a history of chronic cough presented with right vocal fold paralysis and decreased gag reflex. A mediastinal lymph node biopsy revealed non-caseating granulomas. A third reported patient initially presented with unilateral throat and tongue burning sensation as well as vocal cord and tongue paresis. An MRI of the brain showed an enhancing mass in the jugular foramen extending into the right hypoglossal canal and second division of CN-V. Finally, a case of bilateral vagus and glossopharyngeal nerve enhancement was reported in a patient with biopsy-proven sarcoidosis who presented with palatal weakness and vocal folds paralysis[60].

SPINAL ACCESSORY NERVE, OR CRANIAL NERVE-XI

Isolated spinal accessory neuropathy has not been reported as a clinical manifestation of neurosarcoidosis. However, cranial nerve-XI neuropathy has been reported in combination with other cranial neuropathies. Clinical manifestations include ipsilateral sternocleidomastoid and trapezius muscle weakness.

HYPOGLOSSAL NERVE, OR CRANIAL NERVE-XII

Hypoglossal nerve involvement commonly occurs with other cranial neuropathies. As with CN-IX, -X, and -XI, the medulla and subarachnoid space are the most common sites of cranial nerve-XII involvement. The nerve is commonly affected as a result of a meningeal process, such as pachi meningitis, or focal granulomatous disease involving the medial medulla. The main presenting symptom is dysarthria[61,62], but patients can also have tongue deviation and atrophy.

Multiple cranial neuropathies of sarcoidosis

In most patients with neurosarcoidosis, more than one cranial nerve is involved[59]. Loor *et al*[45] reported a 26-year-old woman with an initial presentation of left-sided facial palsy and sensorineural hearing loss. MRI of the brain with gadolinium revealed enhancement of the left CN-VII and bilateral CN-VIII. A chest X-ray demonstrated hilar lymphadenopathy. The patient later developed anosmia, and all her symptoms resolved after a course of steroid treatment[45].

Chapelon *et al*[3] reported a case of a woman with bilateral vestibular symptoms, as well as CN-VII, -IX, -X, and -XI involvement. Another case reported by Chapelon *et al*[3] was of a 21-year-old man with a history of confirmed sarcoidosis who presented with multiple cranial neuropathies (CN-VII, -X, -XI, -XII). As discussed earlier, the predilection of sarcoidosis to the base of the brain is a plausible explanation of multiple cranial neuropathies.

TREATMENT OF NEUROSARCOIDOSIS

Corticosteroids remain the gold standard treatment of patients with neurosarcoidosis, and patients with symptoms should be treated initially with pulse corticosteroid therapy[63]. If the use of steroids is limited secondary to resistance or adverse reactions, immunosuppression therapy is recommended.

According to recent recommendations made by Nozaki *et al*[64] in 2013, prednisone is the first-line of therapy in patients with cranial neuropathy secondary to neurosarcoidosis, particularly if CN-VII is involved, at a daily dose of 20-40 mg. If prednisone cannot be tapered to less than 10 mg per day within 3-6 mo, a higher dose or an alternative agent should be considered. Recurrence of symptoms has been reported when prednisone was tapered to less than 20-25 mg daily[64].

Immunomodulating agents include methotrexate, considered the first agent of choice that allows tapering the prednisone to 10-20 mg per day in one third of neurosarcoidosis patients[64]. Other immunosuppressant agents to be considered include azathioprine, cyclophosphamide, and cyclosporine.

CONCLUSION

Neurosarcoidosis is a rare manifestation of sarcoidosis. The diagnosis can be challenging, as many conditions can mimic neurosarcoidosis both clinically and radiographically. Sarcoidosis mononeuropathy may occur at different stages of the disease and even as the initial clinical manifestation of CNS involvement. Cranial neuropathy can present as an isolated entity of sarcoidosis in the absence of systemic involvement, which makes the diagnosis challenging and dependent on tissue biopsy. In these patients, extensive work-up is warranted to rule out infections and demyelinating conditions, as well as inflammatory and autoimmune diseases.

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MINIREVIEWS

Cerium oxide nanoparticles as promising ophthalmic therapeutics for the treatment of retinal diseases

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Abstract

Nanotechnology offers exciting new approaches for biology and medicine. In recent years, nanoparticles, particularly those of the rare metal cerium, are showing potential for a wide range of applications in medicine. Cerium oxide nanoparticles or nanoceria are antioxidants and possess catalytic activities that mimic those of super oxide dismutase and catalase, thereby protecting cells from oxidative stress. The retina is highly susceptible to oxidative stress because of its high oxygen consumption and high metabolic activity associated with exposure to light. Many retinal diseases progress through oxidative stress as a result of a chronic or acute rise in reactive oxygen species. Diseases of the retina are the leading causes of blindness throughout the world. Although some treatments may delay or slow the development of retinal diseases, there are no cures for most forms of blinding diseases. In this review is summarized evidence that cerium oxide nanoparticles can function as catalytic antioxidants *in vivo* in rodent models of age-related macular degeneration and inherited retinal degeneration and may represent a novel therapeutic strategy for the treatment of human eye diseases. This may shift current research and clinical practice towards the use of nanoceria, alone or in combination with other therapeutics.

Key words: Nanoceria; Age-related macular degeneration; Inherited retinal degeneration; Oxidative stress; Antioxidant

Core tip: This review outlines the recent findings that cerium oxide nanoparticles (nanoceria) may represent novel and broad spectrum therapeutic agents to treat retinal diseases including age-related macular degeneration, retinal angiomatous, inherited retinal degeneration, and fight inflammation and pathologies associated with oxidative stress.

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INTRODUCTION

Many retinal diseases including retinopathy of prematurity, inherited retinal degeneration, diabetic retinopathy, retinitis pigmentosa, glaucoma, and age-related macular degeneration are the leading causes of blindness in infants, adults, and the elderly, respectively. The etiology or development of many retinal diseases involves oxidative stress[1-4]. An imbalance between the production of reactive oxygen species (ROS) and the detoxification of their reactive intermediates causes oxidative stress[5]. Excessive ROS levels can damage lipids, proteins, and nucleic acids. This process subsequently leads to cell death unless it is neutralized by the oxidant defense system. The retina possesses the highest rate of oxygen metabolism and therefore is at higher risk of oxidative damage due to redox imbalance.

Besides traditional antioxidant agents, in recent years special attention has been given to cerium oxide nanoparticles or nanoceria as antioxidants in biological systems[6,7]. Cerium (Ce) is a rare earth element in the lanthanide series of the periodic table. Cerium oxide (CeO_2) nanoparticles are used extensively in a variety of applications such as oxygen sensors[8,9]. The underlying molecular mechanism for the action of cerium oxide nanoparticles is generally thought to be their dual oxidation state, depending on the reaction conditions[10,11]. Nanoceria switch between Ce^{4+} and Ce^{3+} states creating an oxygen vacancy. This capability of these nanoparticles is similar to that of biological antioxidants[12]. Because of these unique antioxidant properties nanoceria act as free-radical scavenger. Free radical scavenging by nanoceria functions by decreasing ROS and has potential uses in various biological applications[7]. It has been recently reported that cerium oxide nanoparticles possess neuroprotective[13,14], radioprotective[15], cardioprotective[16], anti-inflammatory[17], anti-invasive[18], pro-oxidative and antioxidative[19-23], anti-angiogenic[24], pro-apoptotic and anti-apoptotic[21,12] properties. During the past few years, much attention and efforts has been made at addressing the potential use of nanoceria as therapeutic antioxidants for the treatment of oxidative stress related diseases[25-27]. Due to their smaller particle size at about 5 nm in diameter, which allows for easier passage through cell membranes, non-toxic nature and excellent biocompatibility, cerium oxide nanoparticles also have the potential to be used as drug carriers and delivery agents.

In the last few years, our group is involved in developing cerium oxide nanoparticles as therapeutic agents for treatment of retinal diseases. We demonstrated for the first time that these nanoparticles are able to prevent the increases of intracellular ROS concentrations *in vitro* using primary cell cultures of rat retina and could protect retinal morphology and function *in vivo* using an albino rat light-damage model[28]. Next, in the homozygous *tubby* mutant mouse, which displays inherited early progressive cochlear and retinal degeneration that are similar to those of human Usher syndrome, we showed that cerium oxide nanoparticles preserve the retina by decreasing the con-

centrations of ROS, up-regulating the neuroprotection-associated genes expression; down-regulating apoptosis signaling pathways and/or up-regulating survival signaling pathways[29]. Furthermore, in an age-related macular degeneration (AMD) model and in particular for retinal angiomatous proliferation (RAP), the very low-density lipoprotein receptor knockout mouse (*vldlr$^{-/-}$*), we have reported that cerium oxide nanoparticles stopped the development and regression of pathological neovascularization[30]. Our data also demonstrated that nanoceria inhibited the expression of genes associated with inflammation, angiogenesis, and down-regulated MAP kinases, Akt, ASK1 and NF-κB signaling pathways[31,32]. This review aims to provide the recent findings and potential applications of nanoceria for the treatment of retinal diseases.

OXIDATIVE STRESS AND RETINAL DISEASES

Oxidative stress is defined as a disturbance in the balance between the production of ROS, which include hydrogen peroxide, superoxide anion, and hydroxyl radicals, and antioxidant defenses. Although ROS have important roles in regulating signal transduction and cellular function[33], their overproduction can damage lipids, proteins, and DNA, thus affecting many cellular and physiological mechanisms. Numerous pathological conditions have an oxidative stress component, including cardiovascular diseases[34], neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's diseases[35-37], and cancer[38]. Oxidative stress has also been implicated in retinal diseases such as AMD, inherited retinal degeneration, diabetic retinopathy, retinitis pigmentosa, glaucoma and uveitis[1-4,39]. The retina is extremely vulnerable to ROS damage[40]. ROS can be formed in many ways including as a product of the respiratory chain in mitochondria, photochemical and enzymatic reactions as a result of the exposure to ultraviolet light, ionizing radiation, or heavy metal ions[41-47]. Retinal cells have the highest rate of oxygen metabolism of any cells and are frequently exposed to the damaging effects of oxidative stress due to the the excessive exposure to light.

AMD is the leading cause of severe and irreversible loss of vision in the elderly in the world. AMD is divided into two broad types: "dry" and "wet" that account for about 85% and 15% of cases, respectively. "Wet" or exudative AMD, is the most severe form of AMD and is associated with subretinal neovascularization. By contrast, "dry" also known as atrophic or non-exudative AMD, tends to exhibit a slow progression of the disease. This complex disease has both genetic and environmental risk factors with a number of gene polymorphisms being identified which increase susceptibility to environmental risk factors such as smoking, hypertension, diet, obesity, prolonged sun exposure, and oxidative stress[4,48,49]. While there is currently no cure for AMD, some treatments can prevent severe vision loss or decrease the progression of the disease considerably. AMD treatments include anti-

vascular endothelial growth factor (VEGF) therapy, laser surgery, photodynamic therapy, vitamins and nutritional supplements[50-53]. The abundance and complex interactions between the risk factors for AMD limit the effectiveness of therapeutic options. Therefore, new therapeutics is needed to target multiple pathophysiological aspects that contribute to development of AMD, most importantly oxidative stress.

There are other inherited and acquired diseases or disorders that may affect the retina. Retinitis pigmentosa (RP) is a heterogeneous group of inherited ocular diseases that result in a progressive retinal degeneration. RP is the largest Mendelian genetic cause of blindness affecting 1 in 3000 to 5000 people worldwide[54]. This disease exhibits abnormalities in the photoreceptors or in the retinal pigment epithelium of the retina, which lead to progressive visual loss. RP can be inherited in an autosomal dominant, autosomal recessive or X-linked manner[55]. RP may also occur as part of Usher syndrome and Bardet-Biedl syndrome[56]. Usher syndrome is the most common hereditary form of combined deafness and blindness in humans[55]. The oxidative stress hypothesis is supported by several lines of evidence in experimental models of Retinitis pigmentosa[57-60]. In addition, it has been found that Retinitis pigmentosa patients have reduced ocular antioxidant status and antioxidant imbalance in the peripheral blood[60]. Although there is no cure for RP, treatments are available for managing some aspects of its clinical manifestations[61].

CERIUM OXIDE NANOPARTICLES

Cerium belongs to the lanthanide series of rare earth elements. Although most of the rare earth elements of the periodic table exist in the trivalent state, cerium in an oxide nanoparticle can occur in either a 3+ (fully reduced) or 4+ (fully oxidized) state and may flip-flop between the two in a redox reaction. As a result of this, cerium oxides form oxygen vacancies or defects in the lattice structure[11,62]. It is these defects or reactive sites on the cerium oxide nanoparticles that serve as sites for free radical scavenging. Cerium oxide nanoparticles react catalytically with ROS, including hydroxyl radical, superoxide radical and hydrogen peroxide, providing antioxidant properties[12,63]. It has been demonstrated that cerium oxide nanoparticles act as a catalyst that mimics enzymatic antioxidants including superoxide dismutase (most apparent when cerium is in the 4+ state)[64] and catalase (most apparent when cerium is in the 3+ state)[65]. Various techniques including frame spray pyrolysis[66] and wet chemical methods[12,17] have been reported to synthesize cerium oxide nanoparticles. The radical scavenging activities of cerium oxide are even further increased when synthesized as a nanoparticle. Moreover, as the size of the cerium oxide nanoparticle decreases, there is a concurrent increase of cerium in the +3 state, which may further enhance reducing power[67]. Smaller diameter nanocrystals containing more cerium (+3) were found to be more reactive toward hydrogen peroxide[68]. In addition, the presence of a surface coating did not prevent the reaction between the nanocrystal surface cerium (3+) and hydrogen peroxide[68]. Therefore, the most reactive nanoparticles are at about 5-10 nm diameter with the thinnest surface coating (*e.g.*, oleic acid). The radical scavenging properties of cerium oxide can be drastically increased during the reduction to the nanoscale.

Cerium oxide nanoparticles used in our studies were synthesized using wet chemical method as described previously[12]. Briefly, cerium nitrate hexahydrate was dissolved in distilled water and the solution was oxidized using excess of hydrogen peroxide. To maintain the synthesized nanoparticles in suspension, the pH of the solution was kept below 3.0. These cerium oxide nanoparticles contain individual crystallites of 3-5 nm and can be diluted in aqueous and cellular media. The size and shape of the particles was characterized using transmission electron microscope, zeta potential of the suspension was monitoring using dynamic light scattering and X-ray photoelectron spectroscopy was used to determine the surface oxidation state of the nanoparticles as reported previously by us[69].

BIOLOGICAL PROPERTIES OF CERIUM OXIDE NANOPARTICLES

Although cerium oxide nanoparticles have been widely used as oxygen sensors[9] and automotive catalytic converters[70], they have recently begun to be used in biological systems[6,7]. The ability of these nanoparticles to switch oxidation states and their antioxidant activity has a unique advantage for therapeutic implications. The biological properties using *in vivo* mice models of AMD and inherited retinal degeneration and potential applications of cerium oxide nanoparticles as ophthalmic therapeutics are discussed below.

Antioxidant properties

The antioxidant properties of nanoceria were investigated first in primary cell cultures of dissociated rat retinas. Chen *et al*[28] demonstrated by flow cytometric analysis of dichlorofluorescein (DCF) stained retinal cells that nanoceria particles (1, 3, 5, 10 or 20 nmol/L) can effectively inhibit hydrogen peroxide-induced rise of intracellular ROS. Next, we showed that cerium oxide nanoparticles possessed radical scavenging activity *in vivo* by preventing increases in retinal ROS in an albino rat light-damage model[28]. Furthermore, we explored the *Vldlr* knockout mouse, which carries a loss-of-function mutation in the *Vldlr* gene[71]. Studies have revealed that the *Vldlr*^{-/-} mouse recapitulates many key characteristics in patients with AMD who have Retinal Angiomatous Proliferation, a form of wet AMD, and can serve as a unique mouse model of neovascularization-associated oxidative stress[72-74]. Our studies have revealed that a single intravitreal injection of 1 μL of 1 mmol/L (172 ng) nanoceria suspended in saline at postnatal day (P)7 greatly reduced the amount of ROS, measured by

two independent methods, DCF and dihydroethidium (DHE), in the *Vldlr*^{-/-} retinas three weeks later at P28[28]. Similar results were obtained with three other biomarkers of oxidative damage, NADPH oxidase (p47phox), nitrotyrosine and 8-hydroxy-2-deoxyguanosine (8-OHdG). We further confirmed our previous observation by demonstrating that acrolein, a commonly used oxidative stress marker for detecting lipid peroxidation, is higher in *Vldlr*^{-/-} retinas and nanoceria greatly reduced the level of acrolein[32].

Key mediators of the biological effects downstream of ROS include several signaling pathways such as MAP kinases, ASK1, and PI3K/Akt[75,76]. We hypothesized that if ROS were destroyed by cerium oxide nanoparticles, the downstream effects should be decreased. Therefore, we determined whether MAP kinases and Akt are elevated in the retinas of *Vldlr*^{-/-} mice and whether nanoceria can inhibit their activation. Both kinases are elevated in *Vldlr*^{-/-} retinas and a single intravitreal injection of cerium oxide nanoparticles for 1 wk inhibits the phosphorylation of ERK, JNK, and the p38 MAPKs, as well as Akt almost to control wild type (WT) mice treated with nanoceria[31]. We further examined the long-term therapeutic effects of cerium oxide nanoparticles in *Vldlr*^{-/-} retinas and showed that phosphorylated ASK1, JNK and p38, as well as NF-κB are remarkably reduced by nanoceria treatment up to 6 wk post injection[32].

In another experimental paradigm, the *tubby* mouse was used as a model of inherited retinal degeneration to test the ability of cerium oxide nanoparticles to act as direct *in vivo* antioxidants. *Tubby* mice are homozygous for a mutation in the *Tub* gene and have hearing loss and retinal degenerations, major hallmarks of Usher syndrome[77]. To examine the ability of nanoceria to alter ROS, we determine the amounts of ROS by DCF and DHE methods in the retina of *tubby* mice at P18 injected intracardially with 20 μL of 1 mmol/L cerium oxide nanoparticles[29]. The levels of ROS in injected with nanoceria retinas were decreased to control levels. Moreover, we demonstrated that the expression of antioxidant-associated proteins, thioredoxin (Trx) and nuclear factor erythroid 2-related factor (Nrf2) is increased after nanoceria treatment. These results clearly suggest that cerium oxide nanoparticles can scavenge ROS in the retina and thereby inhibit oxidative stress in mice models of AMD and inherited retinal degeneration.

Anti-angiogenic properties
Angiogenesis is a process of forming new blood vessels that is a hallmark in the pathology of many diseases including AMD, diabetic retinopathy, and retinopathy of prematurity. Activators of angiogenesis include the VEGF, angiopoietins and members of the fibroblast growth factor (FGF) family. There is considerable evidence that increased production of ROS in the retina participates in retinal angiogenesis. We have shown that upregulation of retinal VEGF can be detected as early as P14 in *Vldlr*-/- mice[30]. To examine if nanoceria treatment could reduced angiogenesis by inhibiting VEGF, we

determined the effect of nanoceria on VEGF protein expression in *Vldlr*^{-/-} retinas at P14 and P28. We observed a significant decreased of VEGF in retinas of *Vldlr*^{-/-} mice after a single injection of nanoceria at P7. We examined the localization of VEGF and found that cerium oxide nanoparticles inhibit the ectopic expression of VEGF in the outer nuclear cell layer (ONL) of the *Vldlr*^{-/-} retina. Furthermore, using real-time PCR we demonstrated that cerium oxide nanoparticles dramatically decreased the levels of *Vegfa* expression in *Vldlr*-/- retinas[31]. Our PCR array results also showed that the expression of most of the *Fgf* genes, including *Fgf* 1, 2, 3, 5, 7, 9, 11, 21, and 22, are increased in the retina of *Vldlr*^{-/-} mice and cerium oxide nanoparticles were able to decrease significantly their expression. These results clearly support our hypothesis that the rise in retinal VEGF in *Vldlr*^{-/-} mice can be prevented by the scavenging activity of cerium oxide nanoparticles.

Anti-inflammatory properties
Oxidative stress is well known to increase not only angiogenesis, but to drive the onset of inflammation. There is substantial evidence to show that inflammation play a role in AMD[78]. Although some reports have shown that several inflammatory cytokines are elevated in *Vldlr*^{-/-} retinas[72,79] the expression pattern of cytokines and their functions in the *Vldlr*^{-/-} mice have not been thoroughly determined. Therefore, we examined the cytokine expression in the *Vldlr*^{-/-} retina using a mouse cytokine PCR array that profiles 88 key cytokine genes[31]. We found that 37 cytokines were up-regulated and after one week of nanoceria injection 23 cytokines were down-regulated. Nanoceria markedly reduced the overexpression of Tlsp, Lif, IL-3, IL-7, IL-9, IL-12b, Lep, Ifn1, and others. This study suggests that cerium oxide nanoparticles have significant potential as anti-inflammatory agents.

Anti-apoptotic properties
Excessive production of ROS is the key event leading to cell death or apoptosis. The principle mechanism underlying retinal cell death and consequent blindness in several diseases is apoptosis. Apoptosis of neuronal cells is common to all mutations in *tubby* gene family members[80]. To determine the effect of cerium oxide nanoparticles on apoptosis in the retina of *tubby* mouse, the TUNEL assay was conducted[29]. The *tubby* retina demonstrated many more TUNEL positive cells that control retina. In this study, we also demonstrated that intracardial injection with cerium oxide nanoparticles significantly down-regulated caspase-3, 8, 9 and Bak1 expression. Likewise, we found that nanoceria markedly reduced the levels of caspase-3 in the retina of the *Vldlr*^{-/-} mouse[32]. Taken together, it is obvious that cerium oxide nanoparticles down-regulate caspase-induced apoptosis in the retina of mouse models of AMD and inherited retinal degeneration.

Protection of retinal function
To examine the ability of cerium oxide nanoparticles to protect retinal function, retinal responses to the

light stimulus were determined by full field and serial intensity electroretinography (ERG) in tubby mice at P34[29]. Full field ERG showed that injections with cerium oxide nanoparticles improved rod function in *tubby* mice compared to control, saline injected group. Serial intensity ERG of scotopic a- and b-waves showed that both amplitudes were significantly increased in nanoceria injected *tubby* eyes. Moreover, no changes in retinal functions was detected in nanoceria or saline injected rats for 9 d and even after 4 mo post injection[69]. There were no changes in scotopic a- and b-waves, photopic b-wave, and flicker. These data suggest that cerium oxide nanoparticles did not have side effects in the healthy retina.

Toxicity

There is always a concern regarding the potential toxicity of nanomaterials for biological applications. Several reports have shown that cerium oxide nanoparticles (< 10 nm) are well tolerated by animals and are not toxic[25,81], while others provide conflicting data about toxicity[82,83]. Most likely this discrepancy could be due to variation in methods of synthesis or due to differences in physiochemical properties of nanoparticles, surface charge, aggregation of the particles. Nanoceria used in our studies were small in size (3-5 nm) and well dispersed. To determine the safety of cerium oxide nanoparticles for therapeutic use, the cytotoxic effects of the particles intravitreally injected in rat retina after 9, 60 and 120 d was examined[69]. We performed quantitative analyses on superior and inferior central retina, superior and inferior peripheral retina and we did not determine any reduction in thickness in the layers examined for injected with cerium oxide nanoparticles eyes. As mentioned above there were no changes in retinal function between nanoceria or saline injected rats. These results indicate that cerium oxide nanoparticles synthesized according to our procedure[12,69], are not toxic to the rat retina as evaluated by morphology and function up to 12 mo post injection.

Bio-distribution

We determined nanoceria distribution and clearance in the eye using inductively coupled plasma mass spectrometry[12,69]. We observed the highest concentration of cerium oxide nanoparticles in retinal portion of the eye. A small amount of cerium oxide nanoparticles 1 h post injection were detected in the lens and the rest of the eye cup. We determined that approximately 70% of injected cerium oxide nanoparticles were retained in the rat retina more than 120 d and the elimination half-life is calculated to be 414 d. Only trace amounts of cerium oxide nanoparticles were detected in the liver and kidney from 120 d injected rats. These results strongly suggest that cerium oxide nanoparticles are rapidly and preferentially taken up by retinal cells and the rate of elimination is very slow. It is not yet known the mechanism of uptake of nanoceria in retinal cells. Three possible endocytosis pathways may be involved in uptake of nanoparticles into cells, including caveolae-, clathrin- mediated endocytosis, and macropinocytosis. It has been reported that fluorescein-conjugated nanoceria were taken up by keratinocytes *via* clathrin- and calveolae-mediated endocytic pathways[84]. Recently another study indicated that nanoceria could be also taken up into cells through caveolae- and clathrin-mediated endocytosis. Nanoceria were distributed throughout the cytoplasm but not into nucleus[85].

CONCLUSION

Cerium oxide nanoparticles extended the life of photoreceptor cells and preserved vision for up to 4 mo in a mouse with inherited retinal degeneration. Nanoceria prevent development of pathological neovascularizations in the *Vldlr*^{-/-} mouse (a model for Wet AMD) and also regress vascular lesions existing at the time of injection. Nanoceria have a half-life in the retina of 417 d and had no toxic effect on retinal structure and function when present for over a year. Nanoceria affect multiple signal transduction pathways by upregulating neuroprotective genes and downregulating pro-apoptotic and pro-inflammatory genes. Most recently, we showed that cerium oxide nanoparticles inhibit the growth of inherited retinoblastoma malignancies *in vivo* and shrink the volume of tumors present at the time of injection. Collectively, these data suggest that nanoceria are global antioxidants, which have "pan-disease" effectiveness against a number of degenerative eye diseases in multiple animal models and may be just as effective in the therapeutic treatment of many human eye diseases.

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MINIREVIEWS

Recent advances in management of retinoblastoma: A review

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routes are being increasingly employed world-wide for globe preservation. The advent of new radiotherapy techniques has led to improved radiation delivery to the target and more conformal treatment plans with better normal tissue sparing. This review aims to highlight newer advancements in the field of diagnosis and management of retinoblastoma that have been introduced in recent times, with a special emphasis on globe-preserving therapy.

Key words: Retinoblastoma; Recent advances; Chemotherapy; Radiotherapy

Core tip: The management of retinoblastoma has improved significantly over the past few decades. There has been a paradigm shift from enucleation towards conservative treatment modalities that aim at vision and globe salvage. The purpose of this article is to review the literature on various key developments in the field of retinoblastoma, with particular emphasis on globe-conserving treatment.

Abstract

The management of retinoblastoma has evolved significantly over recent years. Current treatment options aim to preserve the globe as well as vision with minimum morbidity. High resolution imaging has improved tumor detection and is useful for prognosticating cases and monitoring response to treatment. Targeted chemotherapy such as intra-arterial and intra-vitreal chemotherapy has shown promising results and these

INTRODUCTION

The diagnosis and management of retinoblastoma (RB) often presents as a challenge to the ophthalmologist. Recent advances have contributed towards improving the clinical outcome of the most common intraocular malignancy seen in children. Evolution in imaging techniques has facilitated accurate diagnosis and staging

of RB. There has been a paradigm shift from enucleation towards conservative treatment modalities that aim at vision and globe salvage. The introduction of intra-arterial and intra-vitreal chemotherapy in recent times has shown encouraging results. The advent of newer radiotherapy techniques have led to greatly improved radiation delivery to the target and more conformal treatment plans with better normal tissue sparing. The purpose of this article is to review the literature on various key developments in the field of RB, with particular emphasis on globe-conserving therapies. A brief overview of these recent advances is highlighted below.

IMAGING

Imaging plays a key role in the diagnosis of RB. With the introduction of high-resolution three-dimensional (3D) Fast Spin Echo (FSE) magnetic resonance imaging (MRI) and high resolution ultrasound, the diagnosis of RB is no longer a dilemma. Although computed tomograph scan is very useful in detecting calcification which can sometimes be missed on ultrasonography, it has been reported that high-resolution three-dimensional (3D) FSE T2 weighted imaging with thin sections (0.4 mm) and high Signal to Noise Ratio (SNR) can also detect calcification[1]. Gradient-echo T2 weighted MRI is also effective in detecting calcified structures[1]. Recently, it has been observed that the difference in Apparent Diffusion Co-efficient values on diffusion-weighted MRI can be helpful in differentiating between viable and necrotic tumor[2]. In addition, this modality can also be used to monitor the response of tumor to chemotherapy in cases of trilateral RB as well as in those eyes that are treated with globe salvaging therapies[2,3]. The presence of vitreous haemorrhage can pose difficulty in delineating the tumor, which can be overcome by T1-weighted MR images without the use of gadolinium-based contrast material[4]. Apart from its diagnostic value, MRI is also an established imaging modality for staging of RB[5]. Contrast-enhanced T1-weighted MR imaging with fat saturation is recommended to rule out optic nerve involvement as well as extra scleral involvement[6]. The sensitivity and specificity of MR imaging for depicting post-laminar optic nerve invasion has been reported to range from 50%-90%[4,5]. A retrospective study by Song *et al*[7] in cases of unilateral RB concluded that focal strong enhancement and enlarged optic nerve on MR films had better correlation with optic nerve invasion than optic nerve enhancement, tumor size and tumor location[7]. It is noteworthy that in some children, this enhancement can be due to aseptic cellutis or inflammation of soft tissues rather than true invasion[8]. A short course of systemic steroids and repeat MR imaging facilitates accurate staging in such cases and has been found to be useful in guiding further management[8].

Another application of imaging in RB is the use of high resolution ultrasound to detect the tumor in the fetus at its earliest stage[9]. Investigators have used high resolution ultrasound at 37 wk of gestation to detect a 2-3 mm elevated lesion in a fetus at risk of heritable RB[9].

Being a rapidly growing tumor, doubling time for RB is considered approximately 15 d[10]. Therefore, it has been suggested that infants proven to carry the family's *RB1* mutant allele can be delivered a few weeks early, to optimize the chances of retaining good vision with minimally invasive therapy[11].

CHEMOTHERAPY

Although enucleation is accepted as the standard treatment for advanced tumors, local and site selective delivery of chemotherapeutic drugs has shown encouraging results in salvaging the globe as well as vision in many eyes otherwise destined for enucleation. These newer therapeutic approaches are discussed briefly.

Super-selective intra-arterial chemotherapy

This novel approach has evolved rapidly over the last few years and has shown encouraging results in both early and advanced tumors[12,13]. Being a site directed therapy, it has considerably fewer systemic side-effects in comparison to conventional intra-venous chemotherapy. Over the last few decades, the selectivity of the technique has improved from using sites such as the internal carotid artery, supra-orbital artery and superficial temporal artery, to the currently used ophthalmic artery[14-18]. Melphalan is the drug of choice for intra-arterial chemotherapy and heparin (70 U/kg) is the anticoagulant used. There is no standardised dosing schedule, however, the conventional dose ranges from 3-5 mg per sitting[13,16,17]. Recently, Abramson *et al*[16] and Gobin *et al*[17] have recommended intra-arterial chemotherapy as a safe and effective treatment for advanced intra-ocular RB. Although intra-arterial chemotherapy has the advantage of fewer systemic side effects as compared to intravenous chemotherapy, some investigators consider melphalan as a more toxic agent than those drugs which are used for intravenous chemotherapy[19]. Exposure to fluoroscopy related radiation and ophthalmic artery occlusion are other concerns[19]. It has been suggested that a selective ophthalmic artery angiogram instead of carotid angiogram can be used to minimise radiation exposure[13]. Though not yet established as a primary treatment, intra-arterial chemotherapy has also been used as a first line treatment in less advanced cases of intraocular RB[12]. There are other investigators who consider it as a part of a multi-modal therapeutic approach[13,18]. Intra-arterial chemotherapy has been reported to be associated with an overall success rate of 55%-100% in salvaging the globe, in addition to the advantage of very low systemic toxicity[12,13]. Recently, Francis *et al*[20] have demonstrated that Carboplatin ± topotecan ophthalmic artery chemosurgery (OAC) can allow for prompt regression of tumors and can be curative as a single agent in combination with focal techniques, with ocular survival of 89.9% at two years. Furthermore, Carboplatin ± topotecan infusions have low hematologic and ocular toxicity and no statistically significant influence on electroretinogram responses, and can be used in conjunction with melphalan-containing OAC[20]. It has been recommended that children, especially

less than 6 mo of age at the start of treatment with carboplatin, should routinely undergo thorough long-term audiologic monitoring[21]. Recently, a single-centre retrospective study has compared the relative incidence of new intraocular lesions after treatment with carboplatin through intravenous (systemic) and OAC in naïve eyes, or those with prior treatment (systemic chemotherapy/external beam radiotherapy)[22]. The incidence reported were 56%, 2.4% and 8% respectively[22]. The systemic chemotherapy treated patients had multiple new lesions within months of treatment, as compared to fewer new lesions in the OAC group[22]. It was noted that previously irradiated eyes showed delayed appearance of new lesions. The new lesions were more common at a younger age and were usually located in the peripheral retina, which can be explained by the centrifugal development of retina[22].

Intravitreal chemotherapy

Another local route for drug delivery that has shown promising results in RB is intra-vitreal chemotherapy (IViC)[23,24]. However, this route is recommended only as salvage therapy for recurrent or recalcitrant vitreous seeds and should not be considered as a primary treatment[19]. In a study by Munier *et al*[23] in RB cases with recalcitrant vitreous seeds, melphalan was injected intravitreally in a dose of 20-30 μg (0.1 mL of 0.2 mg/mL) using anti-reflux procedure, followed by triple freeze-thaw cryoapplication to sterilize the needle track[23]. The procedure was carried out every 7-10 d and was repeated upto eight injections if a response could be documented, until complete seed fragmentation was observed or complete response was achieved[23]. Complete response was established if the seeds (1) completely disappeared (vitreous seeding regression type 0); or were converted into (2) refringent and/or calcified residues (vitreous seeding regression type Ⅰ); (3) amorphous, often non-spherical, inactive residues (vitreous seeding regression type Ⅱ); or (4) a combination of the last two (vitreous seeding regression type Ⅲ)[23]. The authors recommended that IViC could be repeated if vitreous recurrence occurred[23]. In their study, a success rate of 84.14% at 2 years was achieved[23]. A localised peripheral salt-and-pepper retinopathy at the injection site was the only complication noted in 10 eyes (43%)[23]. Another retrospective study on intra-vitreal chemotherapy by Shields *et al*[24] showed 100% (11/11) success rate with 1 to 4 cycles of monthly IViC (melphalan 20-30 μg) at 2 year follow-up[24].

Sub-conjunctival /sub-tenon chemotherapy

It has been observed that systemic chemotherapy alone may not be sufficient to treat Group C (eyes with focal vitreous or subretinal seeding and discrete retinal tumors of any size and location) and Group D (eyes with diffuse vitreous or subretinal seeding and/or massive, nondiscrete endophytic or exophytic disease) cases[25,26]. Local injections of chemo-therapeutic agents like sub-tenon or sub-conjunctival carboplatin have been used with varying degrees of success, usually as an adjuvant to systemic chemotherapy to avoid enucleation and

external beam radiotherapy in cases of group C and group D retinoblastoma with vitreous/subretinal seeds. The Children's Oncology Group recommends use of 20 mg sub-tenon carboplatin along with chemoreduction and focal consolidation for Group C and D tumors[27]. Leng *et al*[28] have reported a favourable outcome with the use of sub-conjunctival carboplatin in RB tumors that progressed despite ablative therapy[28].

RADIATION THERAPY

Despite the established role of radiotherapy (RT) in RB, treatment modalities were shifted to primary chemotherapy combined with local treatment options such photocoagulation, cryotherapy and thermotherapy[29,30]. The high incidence of radiation induced growth deformities and second malignancies was attributed to external beam radiotherapy and RT was therefore reserved for tumours refractory to chemotherapy and local therapies. However, the assessments of risk by RT were based on outcomes of radiation delivery in the old era[31,32]. In recent times, there has been substantial advancement in radiation therapy and the advent of newer radiotherapy techniques has led to greatly improved radiation delivery to the target and more conformal treatment plans with better normal tissue sparing. These newer radiotherapy techniques which include intensity modulated radiotherapy, stereotactic radiotherapy volumetric modulated arc therapy (VMAT), proton therapy, and helical tomotherapy (HT) provide highly accurate radiation delivery[33].

Proton beam therapy provides uniform dose coverage of the target and unlike photon beams, has no exit dose and distributes no energy beyond the target. These unique properties reduce the incidence of late effects of radiation. A study by Sethi *et al*[34] compared the risk of second malignancies in survivors of RB treated with photon and proton radiation therapy[34]. The observed 10 year cumulative incidence of RT induced second malignancies were significantly different in proton and photon modalities ($P = 0.015$)[34]. However, proton therapy is expensive and is currently not widely available. In another study on the dosimetric comparison of various RT techniques by Eldebawy *et al*[33], it was concluded that inverse image guided radiotherapy using VMAT or HT provides superior conformity index and improved orbital bone and brain sparing[33].

Plaque Brachytherapy is commonly used for recurrent and residual disease after failure with chemotherapy and local therapy. The American Brachytherapy Society Ophthalmic Oncology Task Force (ABS-OOTF) recommends primary brachytherapy for unilateral anterior lesions[35]. Small tumours less than 15 mm in base and up to 10 mm in thickness in the absence of vitreous seeding are eligible[35]. The choice of radionuclide is decided according to local availability and intraocular dose distribution. I^{125} and Pd^{103} are used in North America, whereas I^{125} and Ru^{106} are used in Europe. Dosimetry of plaques presents a unique challenge which is due to the steep dose gradient within the tumour and presence of criti-

cal structures within few millimetres of the radioactive source. However, the TG-129 reports that adoption of heterogeneous dose calculation methods in clinical practice would result in dose variation of > 10% and requires careful assessment[36].

GUIDELINES FOR PATIENT FOLLOW-UP

After completion of therapy, regular follow-up is extremely important in these children in order to detect any recurrence of tumor, new lesion, or metastatic disease. It is recommended to follow-up all affected cases till the age of 16 years and to conduct screening of unaffected relatives or mutation carriers till the age of five (reference: 2013 Copyright American Cancer Society) or seven years (reference: NHS England/E04/S(HSS)/a, Copyright NHS Commissioning Board, 2013).

To summarize, the management of RB has evolved significantly over the last few years. Worldwide, there is an increasing trend towards preservation of the globe and vision in RB affected children. Newer advancements in diagnostic and therapeutic modalities have resulted in improved treatment outcomes in these children. Familiarity with these diagnostic and treatment modalities is essential for optimum management.

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