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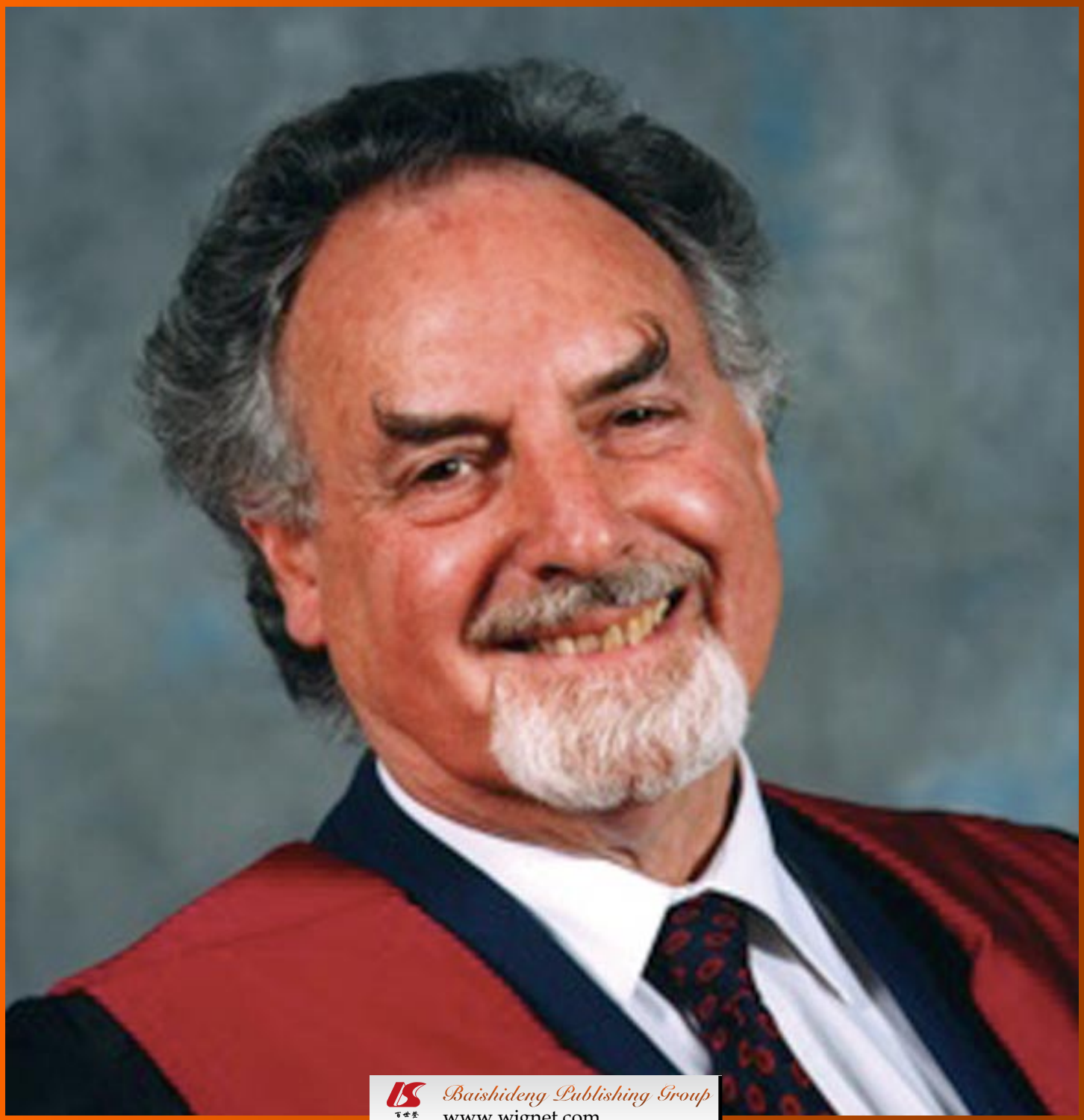

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EDITORIAL

What is the purpose of launching the *World Journal of Pharmacology*?

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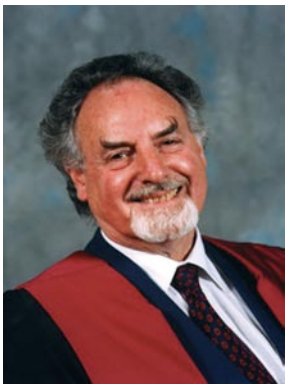

Figure 1 Editor-in-Chief of *World Journal of Pharmacology*. Geoffrey Burnstock, PhD, DSc, FAA, FRCS (Hon), FRCP (Hon), FmedSci, FRS, Professor, Autonomic Neuroscience Centre, University College Medical School, Royal Free Campus, Rowland Hill Street, London NW3 2PF, United Kingdom.

Abstract

The first issue of the *World Journal of Pharmacology* (*WJP*), whose preparatory work was initiated on January 10, 2011, will be published on February 9, 2012. The *WJP* editorial board has now been established and consists of 100 distinguished experts from 23 countries. Our purpose of launching the *WJP* is to publish peer-reviewed, high-quality articles *via* an open-access online publishing model, thereby acting as a platform for communication between peers and the wider public, and maximizing the benefits to editorial board members, authors and readers.

Key words: Maximization of personal benefits; Editorial board members; Authors; Readers; Employees; Open-access; Pharmacology

Peer reviewer: Oleg A Barski, PhD, Assistant Professor, Department of Medicine, Cardiology, University of Louisville, 580 S. Preston St., Rm. # 421, Louisville, KY 40241, United States

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INTRODUCTION

I am very pleased to announce that the first issue of the *World Journal of Pharmacology* (*World J Pharmacol*, *WJP*, online ISSN 2220-3192, DOI: 10.5497) on which preparation was initiated on January 10, 2011, is officially published on February 9, 2012. The *WJP* editorial board has now been established and consists of 100 distinguished experts from 23 countries. It is my great honor to have the world renowned pharmacologist, Geoffrey Burnstock, PhD, DSc, FAA, FRCS (Hon), FRCP (Hon), FmedSci, FRS, Professor, as the first Editor-in-Chief of the *WJP* (Figure 1). What is the purpose of launching the *WJP*? What is the scope and how are the columns designed?

The role of academic journals is to exhibit the scientific levels of a country, a university, a center, a department and even a scientist, and build an important bridge for communication between scientists and the public. As we all know, the significance of the publication of scientific articles lies, not only in disseminating and communicating innovative scientific achievements and academic views, as well as promoting the application of scientific achievements, but also in formally recognizing the "priority" and "copyright" of innovative achievements published, as well as evaluating research performance and academic levels. To realize these desired attributes of a journal and create

a well-recognized journal, the following four types of personal benefits should be maximized.

MAXIMIZATION OF PERSONAL BENEFITS

The maximization of personal benefits refers to the pursuit of the maximum personal benefits in a well-considered optimal manner without violation of laws, ethical rules and the benefits of others.

Maximization of the benefits of editorial board members

The primary task of editorial board members is to give a peer review of an unpublished scientific article *via* an online office system to evaluate its innovativeness, scientific and practical values and determine whether it should be published or not. During peer review, editorial board members can also obtain cutting-edge information in that field at first hand. As leaders in their field, they have priority to be invited to write articles and publish commentary articles. We will put peer reviewers' names and affiliations along with the article they reviewed in the journal to acknowledge their contribution.

Maximization of the benefits of authors

Since the *WJP* is an open-access journal, readers around the world can immediately download and read, free of charge, high-quality, peer-reviewed articles from the *WJP* official website, thereby realizing the goals and significance of the communication between authors and peers as well as public reading.

Maximization of the benefits of readers

Readers can read or use, free of charge, high-quality peer-reviewed articles without any limits, and cite the arguments, viewpoints, concepts, theories, methods, results, conclusion or facts and data of pertinent literature so as to validate the innovativeness, scientific and practical values of their own research achievements, thus ensuring that their articles have novel arguments or viewpoints, solid evidence and correct conclusions[1].

Maximization of the benefits of employees

It is an iron law that a first-class journal is unable to exist without first-class editors and only first-class editors can create a first-class academic journal[2,3]. We insist on strengthening our team cultivation and construction so that every employee, in an open, fair and transparent environment, are able to contribute their wisdom to edit and publish high-quality articles, thereby realizing the maximization of the personal benefits of editorial board members, authors and readers, and yielding the greatest social and economic benefits.

CONTENTS OF PEER REVIEW

In order to guarantee the quality of articles published in the journal, the *WJP* usually invites three experts to comment on the submitted papers. The contents of peer review include: (1) whether the contents of the manuscript are of great importance and novelty; (2) whether the experiment is complete and described clearly; (3) whether the discussion and conclusion are justified; (4) whether the citations of references are necessary and reasonable; and (5) whether the presentation and use of tables and figures are correct and complete.

SCOPE

The aim of the *WJP* is to report rapidly new theories, methods and techniques for prevention, diagnosis, treatment, rehabilitation and nursing in the field of pharmacology. *WJP* covers topics concerning neuropsychiatric pharmacology, cerebrovascular pharmacology, geriatric pharmacology, anti-inflammatory and immunological pharmacology, antitumor pharmacology, anti-infective pharmacology, metabolic pharmacology, gastrointestinal and hepatic pharmacology, respiratory pharmacology, blood pharmacology, urinary and reproductive pharmacology, pharmacokinetics and pharmacodynamics, clinical pharmacology, drug toxicology, pharmacology-related traditional medicine, and integrated Chinese and Western medicine. The journal also publishes original articles and reviews that report the results of pharmacology-related applied and basic research in fields such as immunology, physiopathology, cell biology, medical genetics and pharmacology of Chinese herbs.

COLUMNS

The columns in the issues of the *WJP* will include: (1) Editorial: to introduce and comment on the substantial advance and its importance in the fast-developing areas; (2) Frontier: to review the most representative achievements and comment on the current research status in the important fields and propose directions for the future research; (3) Topic Highlight: this column consists of three formats, including (A) 10 invited review articles on a hot topic, (B) a commentary on common issues of this hot topic, and (C) a commentary on the 10 individual articles; (4) Observation: to update the development of old and new questions, highlight unsolved problems and provide strategies on how to solve the questions; (5) Guidelines for Clinical Practice: to provide guidelines for clinical diagnosis and treatment; (6) Review: to systematically review the most representative progress and unsolved problems in the major scientific disciplines, comment on the current research status and make suggestions on future work; (7) Original Articles: to originally report the innovative and valuable findings in pharmacology; (8) Brief Articles: to briefly report novel and innovative findings in pharmacology; (9) Case Report: to report a rare or typical case; (10) Letters to the Editor: to discuss and make reply to the contributions published in the *WJP*, or to introduce and comment on a controversial issue of general interest; (11) Book Reviews: to introduce and comment on quality monographs of pharmacology; and (12) Guidelines: to introduce consensuses and guidelines

reached by international and national academic authorities worldwide on research in pharmacology.

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EDITORIAL

Design flaws in randomized, placebo controlled, double blind clinical trials

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Abstract

The hypothesis in drug clinical trials is that the drug is better than a placebo in patients suffering from a disease. The unstated assumption is that the drug cures the disease or is a powerful treatment for the disease. This is an incorrect assumption. Drugs do not cure or treat diseases. The body heals itself; drugs promote this ability of the body to heal itself. Placebos are assumed to be inactive; however, placebos can also promote the ability of the body to heal itself. Placebos are actually treatments that can stimulate endogenous healing mechanisms. The possible place of placebos in health management is controversial. Clinical trial design should be altered. The hypothesis of clinical trials should be that the drug speeds up or improves the healing of the patient, putting patient healing as the first objective. Placebos should not be used as controls but could be tested as drugs in their own right. The control in clinical trials should be no treatment. Alternatively, new drugs could be compared to existing drugs in clinical trials.

Key words: Randomized clinical trials; Placebo effect; Drug efficacy; Healing

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BACKGROUND

The basis of clinical pharmacology is the randomized, placebo controlled, double blind clinical trial (RCT). Design recommendations for RCTs can be found at www.consort-statement.org. The hypothesis in RCTs is that the drug will produce a greater response than a placebo in patients suffering from the same symptoms caused by the same disease. RCTs are designed to prove the power of new drugs. This design comes directly from laboratory pharmacology experiments, such as when receptor activation by a drug is tested in comparison to a placebo. These laboratory experiments can be carefully controlled, especially in purified receptor preparations. There are many difficulties in extrapolating from experimental design in purified receptor preparations to RCTs in diseased patients. Purified receptor studies do not contain endogenous agonists and antagonists that confound RCTs. A basic assumption in RCTs is that healing is the same as efficacy in comparison to a placebo. This assumption is frequently not true[1].

HEALING THE PATIENT IS THE FIRST PRIORITY

RCTs seek first to prove the efficacy of a drug but do not seek to heal the patient. In fact, some RCTs seek receptor interactions or symptom reduction as the end point, not

healing. It is assumed that activating receptors is enough to heal the patient. This is not always true. Several drugs have been approved for use in the USA by the FDA after extensive RCTs, only to be shown to be ineffective at healing patients in post marketing studies. Examples of this are: gemtuzumab ozogamicin, erityl tetranitrate, propoxyphene (not recommended in elderly patients in California), trimethobenzamide hydrochloride and midodrine.

BODY HEALS ITSELF

Each receptor in the body has one or more endogenous agonists and endogenous antagonists[2]. These agonists and antagonists exist in a balance that is called health. When this balance is corrupted, disease may occur. This is similar to the Chinese concept of balancing yin and yang in health. For instance, insulin is an endogenous agonist that binds to and activates the insulin receptor and increases the uptake of glucose into cells. Insulin receptor activation is inhibited by a variety of endogenous antagonists including: ceramide, TNFα, visfatin, IL-6, resistin and RELMs[3-5]. When the balance of agonists and antagonists is altered, insulin resistant diabetes can occur. In hypertension, resistin, an endogenous antagonist, inhibits bradykinin, an endogenous agonist, induced vasodilation[6]. Vascular tone is decreased by a variety of endogenous agonists, including PGI_2, nitric oxide and acetylcholine. Blood pressure is increased by several endogenous compounds such as endothelin, angiotensin, renin, aldosterone and other factors. Anxiety is another example, where receptor dysfunction is learned by the patient. In other words, a cognitive act of the patient causes an imbalance in the agonist antagonist balance in the brain and results in receptor dysfunction. The receptors involved include: norepinephrine, arginine vasopressin, neuropeptide Y, galanin, dopamine, serotonin and GABA[7,8]. Anxiety may decrease the healing of some diseases.

Hormones and other endogenous compounds have releasing agents and release inhibition agents that exist in a balance required for health, such as somatostatin and somatocrinin. In disease, the balance is prevented by increased production of endogenous compounds that promote disease. For instance, endocannabinoids increase in obesity, inhibit the secretion of anti-inflammatory adiponectin and increase the secretion of inflammatory adipokines[9] that are involved in atherosclerosis, arthritis, diabetes, hypertension and other chronic conditions[10].

PLACEBOS ARE TREATMENTS THAT STIMULATE ENDOGENOUS MECHANISMS

Recent authors defined "a placebo as any treatment that is used for its ameliorative effect on a symptom or disease but that is ineffective for the condition being treated"[11]. However, many substances can be effective for the condition being treated since they stimulate the body's ability to heal itself through endogenous mechanisms. In contrast, a drug is itself capable of interacting with receptors that stimulate endogenous mechanisms and promote the body's ability to heal itself. It might be more appropriate to define a placebo as an agent that acts only by stimulating endogenous agonist antagonist mechanisms. Even this definition is troublesome. For instance, morphine is an endogenous agonist that is made in the human body. Therefore, administering morphine stimulates endogenous agonist antagonist mechanisms.

Most studies use a solvent, capsule or tablet that does not contain the drug as a placebo. A recent review discussed data showing that placebos increase the healing of many diseases, or at least decrease the symptoms of many diseases[12]. Placebo effects cannot be dismissed and may be clinically significant. Recent guidelines advise using the placebo effect to augment analgesia[13]. However, a recent meta analysis of 202 RCTs found that placebos did not have clinically significant effects[14]. Placebos are very good at increasing dopamine release, decreasing pain, decreasing headaches, increasing endogenous opioid release, decreasing β-adrenergic activity, anxiety relief, immunosuppression and other specific pharmacological effects[12,15-18]. It is very possible that treating anxiety may help with healing of many diseases[12]. Similarly, treating pain may improve healing of some diseases. Placebos, like drugs, can facilitate the ability of the body to heal itself through endogenous agonist and antagonist mechanisms. The exact mechanism (s) of action of placebos is not known. In other words, it is not known how placebos reestablish endogenous agonist and antagonist balance.

A confounding factor in placebo mechanisms is that, since they stimulate endogenous opioid release, some patients like them. This has prompted some scientists to conjecture that anything that stimulates endogenous opioid release is a placebo. This argument is used as proof that acupuncture, which stimulates endogenous opioid release, is a placebo treatment. However, acupuncture also stimulates type Ⅱ and Ⅲ small diameter, myelinated afferent nerve fibers in muscles that send impulses to the spinal cord and activate analgesia centers in the spinal cord, midbrain and hypothalamus-pituitary[19]. The spinal cord neurons are endorphinergic and release enkephalin or dynorphin to block pain transmission. Periaqueductal gray matter cells in the midbrain secrete enkephalin, which results in serotonin and norepinephrine release in the spinal cord to inhibit pain transmission. The pituitary gland releases β-endorphin into the blood to cause analgesia at remote sites. Acupuncture is useful therapy in many patients[20], is FDA approved and is covered by many medical insurance companies. It should also be remembered that many drugs stimulate the release of endogenous opioids, such as capsaicin[21], alcohol[22], cocaine[23], propofol[24], ibuprofen[25], clonidine[26] and serotonin releasing drugs[27]. If endogenous opioid release

is specifically characteristic of placebos, then some drugs are placebos.

Several studies have sought ways to predict which patients will have placebo responses in order to eliminate these patients from selection. Some studies have suggested that the placebo response is predicted by beliefs and expectations of patients[28-30]. Other studies have found placebo response correlates with Caucasian patients, study duration, disease severity, dosing regimen, type of RCT, doctor patient communication and other factors[31-33].

CARPENTER APPROACH

One of the purposes of RCTs is to find more powerful or more specific drugs. Medicine is dominated by the carpenter approach; if the hammer does not work, get a bigger hammer. This is especially evident in pain patients where initial use of nonsteroidal anti inflammatory agents can lead quickly to opioids and the fentanyl patch. Some doctors tell patients that they should not be in pain. However, pain is a necessary part of life. Pain protects patients from damaging themselves from burns, bruises and other problems. Typically, pain patients become tolerant to opioids and increase the dose. Toxicity may occur, including respiratory depression and seizures. As of 2011, there are approximately 10 000 US patients dying yearly from prescription opioid overdose. The body has natural pain relieving agonists that are produced as needed in the brain, act locally and have short half lives. These agonists, endorphins, enkephalins and dynorphins, are very safe and are in a balance between synthesis, release, catabolism and natural antagonists. They are greatly superior to an administered opioid that must penetrate into the body and reside in the body for a convenient length of time. Administered opioids shut down the synthesis of natural opioid agonists[34]. When administered opioids, including the fentanyl patch, are gradually removed from the patient, the body may not respond quickly to reestablish the natural pain relieving mechanisms due to long term opioid receptor desensitization[35]. This may leave the patient in much more pain than was experienced before the opioid interventions.

Another example of the carpenter approach is the overuse of antibiotics. The body has an endogenous immune system to fight bacterial infections. Over prescription of antibiotics in otitis media has led to bacterial resistance such that the body can no longer heal itself from infections[36]. In addition, antibiotic toxicity is becoming more of a problem as the doses used and the number of antibiotics used at the same time increase. It is better to use a couple of drops of olive oil in the auditory canal for otitis media[37].

SHARPLY FOCUSED APPROACH

Funding agencies have expressed a need for a more sharply focused approach to RCTs and the use of specific biomarkers to prove the efficacy of new drugs[38]. This approach makes biomarkers more important than patient healing. This may be the result of reports that RCTs examining the same drugs find conflicting results[39], doctors use placebos in their patients[40], RCT design and placebo responses have changed over the years[41-43] and funding sources affect the outcomes of RCTs[44]. Many drugs have been tested in extensive RCTs and have been FDA approved in the USA inappropriately. Examples of this are rofecoxib and valdecoxib, which were approved even although they caused severe toxicity (myocardial infarction and stroke). These drugs were developed as the result of intensive, sharply focused investigations to find COX-2 inhibitors for use in pain patients. Other examples of FDA approved drugs that were removed because of toxicity problems include: azaribine (stroke), ticrynafen (liver toxicity), benoxaprofen (liver toxicity), zomepirac (fatal allergic reaction), nomifensine (hemolytic anemia), suprofen (flank pain syndrome), encainide (fatal arrhythmia), temafloxacin (kidney failure), flosequinan (increased deaths), fenfluramine (heart valve disease), bromfenac (liver toxicity), mibefradil (fatal arrhythmia), grepafloxacin (fatal arrhythmia), cisapride (fatal arrhythmia), troglitazone (liver toxicity), cerivastatin (muscle damage leading to kidney failure), rapacuronium (severe breathing difficulty), etretinate (birth defects), levomethadyl (fatal arrhythmia), gemtuzumab ozogamicin (myelosuppression and no efficacy), terfenadine (fatal arrhythmias), astemizole (fatal arrhythmias), propoxyphene (fatal arrhythmias) and conjugated estrogens (heart attack, stroke, breast cancer, Alzheimer's disease).

The approval of these drugs is symptomatic of the over anxious need for ever more powerful drugs and ever more specific drugs. Clearly, as toxic lifestyles produce more chronic diseases, there is an increasing insistence from patients that drugs should be produced to cure them of these diseases. However, the danger of toxicity from more powerful and more specific drugs must not be overlooked. What is routinely overlooked is that prevention of these chronic diseases should be the first priority[10,45-47].

PROTECTING THE PUBLIC FROM FRAUD

One of the stated purposes of RCTs is to demonstrate the power of drugs such that the public can be protected from products that lack efficacy but are available on the market. The National Center for Complementary and Alternative Medicine is especially vigilant in this regard and seeks to protect the public from plant derived medicines that lack efficacy but may be toxic[48]. Prior to 1960, most drugs were derived from plants and natural sources. *Homo sapiens* has survived for 200 000 years by using plants as medicines. There has been an enormous natural selection where people who responded to plant medicines survived. Today most drugs come directly or indirectly from plants or natural sources, including cancer drugs, most

antibiotics, vitamins, minerals and other prescription drugs.

TRILLION DOLLAR FRAUD

Patients are led to believe that powerful drugs are available to treat diabetes, cardiovascular disease, congestive heart failure, arthritis and other chronic diseases. Many drugs have been tested in RCTs and have been approved for use in these diseases. There is no drug that cures diabetes, cardiovascular disease, congestive heart failure or arthritis. Drugs are available to manage these diseases and allow patients to live with their pathology. These chronic disease processes can be partially slowed down by drugs; however, even with the best drugs, these chronic diseases progress. One of the problems with treatment of these diseases is that the drugs used are too specific and usually treat only one symptom. The goal of medicine should be to heal the patient. Unfortunately, with many chronic diseases, the goal has become keeping the patient alive. These diseases are caused by adopting toxic lifestyles that produce weight gain, muscle loss, fat accumulation, toxic adipokine secretion, toxic lipid accumulation and other detrimental changes[3,9,10,47,49,50]. Patients are advised to change their lifestyles, including lose weight, exercise more and eat healthy diets, in order to help them manage these chronic diseases. There is evidence that lifestyle changes can greatly improve the management of these chronic diseases[45,46,51,52]. Yet, many patients do not make lifestyle changes and prefer to rely on drugs. The emphasis in healthcare should be prevention of these chronic diseases by teaching patients to avoid toxic lifestyles[45-47].

Of course, there are some diseases for which prevention is not possible. These diseases include genetic diseases and type I diabetes; although, recent work has shown that the onset of type I diabetes can delayed by nicotinamide[53].

DESIGNING RCTs IN THE FUTURE

RCTs should be designed with healing the patient as the primary goal. The hypothesis should be that a drug will promote healing better than no treatment. This removes placebos as a confounding variable. It is important to remember that some patients get a placebo response just from visiting the doctor's office, even without seeing the doctor[12]. These patients may have to be removed from statistical analysis of the data. Comparing a drug to no treatment means that double blinding cannot be possible. Such trials can still be randomized and can still be statistically valid. The results from patients tested with new drugs can also be compared to historical patients receiving no treatment in the same hospital.

Of course, the danger in comparing a drug to no treatment is that a drug that works only through placebo mechanisms may become approved. As demonstrated above, several drugs that lack efficacy have already been approved. As more drugs become available, new drugs should be compared to existing drugs, rather than placebos or no treatment. Such trials can be performed in randomized, double blinded designs.

In patients afflicted with chronic, incurable diseases, lifestyle changes, not drug therapy, should be the primary goal. Toxic lifestyles prevent the body from healing itself or at least reestablishing normal agonist antagonist balance. These chronic diseases include hypertension, cardiovascular disease, congestive heart failure, arthritis, insulin resistant diabetes and others. In patients with long standing chronic illnesses, healing may be impossible due to extensive pathology, in which case, disease management becomes the secondary goal. Pain patients can be difficult to heal, especially in diseases where the cause of the pain is not completely known, like neuropathic pain or fibromyalgia. These patients can be tested in clinical trials where no treatment is compared to a treatment[54]. Of course, in patients that have life threatening symptoms from chronic diseases, symptom management must be a secondary goal. Placebos or no treatment are not ethical in diseases with life threatening symptoms that have effective drug therapy[55]. An alternative hypothesis in RCTs could be that a new drug promotes healing better than a conventional drug or has equal ability to promote healing compared to a conventional drug. Comparing drugs removes confounding effects of placebos.

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OBSERVATION

How and why chemicals from tobacco smoke can induce a rise in blood pressure

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Abstract

The primary objective of this article is to analyze the role of tobacco smoke compounds able to damage the cardiovascular system and, in particular, to interfere with blood pressure. They are products of tobacco plant leaves, like nicotine, thiocyanate and aromatic amines, and a chemical derived from cigarette combustion, carbon monoxide. Of the other thousands of chemicals, there is no clear evidence of cardiovascular damage. Nicotine and its major metabolite, cotinine, usually increase blood pressure by a direct action and an action stimulating neuro-humoral metabolites of the body as well as sympathetic stimulation. An indirect mechanism of damage exerted by elevated carboxyhemoglobin concentrations is mediated by carbon monoxide, which, mainly induces arterial wall damage and, consequently, late rising in blood pressure by a toxic direct action on endothelial and blood cells. Thiocyanate, in turn, reinforces the hypoxic effects determined by carbon monoxide. Aromatic amines, depending on their chemical structure, may exert toxic effects on the cardiovascular system although they have little effect on blood pressure. A rise in blood pressure determined by smoking compounds is a consequence of both their direct toxicity and the characteristics of their chemical chains that are strongly reactive with a large number of molecules for their spatial shape. In addition, a rise in blood pressure has been documented in individuals smoking a cigarette, acutely and chronically, with irreversible artery wall alterations several years after beginning smoking. Since cigarette smoking has a worldwide diffusion, the evidence of this topic meets the interest of both the scientific community and those individuals aiming to control smoking.

Key words: Smoking chemicals; Blood pressure; Nicotine; Carbon monoxide; Arterial damage

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INTRODUCTION

Growing evidence indicates that over 4000 chemical compounds are usually concentrated and condensed into tobacco mixture[1]. A large majority of these have carcinogenic effects but there are many with cardiovascular toxicity, which depends on several factors, partly related to tobacco smoke and partly due to the environment and lifestyle of individuals exposed to smoking.

The harmful health effects of tobacco smoke adversely target the cardiovascular system and there is also evidence that death rates are uniformly higher among smokers than non-smokers in both sexes and whatever the age at the death. In addition, reports[2,3] indicate that the excess mortality in smokers mainly affects smokers

aged from 45 to 54 years more than those younger or older in age. It is well known that older age is related to arterial hypertension[4,5] and, consequently, there is evidence that smoking usually precedes elevated blood pressure. Moreover, the strong relationship which links smoking exposure to type of response of heart and blood vessels[6-10] may present a variety of patterns and different severe manifestations.

In spite of the great number of findings which show the adverse role of smoking compounds on blood pressure without doubt, current opinions on that are not yet unanimous. There is a discrepancy in opinions that may be attributed to the lack of reproducible data, particularly in epidemiological studies. On the other hand, experimental findings conducted on both humans and animals give evidence of reproducible results for cardiovascular events and events related to hypertension. In addition, high blood pressure has consistently been found to be a strongly predictive factor for coronary artery disease and stroke[4-6]. Undoubtedly, careful control of major cardiovascular risk factors[10], including hypertension, which should be unconditionally lowered worldwide, is able to reduce significantly the rate of stroke and coronary events.

The purpose of this review is to describe the mechanisms by which the chemicals of tobacco smoke can cause changes in arterial blood pressure, as well as explaining the reasons for selecting this subject by an analysis of chemical and pharmacological properties of tobacco smoke toxins which adversely affect the cardiovascular system, in an attempt to clarify the real potential of the topic in regard to the scientific community and health professionals concerned.

TOBACCO COMPOUNDS AND CHEMICAL CHAINS

A chemical chain of a substance often influences and determines its mechanism of action. In chemistry, a chemical chain is a series of linked atoms that form a molecule, usually of the organic but also the inorganic type. Different properties and reactions characterize the chemical chains according to structural shape. There are closed-ring chains where the atoms in a molecule form a closed loop, which, consequently, is often a stable and scarcely reactive chemical; long-chains with relatively long bindings of atoms in the same molecule; and open chains ending with an open binding which may interact actively with atoms of various molecules. In addition, chemical chains spatially build the geometrical aggregation of constituent elements of a molecule that take part in isomeric substance composition. Often, more than one reaction is possible given the same starting materials. The reactions may differ in their stoichiometry according to the number and atomic concentrations of the molecules, as well as the substrates that are prevailing. So, a chemical atom may react actively with another to give a specific compound if a different atom is not present, while the same atom may react differently in the presence of other atoms, choosing to form a compound which differs from that of the chemical reaction performed in the absence of other atoms. There is evidence that the knowledge of these properties of chemical binding will contribute to better understanding the damaging mechanism of tobacco compounds, including primarily nicotine and its metabolites[11] and carbon monoxide. These substances may react differently according to their affinity towards environmental substrates and, consequently, determine various levels or type of individual responses.

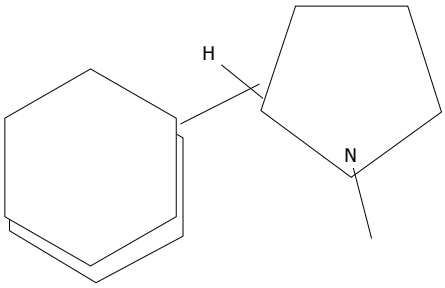

Figure 1 Chemical formula of nicotine. One can see that the molecule is formed by a closed chain with a binding to an open chain. The latter has an N reactive atom. Open chain is responsible for major chemical reactions of the molecule.

NICOTINE AND ITS METABOLITES

Chemistry and pharmacodynamics

Nicotine is a natural alkaloid[12] obtained from the dried leaves and stems of tobacco plants. Chemically, the alkaloid has a basic charge which is responsible for the mechanisms of chemical reaction. Nicotine concentration in the tobacco plant ranges from 0.5% to 8%.

Nicotine has always been identified as the most powerful toxin of cigarette smoking since its harmful action, either functionally or structurally, can be widely demonstrated at a relative low concentration in both clinical and experimental findings involving all body organs. Nicotine biosynthesis takes place in the roots of the tobacco plant. Then, it accumulates in the leaves, the particular shape of which is a basic factor for the harvesting and extraction of the alkaloid. After manufacturing, each cigarette may reach nicotine concentrations from 1.5 to 2.5 mg. However, not all of the smoked substance enters the blood, which is, in any case, damaged by absorbed nicotine[13].

Chemistry

The chemical formula of nicotine is C10H14N2, equivalent to 3 (1-Methyl-2-pyrrolidinyl) pyridine. Its spatial shape (Figure 1) shows two chains, one closed chain and one open chain with an N reactive atom. This structure is basic to understanding some chemical properties, like nicotine addiction and toxicity, since it permits identification of two types of spatial aggregation of those constituents taking part in nicotine composition[13]: nicotineS (-) isomer and nicotineS (+) isomer. NicotineS (-) isomer is the main nicotine isomer[11], tasted as pleasant in cigarette

by smokers but not by non-smokers, whereas the nicotineS (+) isomer is unpleasant for both smokers and non-smokers. This chemical composition helps to explain the reason for the bad taste for the smoker who starts smoking, even if it changes rapidly when pleasant nicotineS (-) isomer utilizes its effect. There is evidence that tobacco manufacturing industries try to reinforce the response linked to nicotineS (-) isomer.

Nicotine is a hygroscopic liquid miscible in its basic charge with water. This property makes the alkaloid diffusible on the whole tobacco leaf so that harvesting tobacco leaves fills up with the greatest amount of nicotine. Its chemical formula of a nitrogenous base permits an interaction between nicotine and acid compounds, forming salts usually soluble in water and, consequently diffusible, some of these characterized by high toxicity.

Pharmacodynamics and metabolism

Absorption of nicotine by individuals occurs very quickly through several tissues of the body. The oral cavity absorbs from 4% to 45% of the total dose[14]. In addition, as nicotine enters the body[15], it reaches the blood, acting on specific receptors. Nicotine-acetylcholine receptors that feel the level of blood nicotine concentration are deputed to permit the biochemical action of the alkaloid. In the presence of low concentrations of nicotine, the activity of nicotine-acetylcholine receptors increases, whereas high concentrations inhibit receptor activity. Therefore, different pharmacological responses to nicotine may be seen, from stimulant to depressant effects, on those structures involved in alkaloid activity.

Nicotine effects are mediated through the interaction with the sympathetic system, catecholamine release, endothelial function and metabolic profile of the individuals[1,16-20].

Nicotine exerts direct and repeated effects on the sympathetic system which are, initially, transient and stimulant, but, in the long run, they become of depressant type with severe functional and structural changes in those organs involved with the nicotine action. For the heart and blood vessels, there is evidence that nicotine triggers cardiovascular responses through sympathetic stimulation and direct and mediated catecholamine release[17,20]. In addition, nicotine specifically stimulates release of norepinephrine from the hypothalamus and antidiuretic hormone from the pituitary gland[21], as well as chemoreceptors in the carotid arteries[22], triggering different reflexes, which may lead to multiple adverse responses.

Liver, kidney, lung and oral mucosa are those body organs involved in regulating nicotine metabolism which damages the cardiovascular system and endocrine glands.

Metabolites of nicotine, the main one of which is cotinine, need to be highlighted since they permit analysis of follow-up studies on selected populations of smokers, more for their toxicity which is similar to that of nicotine. Nevertheless, differences exist between nicotine and its metabolites regarding the mechanism of action. Nicotine usually acts more rapidly and therefore may be dosed earlier in biological liquids. On the other hand, chronic toxicity is better estimated by dosing cotinine in biological liquids. Large-scale trials assessing the results of antismoking campaigns dose urinary cotinine. Indeed, it is less expensive than nicotine[23,24]. Similar to nicotine, cotinine is also mainly metabolized in the liver.

CARBON MONOXIDE

Carbon monoxide is a gas with the highest toxicity, depending mainly on concentrations in the environment and body organs. This chemical is not contained in the fresh leaf of tobacco but produced by a chemical reaction, characterized by decomposition in the burned cone of a cigarette between environmental oxygen and the paper of the smoked cigarette. Carbon monoxide derived from a single smoked cigarette reaches small concentrations which can acutely induce functional but transient responses, particularly in the lungs and cardiovascular system, whereas dated chronic smoking often causes irreversible alterations. However, carbon monoxide alone from tobacco smoke may be considered, chronically, a potentially silent killer even if different levels of carboxyhemoglobin are reached after acute smoke exposure[25].

Chemistry and toxicology

Carbon monoxide is a colorless, odorless and tasteless gas with high and potentially lethal toxicity. The blood level of the gas regulates toxic responses.

The chemical formula of the gas is CO, a diatomic molecule with binding of two atoms, carbon and oxygen[13]. Spatially, carbon monoxide outlines a diatomic linear chain which permits an easy dissociation and, consequently, re-composition in atoms of carbon and oxygen able to react again together or with other atoms originating from several different compounds. In addition, new linking with substances with major chemical affinity, such as hemoglobin, may occur. Usually, carbon monoxide is less dense than air and, therefore, is capable of spreading out more quickly. It is also soluble in water and burns in air, producing carbon dioxide, a metabolite largely diffused in the environment and the end-point of catabolic reactions associated with intracellular respiratory metabolism.

However, a little amount of carbon monoxide in a concentration not harmful for life arises spontaneously into the body through a chemical reaction involving heme and an enzyme named heme-oxygenase. Heme is an iron component of hemoglobin, a molecule strongly reactive with carbon monoxide.

The toxicity of carbon monoxide produced by cigarette smoking recognizes several complex reactions consisting of oxygen removal from hemoglobin and its replacement with carbon monoxide, formation of carboxyhemoglobin, tissue hypoxia and impairment of cellular metabolism. These mechanisms are the result of carboxyhemoglobin production, but carbon monoxide also acts directly, depending on

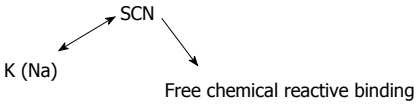

Figure 2 Chemical formula of thiocyanate. There is evidence that the toxin has a free binding able to react chemically with other molecules to reinforce adverse cardiovascular effects. SCN: Seattle community network.

its chemical molecule, to morphologically damage the heart and blood vessels[26-31].

THIOCYANATE

Thiocyanate is the third chemical of tobacco smoke that exerts adverse cardiovascular effects with consequent increased damage caused by nicotine and carbon monoxide.

The substance is largely diffused in nature in plants of the genus Brassica (cabbages) and is also a component of biological liquids like blood, saliva and urine. In addition, it reacts at different steps of body metabolism[32-34], including mainly iodine metabolism. Thiocyanate develops in the vapour phase of tobacco smoke, acting particularly as a compound able to reinforce chronic damage derived from tobacco smoke.

Chemistry

Thiocyanate is a colorless, odorless crystalline powder with a chemical formula that may vary according to the salt linked with its structure. Spatially, the basic chemical structure of thiocyanate is shown in Figure 2. It shows that there can be variability in the type of salt which bonds the thiocyanate main chain, usually K or Na, and is free binding, able to react with several other molecules[35].

Toxicity

The main metabolites of thiocyanate formed in the body have high toxicity, primarily hydrogen cyanide. However, the metabolite concentration in the vapor phase of smoking usually does not reach values acutely harmful for the individual's life. When cyanide enters the blood, it forms a stable complex with enzymatic chains, particularly with those cytocrome oxidases involved in the synthesis of adenosine triphosphate (ATP). The result of the chemical reaction is a reduced ATP synthesis which causes problems of intracellular respiratory chains and different degrees of hypoxia. Therefore, thiocyanate increases the hypoxia due to carbon monoxide and nicotine from smoking. There is evidence that a deep interaction exists among the main chemical compounds of smoke to cause damage of heart and blood vessels[36].

AROMATIC AMINES

Aromatic amines are chemical compounds with a chemical formula (Figure 3) containing one or more closed benzene rings added to aromatic constituents like NH2, NH or other nitrogen groups. These classes of chemicals, largely concentrated and diffused into the environment from burned tobacco, exert mainly carcinogenic effects[1,37], even if morphological alterations of heart and blood vessels have been described. However, their toxic effect on blood pressure is still to be demonstrated.

Table 1 Factors involved in blood pressure control

Factors	Controlled parameters
Cardiac inotropism	Heart rate, cardiac output
Arterial resistance	Arterial stiffness, arterial vasoconstriction, arterial dilation, endothelial function, anatomical structure of the arteries
Blood volume	Salt uptake
Blood viscosity	Red blood cell numbers

Table 2 Main factors influencing those parameters that regulate blood pressure

Factors	Influenced parameter
Dietary factors	
Salt intake-sodium, potassium	
Calcium, magnesium	Blood flow and volume
Vegetarian diet, alcohol	
Bio-humoral and hormonal factors	
Angiotensin	
Vasopressin	Arterial wall
Vasodilators	Blood flow and volume
Vasoconstrictors	Arterial resistance
Catecholamines	
Neural factors	
Central nervous system	
Sympathetic system	Sympathetic stimulation Catecholamine release
Genetic and metabolic factors	Glucose and lipid metabolism Genetic code
Lifestyle	Preventive measures

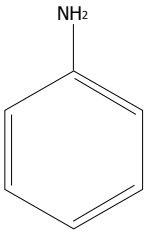

Figure 3 Benzene ring completed by an amine group, like in aniline. The group NH_2 may be changed by different nitrogen groups to form different classes of aromatic amines.

FACTORS REGULATING BLOOD PRESSURE

There are four main factors that regulate blood pressure; in their turn, they are influenced by a large number of physiological and pathological responses that determine the level of blood pressure. Tables 1 and 2 summarize the main influencing events.

Blood pressure regulation is under the control of cardiac inotropism, arterial resistance, blood volume and

viscosity or thickness of circulating fluid. These factors cause blood pressure levels as a result of a meeting or engagement between different types of lifestyle and a genetic code, characteristics of a single individual or his family. The result may be a normal blood pressure level or changes in blood pressure, even in the absence of identifiable triggering causes. This concept of a different distribution of blood pressure values within populations as well as establishing the appearance of complications related to abnormal blood pressure is a basic point of preventive antihypertensive measures[38].

Cardiac inotropism, usually evaluated by assessment of the amount of blood pumped out in every single beat by the heart, regulates cardiac output, which is the result of the heart rate multiplied by the number of each cardiac systole. As one can easily deduce, this regulating parameter of blood pressure may often be influenced by several factors and, therefore, it is difficult to maintain stable levels.

Arterial resistance plays a strong role in the control of blood pressure. There is evidence that increased arterial stiffness[39], which is a parameter strongly influenced by smoking compounds, causes adverse effects on arterial elasticity. Stefanidis and co-workers[39] investigated aortic elasticity in 48 male patients, most of whom had coronary heart disease. By means of a sonometric catheter, they measured the diameter of the aorta while simultaneously determining arterial blood pressure at the same location. Increased aortic stiffness, strongly influenced by exposure to passive smoking, with consequent reduction in aortic elasticity, augmented left ventricular afterload and impaired left ventricular function. Vasoconstriction in systemic arteries, caused by tobacco smoke[40,41], in the presence of a stiffer aorta necessarily also worsens myocardial ischemia in individuals suffering from ischemic heart disease and among those with hypertension whose aortic elasticity is already compromised. Endothelial function is a basic parameter able to control blood pressure, functionally or morphologically. An excellent paper by Deedwania[42] specifically analyses the role and significance of endothelium, stressing the importance of this structure as a new target for cardiovascular therapeutics. It has been well established that vascular endothelium has a pivotal role in maintaining vascular tone. In addition, endothelial dysfunction is an early marker of impending atherosclerosis, also induced by smoke compounds and strongly related to hypertension[43-46]. By its chemical compounds, endothelium modulates artery dilation under normal conditions.

The patent arterial lumen, with its cylinder shape that progressively reduces the caliber along the entire length from the origin to the end, determines a different degree of resistance to blood flow. Blood flow resistance is lower at the origin of the arterial vessel[47] while increasing significantly at the end.

Finally, blood flow and viscosity change their power in the control of blood pressure according to a large number of factors[1], depending on their capacities of influencing the cardiovascular system.

The above parameters are significantly changed by a large number of factors that stimulate or reduce their activity. The main factors which influence the parameters regulating blood pressure may be classified into five groups: dietary factors, bio-humoral and hormonal factors, neural factors, genetic and metabolic factors and factors associated with lifestyle. Table 2 analyzes the most important of them.

Salt intake strongly influences blood pressure by involving several mechanisms like blood flow and volume, kidney function and acid-basic balance[48-51]. Smoking compounds exert little or no effects directly on these parameters, although indirectly their effect is mediated by those changes induced by neuro-humoral stimulation[52].

Among bio-humoral factors, angiotensin and renin-angiotensin-aldactone are primarily associated with development of hypertension, either through isolated or combined activity with other structures, like the sympathetic system and catecholamine release that are stimulated by the renin system.

Angiotensin[53-56] is one of the major stressing substances at any vascular district, increasing vascular tone and consequently arterial resistance. Plasma angiotensin levels are increased by several hormones, like plasma corticosteroids, estrogens, thyroid hormone concentrations as well as its metabolite Angiotensin Ⅱ, which is the most potent pressor known. Among the angiotensin family, which has several compounds like angiotensin Ⅰ, Ⅱ, Ⅲ and Ⅳ, differing in both type and number of constituents of amino-acids, angiotensin Ⅱ is of great importance to vascular tone control, particularly for coronary circulation. Studies[57-61] concluded that angiotensin Ⅱ is one of the most potent substances for increasing vascular tone and arterial resistance by a direct action on the arterial wall mediated by angiotensin Ⅱ receptors. In addition, a significant increase in myocardial oxygen consumption exists because of increased heart rate, systemic blood pressure and left ventricular wall stress. There is clear evidence that a close and complex interaction links angiotensin Ⅱ to the responses of other factors that modulate vascular tone, whether they exert vasoconstrictor or vasodilator effects[62]. Sympathetic stimulation also correlates its effects with angiotensin Ⅱ, strongly reinforcing their power[63].

A large number of substances with vasoconstrictor effects, like vasopressin or some prostaglandins and vasodilator effects like nitric oxide or relaxing factors, directly or indirectly regulate vascular tone causing increased or lowered blood pressure, respectively[64-70].

Some observations on the effects of the sympathetic nervous system and catecholamine release are as follows.

The role of these structures for blood pressure control seems to be limited to a short time, whereas the kidneys have a long-time control[71].

Indeed, the major hypothesis for the development of rising blood pressure involves the kidney with an abnormal excretory function[72]. However, cigarette smoking poorly influences this mechanism of blood pressure control.

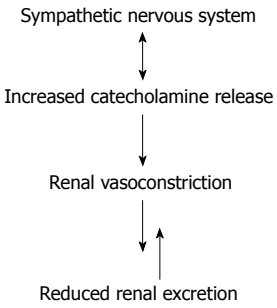

Figure 4 Schematic graphic showing the mechanism by which the interaction between sympathetic stimulation and endocrine response may alter kidney function.

Increased sympathetic nervous system activity has been identified as a factor able to adversely influence renal excretory function and smoking has been recognized as exerting a strong action on the sympathetic system[1,73-75].

In turn, sympathetic activity triggers a wide number of endocrine responses capable of inducing a short-term increase in blood pressure followed by a long-term increase[76-78]. In addition, there is evidence that the interaction between sympathetic stimulation and endocrine metabolism influences renal function, determining renal vasoconstriction and, therefore, reduced renal excretion (Figure 4).

The increased sympathetic activity determines a major renal tubular absorption of sodium and, therefore, sodium retention. In addition, decreased renal blood flow and glomerular filtration rate as a consequence of vasoconstriction, increases renal vascular resistance and, finally, stimulation of the renin-angiotensin-aldosterone system with an increased renin release. That leads to major angiotensin production. The primary result of these combined actions is a decrease in renal excretory function.

The sympathetic nervous system also stimulates aortic baroreceptors capable of inducing an increase in blood pressure.

Catecholamines induce a sympathetic response[75-79]. There are several catecholamines: epinephrine primarily modulates those responses linked with acute stress because of its prompt availability. On the other hand, norepinephrine acts primarily as a neurotransmitter of sympathetic postganglionic neurons. In practice, epinephrine induces acutely elevated blood pressure that, chronically, norepinephrine release contributes to maintain. Therefore, it is hard to establish whether rising blood pressure depends on one or the other hormone, explaining how the interpretation of the regulation of blood pressure linked to two hormones would be very complex. However, plasma concentrations of epinephrine and norepinephrine usually reflect adrenal medullary secretion and sympathetic nerve activity, respectively. Several factors related to exercise, mental stress, electrolyte balance, smoking and age[77,78] can stimulate the feed-back mechanism of catecholamine production, metabolism, uptake and excretion.

A third catecholamine, dopamine, the immediate metabolic precursor of norepinephrine, exerts sympathetic activity[79].

The effects of catecholamine release, primarily epinephrine, are very quick if compared with hormonal activity of other endocrine glands. Therefore, changes in target organ function may occur acutely after exposure to a standard stimulus, such as smoking a cigarette or passive exposure[80-82].

The effects of catecholamine release depend strictly on the type of catecholamine adrenoreceptor involved. Two types of adrenoreceptors play a strong role in modulating catecholamine response: α-adrenoreceptors and β-adrenoreceptors. B-stimulation causes an increase in heart rate and myocardial contractility, whereas α-stimulation is responsible for vasoconstriction. Norepinephrine induces a rise in both systolic and diastolic blood pressure as a consequence of increased vascular tone[79], while epinephrine usually acutely raises only systolic blood pressure. Finally, catecholamine release induces changes in lipid and glucose metabolism[1,10] with evident impact on blood pressure levels. There is clear evidence that tobacco smoke directly affects the metabolic profile of glucose and lipids[10,83,84].

In conclusion, a large number of factors control or are involved in regulating blood pressure, determining changes in baseline values that are the result of deep and complex interactions. External and modifiable stimuli, including cigarette smoking, play a strong role in maintaining blood pressure changes.

SMOKING COMPOUNDS AND BLOOD PRESSURE

Two groups of individuals need to be identified to better interpret how and why cigarette smoking influences blood pressure: active smokers and passive smokers. Past smokers may be included into one of the two groups, depending on when they quit smoking.

Blood pressure in active smokers

Active smokers display blood pressure values which vary widely according to a great number of individual, racial, social and lifestyle factors[85]. In addition, changes in blood pressure characterize the same smoker whether he was smoking a cigarette or not[8]. There is evidence that while a smoker smokes a cigarette, more elevated values than baseline measures in systolic blood pressure and heart rate are usually observed. This fact should prove that a smoking individual triggers transient but effective sympathetic responses, which acutely raise blood pressure levels. There is evidence that smoking is a chemical toxicosis[86] able to cause both acute and chronic detrimental effects and, similar to toxic diseases, exerts a double mechanism of acute damage which may be superimposed onto previous chronic damage caused by smoking itself.

Findings[87,88] documented that cigarette smoking in males was inversely related to systolic blood pressure, with

a reduction of 1.3 mmHg in 1.1% of light smokers, 3.8 mmHg in 3.1% of moderate smokers and 4.6 mmHg in 3.7% of heavy smokers when smokers were compared with non-smokers. On the other hand, diastolic blood pressure did not seem to undergo these changes. Both western and oriental populations participated in these studies so that the observed response did not relate to racial factors and, moreover, reduction probably characterized mainly young smokers since not enough time elapsed from starting smoking. Consequently, vascular damage did not clearly appear. Epidemiological surveys[89-91] would confirm these results, identifying a lowering of blood pressure in smokers compared to non-smokers, although the observations concern particularly young smokers and adolescents, frequently with loss in body weight. Therefore, unanimous opinions do not exist about that assessment. There is evidence, however, that chronic older smokers usually display elevated blood pressure[88,92,93]. The role of active smoking on blood pressure is still being debated but evidence indicates that older smokers display systolic blood pressure values significantly higher than those experienced by systolic hypertension of old age[94].

The different opinions about the behavior of blood pressure in smokers may be explained by the phenomenon of masking the damage as the result of the combined action of nicotine and carbon monoxide on the vessel wall, as suggested by Leone[85] and Landini *et al*[95]. Nicotine, after an early and transient vasoconstriction with a consequent increase of systolic blood pressure and heart rate, has vaso-paralytic effects followed by a decrease of these two parameters. At the same time, carbon monoxide exerts its pathological action of a structural type on the arterial wall resulting, within several years, in irreversibly anatomical damage of the arteries with a steady increase in blood pressure. This is routinely observed in older individuals who have been heavy smokers.

Hypertension exacerbates the cardiovascular risk thus previously thought to be linked only to cigarette smoke, with an obvious increased incidence of stroke and coronary artery disease which sometimes can pre-exist the hypertension. Ex-smokers show a progressive reduction in blood pressure levels only in the event that carbon monoxide has not arrived to determine irreversible damage to the arterial wall. At this level, the damage[1,17,88] is also not tied to functional responses evoked by the vasoconstriction due to activation of the sympathetic nervous system, altered sensitivity of the nicotine-receptors, circulating catecholamines and vasoconstrictor endothelium-mediators, including primarily endothelin.

Therefore, an acute rise in blood pressure observed while an individual is smoking a cigarette is due primarily to nicotine as a consequence of sympathetic stimulation, while carbon monoxide chronically damages arterial wall, causing morphological lesions that, in time, become irreversible and induce a change in arterial blood pressure with the appearance of hypertension[88].

There is evidence that inter individual variability in blood pressure response in active smokers depends on a wide number of factors, which exert their effects, time by time, prevailing one on the other according to anatomical and functional health status.

Blood pressure in passive smokers

Passive smoking causes blood pressure changes which are a result of two main factors: type of exposure and its duration.

Acute exposure usually causes a transient increase in systolic blood pressure[37] due to adrenergic and sympathetic stimulation, as a heart rate increase also demonstrates. These changes accompany transient endothelial dysfunction that is strongly related to passive smoking exposure, even in healthy people and it is the door to atherosclerotic lesions[43,96-98]. In addition, it is assumed that endothelial dysfunction has a strong association with hypertension[99]. Acute exposure to passive smoking does not initially cause morphological alterations of the arterial wall. They are a result of direct action of carbon monoxide on endothelial cells and platelets and will appear later, becoming responsible for the rise in blood pressure. However, when structural lesions induced by smoking appear, they do not differ from those caused by other factors that cause hypertensive disease or its complications, as well as those from pre-existing atherosclerotic diseases. Therefore, anatomically, one can observe the lesions of the atherosclerosis and its complications, even in cases of rising blood pressure.

In summary, blood pressure in passive smokers shows transient and acute increases followed by chronic lowering in its values and, again, late increase when irreversible morphological alterations of the arterial wall appear.

Clinical implications

The chemical structure of smoking compounds influences the functional and pathological response of hypertensive individuals, although clinical implications depend primarily on the degree and severity of cardiovascular damage.

Substantial evidence[95,100,101] indicates that hypertension associated with pleasure-loving habits, including cigarette smoking and alcohol consumption, tend to maintain or increase blood pressure and are also predictive of hypertension in normotensive individuals. Therefore, an incorrect lifestyle associated with hypertension certainly helps to damage body organs with a more elevated risk of complications.

Among the body organs, heart and blood vessels, brain, kidney, eyes and genitals primarily feel the adverse effects caused by association of smoking and hypertension.

Clinically, symptoms related to reduced coronary and cerebral perfusion dependent on vascular narrowing, often a high degree of narrowing of the coronary, cerebral and carotid artery can be demonstrated in hypertensive smokers with a rate significantly higher than those observed in hypertensive non-smokers and former smokers[95]. Therefore, ischemic chest pain, arrhythmias and late clinical signs due to congestive heart failure can be documented in hypertensive smokers[102-104].

Table 3 Main clinical involvement caused by smoking compounds

Body organ	Clinical picture
Heart	Coronary artery disease
	Heart enlargement
	Congestive heart failure
Brain	Transient ischemic attack
	Stroke
	Cognitive decline
Kidney	Arteriolosclerosis
	Chronic renal failure
Eyes	Artery vessel alterations
Genital system	Sexual dysfunction
Bone	Osteoporosis

Clinically, the brain alterations consist of transient ischemic attack, stroke and cognitive disorders, characterized by varying degree of memory disturbances to vascular dementia[105,106].

The kidney normally responds with reduced glomerular filtration responsible for oliguria due to arteriolosclerotic narrowing of resistance arteries[107]. Extreme complications of renal disorders can develop to chronic renal failure which, in a few cases, requires dialysis[108].

This colourful mosaic of alterations that can be isolated or variously combined with each other, lead to a range of clinical, diagnostic and therapeutic implications in patients affected by the above pathological pictures, making it difficult to control. It follows that a correct approach to the pathological aspects that relate smoking and hypertension is far from being achieved and, therefore, all the innovative factors depending on findings that may help to clarify this phenomenon should be accepted and carefully evaluated. In this context, the most recent data on a possible mechanism of damage related to the chemical structure of compounds in cigarette smoking should be taken into account.

Eyes, sexual function and often calcium metabolism in bone, followed by osteoporosis or enhanced pre-existing osteoporosis in both women and men[109], may be altered as a result of the combined action of smoking and hypertension with a development of serious clinical implications requiring a close physician-patient interaction.

Studies conducted to translate obtained data in different findings to all populations worldwide do not always come to the expected results. That also characterizes large scale trials[110-114]. Therefore, evident discrepancies exist between research and clinical practice, so patients do not always get appropriate treatment for their disease. For example, it would seem useless to choose one drug over another if the goal is to achieve a reduction in blood pressure which can be achieved with a different lifestyle and therapy.

Table 3 analyses the main clinical picture which may result from the combined action of smoking and hypertension.

These observations suggest without doubt that a routine assessment of smoking habits in patients suffering from hypertension is warranted in an attempt to avoid or reduce the rate of serious pathological events.

CONCLUSION

In conclusion, a rise in blood pressure in smokers, although there is no clear causal relationship between these two factors, depends on four main factors: the toxic effect of smoking compounds on the arterial wall, sympathetic stimulation, adrenergic stimulation and the spatial shape of chemical chains that constitute smoking compounds able to damage heart and blood vessels.

The toxic effect is primarily due to carbon monoxide that deeply alters arterial wall cells. Sympathetic and adrenergic stimulation mainly activated by nicotine and its metabolites trigger all those responses that characterize atherosclerotic progression.

Finally, the observations that chemical chains of smoking compounds, particularly nicotine, are more strongly reactive according to their spatial shape provide study material on a subject not yet well investigated but potentially of positive impact. In my opinion, changing the molecular reactivity of smoking compounds towards the production of less toxic substances could open unexpected positive results for a better control of damage from smoking.

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REVIEW

Models for depression in drug screening and preclinical studies: Future directions

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Abstract

The basic consideration in the field of antidepressants is that tests to model depression do not exist, as depression etiopathology is unknown. So far, any kind of proposed model for depression needs to satisfy construct, face and predictive validities. In the present editorial, this idea is challenged, based on the fact that "old" methods can only reveal therapeutical "me-too" drugs and that there is no longer a need of therapeutical "me-too" drugs in the field of antidepressants. Since reduction in the number of antidepressant non-responders is a real medical need, the predictive validity of animal models will be challenged in the future, as the new methods should be based on antidepressant-insensitive animals. Moreover, antidepressants exert similar effects in depressed and non-depressed subjects, but mood normalization is only induced in depressed patients. This implies that the use of normal cells and animals only involves pharmacological rather than therapeutical actions of drugs. Therefore, the use of environmental-induced changes, in the hope that these can evidence antidepressant-insensitive animals, will predominantly be used in the future. In the choice of experimental settings, other factors need to be taken into consideration: (1) gender of animals, as depression affects females more than males, (2) natural rhythmicity in drug effects; (3) pharmacokinetics; and (4) possible biomarker(s) to be measured. There are no golden recipes to discover new antidepressants but the experimental long-term strategy should very clearly be declared before starting the experiments.

Key words: Antidepressants; Animal models; Biomarkers; Predictive validity; Stress; Gender; Variability

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PREMISE

Despite the many results published on various mechanisms of action elicited by various compounds or herbal extracts in preclinical settings, which might suggest new potential antidepressant actions, well-established therapeutical antidepressant activity of drugs only derives from placebo-controlled, double-blind, randomized clinical phase Ⅲ results. Such clinical results also need to include long-term antidepressant benefit, where efficacy is also retained during maintenance treatment. Based on the efficacy of short- and long-term clinical phase Ⅲ trials, regulatory authorities give the authorization to commercialize the new medicine. There are several marketed antidepressants: i.e. tricyclics, monoamino-oxidase inhibitors and selective or mixed monoamine (serotonin, noradrenaline or/and dopamine) reuptake inhibitors.

The richness of such armamentarium is very important because physicians may choose a particular drug with a more tolerable profile for a particular patient, above all when severe comorbidity is present. Electroshock therapy is also considered to treat depression[1], mainly in drug-resistance cases[2]. In this editorial, only those drugs with approved labeling as antidepressants will be considered as efficacious medicines. In fact, some compounds that had shown some efficacy in preclinical and early clinical studies may not confirm their activity in larger clinical trials, or have been in clinical studies for too long, casting some doubts on their therapeutical benefit and/or safety window, as in the case of NK-1 (TAK-637; L733060[3]; MK869[4] or GR (mifeprisone), CRH1 (R121919; ORG34517; and NBI34041, SB723620), V1b (SSR149415) antagonists[3]. So, despite the initial scientific excitement, no compound that interferes with the stress system and that showed antidepressant-like activity in animals, exerted consistent antidepressant activity in humans[3]. Furthermore, the 5-HT$_{1A}$ receptor agonists gepirone, ipsapirone, flesinoxan and flibanserin[3,5-8], the peptide analog of melanocyte-inhibiting factor nemifitide[9] or the triple monoamine uptake inhibitor NS2359, as reported in the NeuroSearch web site[10], never reached the market or showed satisfactory antidepressant activity in clinical trials. Moreover, no herbal medicines have been registered as antidepressants. This does not mean that such compounds may not be useful for a particular subpopulation of depressed subjects but, until their efficacy is clearly shown and approved for that particular subpopulation, they are not considered as efficacious antidepressants. This "rigid" way of thinking is only dictated for the sake of clarity and for the scope of the present editorial, i.e. stimulating the search for new therapeutical strategies. What is written in the present editorial only represents personal points of view that may or may not be shared by the reader. Furthermore, as most recent publications often offer a complete overview of the literature, such papers will be quoted rather than the most well-known articles. In DOIng this, there is absolutely no intention to underestimate the very important contribution of some researchers who were pioneers in their field.

BACKGROUND

The field of antidepressants has been characterized by the introduction of more selective and potent medicine[11] into the market, with different side-effects than older antidepressants[12]. However, even if some drugs appear to be therapeutically better than others, it is not established that the new antidepressants have improved the number of responders or remitters better than the older medicines[13], the number of responders and remitters is an important medical need in the field of unipolar depression[14]. Several scientists have tried to analyze the reasons of such research difficulties in drug discovery. Animal models, incapability of detecting patient subpopulations, clinical trial design, unsatisfactory medical end-points, lack of biomarkers, psychological pressure on scientists working in pharmaceutical industries, marketing strategies and difficulty in establishing public-private R&D partnership have, from time to time, been evoked as causes for such failures[15-21]. However, such failure in drug discovery is not a peculiar aspect in the field of antidepressants or drugs for the central nervous system (CNS) as it also happens in other therapeutical areas other than the CNS[22]. More recently, genetic polymorphism has also been implicated in depression and in reduced antidepressant response in patients[23,24]. Various attempts have also been made to better define the role of neuroimaging for both drug-treatment and depressive patients[25-30]. Likewise, some biomarkers have also been suggested to differentiate drug-sensitive from drug-resistant patients[2]. Despite the interesting premises of genetic and neuroimaging findings or of various biomarkers, there is no universally agreed consensus on such indicators for antidepressant-resistance or for the course of the mental illness[1,31]. However, the real reason is that the etiopathogenesis of mood disorders is unknown and modeling what it is unknown is a challenging task; of course, this also applies for other pathologies. Thus, active searching for important biological indicators of antidepressant-resistance and of depression as mental illness seems to be the only way to proceed in this field.

A further difficulty in the field of antidepressants (but not only in this therapeutical class) is that the results of potential antidepressants in clinical trials are not always published[13]. Therefore, whether the failure is due to weak antidepressant activity or other causes (metabolism, side-effects, high placebo response, depressed sub-population, loss of interest by the company, *etc.*) is difficult to ascertain. A further complexity is that antidepressants are used, not only to normalize depressed mood, but also to treat anxiety disorders[32] and chronic pain[33]. Thus, it appears that antidepressants induce different therapeutical effects and to dissect these in different mechanisms of action is complex.

The present editorial does not review animal models or list mechanisms of action that are involved in pharmacological effects of various potential antidepressants, as manuscripts on these topics already exist[18,34-52]. The aim of this editorial is to express a personal point of view, based upon many years of experience, in order to elicit the interest of researchers and let them think about how they use their methodologies. In fact, failure to discover new antidepressants may not solely depend on chosen animal models or preclinical settings but also on how these preclinical methods are used.

As aforementioned, the medical needs in the field of antidepressants are, among others[53], an increase in response and remission rates[14] and to shorten therapeutical onset of action[54,55]. Nevertheless, very few attempts have been made in preclinical settings towards these directions. Thus, current preclinical models to screen potential antidepressants are vitiated by a tautology, as a model is only validated with already known clinically effective drugs.

Since it is difficult to think that new antidepressants may emerge with old methods, the chance to find innovative antidepressants is uniquely based on clinical trials. Researchers should take the courage to embark in alternative experimental strategies. This manuscript deals with this point of view.

BEHAVIORAL STATUS OF ANIMALS

Therapeutically, antidepressants normalize impaired mood function in depressed patients and only induce other (and/or adverse) effects in non-depressed subjects[55-57]. In accord with these findings, and in contrast with what is reported for depressed patients, in healthy subjects monoamine depletion does not change mood parameters[58] and antidepressants, in general, do not seem to modulate mood[59,60]. In healthy volunteers, antidepressants may exert pharmacological effects[61,62] that are similar to those observed in depressed subjects[63]. Thus, the use of normal animals does not seem appropriate for studying the mechanism of "therapeutical" actions of antidepressants. Nevertheless, antidepressants are often given to animals that are considered "normal" and reviews are written by using these data[38,64,65]. The first question is whether antidepressant-induced effects in normal animals may be considered as epiphenomena. Unless it becomes clear that "depressive" subjects have impairment in the function that is restored by antidepressants, the effects in normal animals may be related to the pharmacology of antidepressants rather than to their antidepressive therapeutical properties. This means that, from a therapeutical standpoint, all the results coming from normal animals or *in-vitro* assays from unaltered biological systems are questionable.

Only animals with "altered" biological systems should be used to investigate potential antidepressants; therefore, how to define a biological system as "altered" is important. Only a portion of human subjects develop depression. Thus, those procedures that induce "depression-like" effects in all animals should be avoided. Moreover, antidepressants only partially work clinically. Therefore, only those procedures which allow distinguishing antidepressant-sensitive and antidepressant-insensitive animals should be considered. This leads to another issue, in which animals can be considered as "real" controls. If normal animals serve as control for "altered" animals, in "altered" animals the comparison should be made between antidepressant sensitive and insensitive subjects. Thus, the new potential antidepressant should be tested in antidepressant insensitive animals. Consequently, one of the principles considered important for animal models, the predictive validity, will not be verified anymore.

As a diagnosis of depression is based on interviews, whether the "alterations" provoked in animals are related to human depression is difficult to determine. Nevertheless, antidepressants should be administered after the behavioral changes and not before[66]. This difference may discriminate between antidepressant- and anxiolytic-like effects. Such a concept derives from the fact that some anxiety disorders, such as generalized anxiety, compulsive-obsessive disorders or panic attacks, may be more related to the difficulty of coping with stressful situations rather than feeling despair or anhedonic. However, this concept does not apply to post-traumatic stress disorder (PTSD), where there is a clear traumatic precipitating event. In PTSD, subjects undergo an intense acute stress. Thus, it may be that the use of repeated stressful procedures might be helpful in determining potential antidepressant properties. That chronic stress, which lasts for weeks or months, is a more of a reliable predictor for depressive symptoms than acute has already been suggested[67,68]. Among the various behavioral methods used to detect potential antidepressant activity, some of them, such as learned helplessness, chronic mild stress and competition within a social milieu, seem more promising than others because they are based on repeated stressful conditions[18].

How the test is carried out is an important factor. Learned helplessness, for example, may be provoked by using stress levels that induce failures in the escaping behavior in all animals[69] or only in part of them[70,71]. However, some animals do not develop helplessness, as shown by the fact that it is possible to genetically divide those who develop helpless from those who do not[48,72-74]. Within the frame of competition within a social milieu, the resident-intruder paradigm[42,75-78] and pair-animals forced to feed in a limited time[38,78,79] are interesting, because not all animals develop the same reaction to the stimuli. Furthermore, rodents can be divided in to antidepressant -sensitive and -insensitive animals[39,54,79].

Other animal paradigms that are commonly used, such as the forced swimming test, the tail suspension test, maternal separation, olfactory bulbectomy and operant responses, appear more problematic in the sense that all the animals apparently develop similar behavioral changes and the stress is not delivered chronically, except for bulbectomy where rats may be lesioned from the very beginning[18].

READ-OUTS

The issue is not to reproduce the same symptomatology of depressed subjects in animals, but to interpret the animal behavior. For example, in the learned helplessness procedure, there is discussion whether it is better to consider as read-out the so-called "fixed ratio 1" or FR1, the escape from the compartment where there is the electrical shock to another one devoid of danger[80-83], or the so-called "fixed ratio 2" or FR2, which requires passing through the doorway twice in order to turn off the shock[84-86]. FR2 should better reproduce the wish to avoid a frustrating situation, whereas FR1 seems more difficult to interpret[87,88], even if it is easier to obtain.

Anhedonia, namely lack of pleasure, is a frequent symptom in depressed patients. Typically, in animals, anhedonia is assessed by measuring intracranial electric self-stimulation or sucrose-intake in chronic stressed ani-

mals[18]. Despite the fact that not all the stressed animals reduce their intake of a sweet solution[88], it seems that the reduction in sucrose-intake may not only depend on reduced motivation[77,89]. This point deserves further critical discussion[90].

Interestingly, young animals seem to be resistant to chronic mild stress-induced anhedonia in contrast to adult rats[91], indicating an age-dependant effect of chronic stress.

All read-outs are based on animal movements, such as escaping, swimming, consummatory behavior, aggressiveness and vocalization. Generally, researchers measure "normal" motor activity to support the notion that the observed effects do not depend on changes in capability to move. This experimental procedure may induce misleading interpretation. Animals may have normal motor activity but can change it depending on the test procedure used. For example, flibanserin, a potential antidepressant that did not match the expected outcome in clinical trials[3], reduced spontaneous motor activity in rats[92] but did not change, even at a higher dose, swimming speed in the Morris water maze[92] or inter trial crossings in the learned helplessness test[93]. Flibanserin reduced motor activity in the light-dark test in mice[92] but did not change it in an open-field, even at a higher dose[94]. However, how changes in motor activity may affect the behavior in so-called animal models for depression is difficult to ascertain, as a compound's effect may be test-dependent[92]. So, the effects on motor activity should be interpreted with caution in the therapeutical sense.

As aforementioned, when the results of a new compound are presented, information on its pharmacokinetic/metabolic profile should always be provided, together with its effects on gross animal behavior[95,96].

Differential responses of both sexes to antidepressants should also be taken into account. This has already been reported in the pharmacokinetics and pharmacodynamics (time to response, efficacy and side effects) of antidepressants in depressed patients[97,98]. In animals, Dalla *et al*[99] reviewed this field and concluded that females are more sensitive than males in the chronic mild stress and forced swimming test[100], but they are not as susceptible as males in the learned helplessness model. Sex differences may also be observed in Flinders rats, not only for their serotonergic tone, but also in response to antidepressants, as these drugs tend to alleviate sex differences[99]. Immunomodulation, neurochemical and behavioral responses point to the important role of the immune system in the pathophysiology of depression[99,101,102] and it is possible that the actions of estrogens in the brain may affect the serotonergic system in a sexually dimorphic manner[100]. Pharmacokinetics/metabolic profile between sexes should, however, always be considered before reaching a conclusion on sexual dimorphism[103].

Another aspect to consider is the possible biological rhythmicity in the animal's behavior and/or drug effect[104-112]. On the other hand, this phenomenon has also been observed in antidepressant-treated patients[113]. Thus, to be sure that the read-outs are consistent and reproducible, experiments should be repeated throughout the year and in both males and females. For example, by using the forced swimming test, DBA/2 mice were reported to be sensitive[114,115] or insensitive[116,117] to selective serotonin reuptake inhibitors. Whether these contrasting results were due to testing in different periods of the year still remains to be elucidated. Similar considerations hold for the strain C57BL mice in the tail suspension test, where it was found that they were highly citalopram-sensitive[118] or almost citalopram-insensitive[119].

TRANSLATIONAL MEDICINE

Animal models may serve to provide some information on the possible therapeutical usefulness of new compounds. Once a Pharma Company is convinced to proceed in clinic with a compound, it is necessary to be sure that the administered dose in humans is the appropriate one. Clinical phase I gives information on tolerability and pharmacokinetics/metabolic profile of the new medicine in healthy volunteers. Clinical phase Ⅱ is aimed at evaluating the therapeutic benefit of the new drug in patients. The problem is how to be sure that the drug plasma levels guarantee the desired pharmacological/therapeutical action in depressed subjects, above all if comorbidity or pathologies that may interfere with metabolism of the compound are present (i.e. renal or hepatic malfunctioning). With the lack of biomarker(s), clinical trials are run without any idea about the goodness of the dose. Thus, whether a clinical trial failed because of no satisfactory clinical outcome or for other reasons is often unknown. The biological marker(s) should be checked in ill subjects and not in healthy volunteers. In fact, neurotransmitter brain concentrations or receptor function status may change in the pathological brain[120-124] and, therefore, an image of the brain or other parameters in healthy volunteers may not provide the right information.

Despite the high interest elicited by brain-derived neurotrophic factor (BDNF), which is decreased in serum and leucocytes of depressed patients prior to antidepressant treatment and increased after 12 wk of escitalopram administrations[125], BDNF was also found to be increased in other neuropsychiatric disorders, such as schizophrenia, panic disorder, eating disorders, Alzheimer's and Huntington's disease[126]. Thus, BDNF may be an indicator of some brain vulnerability rather a specific biomarker for depression and antidepressant-sensitivity. Additionally, there is no apparent correlation between BDNF changes and depressive symptoms[127]. Moreover, BDNF is also increased by amitriptyline in whole blood cell culture from volunteers who are healthy and not ill[128]. The analysis of this biomarker is made more difficult, because the effect of the stress on this parameter in animals is age-dependent[91]. Nevertheless, there are many suggestions of possible biomarkers derived from depressed patients[129-134] or "altered" animals[3,135-137], but

so far none of them has completely been recognized as indicative of depression. Of course, this does not hamper having a biomarker that could be useful to assess the pharmacological, not necessarily the therapeutical, activity of the new medicine.

As far as "pharmacological" activity is concerned, there are no well documented reports. However, the interested reader should read the two very recent reviews on this topic: Leuchter *et al*[138] and Ward *et al*[139]. The first one describes what is interesting in examining the structure and function of the brain and genomic, proteomes and metabolomic measures. In contrast, Ward and Irazoqui[138] focused their attention on what antidepressants do not control or cure depressive symptoms. However, as one can see, none of them have the right to be conclusive.

CONCLUSION

The current available models are simply experimental paradigms sensitive to current antidepressants, which were initially discovered by serendipity. While the scientific information on the pharmacological mechanism(s) of action of antidepressants is always important, the strategy to find therapeutically valid antidepressants must drastically change. Since the first animal models were proposed, there has been intense discussion about the criteria that models should have to be considered as suitable animal models[49]. However, despite this, all animal models are generally equally used and preference is given to those that are easier to be performed.

The current methodology has permitted discovery of the mechanism(s) of action of existing antidepressants, such as monoamine uptake blockade and monoamino oxidase inhibition. The methods used so far might also be useful to study how to reduce the therapeutic delay in treating depression[93,139], even if there is clinical difficulty in assessing fast antidepressant action. However, the weaknesses of the actual way of working in the field of antidepressants appear clear. Whereas on the one hand, "altered" animals are used as behavioral models to test the antidepressant-like potential, on the other hand, normal animals are generally used to evaluate neurochemical, electrophysiological, biochemical and molecular mechanism(s) of action of known antidepressants. Moreover, susceptible animals may be used in behavioral studies, whereas all the animals are used in non-behavioral experiments. Thus, there are two variables: "alteration" *vs* "normality" status, and "susceptible" *vs* "all" animals. Therefore, to reconcile all the results in order to formulate a working hypothesis is really a tough job.

The rationale should be based theoretically on the background knowledge and then verified in antidepressant-insensitive animals for that particular model. The construction of a theoretical hypothesis is essential to have an idea of possible biomarkers or their surrogates. Entering clinical phases without having biological marker(s) to investigate, in order to assess whether compound plasma levels may be sufficient to trigger the desired pharmacological/therapeutical effects, seems to be destined to fail.

As previously written, it is difficult to model what is unknown. However, there are already some published behavioral approaches that seem more promising than others. One has recently been published by Carboni *et al*[135], using Flinders rats. As expected, the immobility time in the forced swimming test of the rats belonging to the Flinders Sensitive Line (FSL) was higher than those belonging to the Flinders Resistant Line. Both the antidepressants escitalopram and nortriptyline decreased immobility time in "normal" FSL rats, but not in FSL rats that underwent repeated maternal separation at postnatal age. This appears to be an example on how a behavioral manipulation makes animals resistant to drug treatment. Moreover, gene-environment interactions revealed changes in peripheral levels of analytes that are involved in inflammation and the regulation of metabolic pathways.

Prediction of clinical efficacy of new antidepressant compounds is not easy and needs a very high level of expertise. The process for potential innovative antidepressants should go through the following steps: (1) have a clear "construct" criterion; (2) selection of antidepressant-insensitive animals by using "old" methods (i.e. escape deficits in the learned helplessness test; sucrose intake in the chronic mild stress; social defeat); (3) to test the compounds after and not before behavioral "alterations"; (4) to verify that insensitivity does not depend on biological rhythms or pharmacokinetics/metabolic profile; (5) to use both females and males; and (6) to identify biomarker(s). If such a procedure is not followed, another therapeutical me-too antidepressant is certain to be found.

In order to discover the antidepressant of the future, the problem of non-responders needs to be addressed. It is also necessary to take into consideration that it is difficult to have a unique animal model for depression, as all pieces of evidence "argue against a unified hypothesis of depression"[101]. Experimentally, it means that all antidepressant-sensitive animals should be discarded[140]. Thus, the alternative is the use of behavioral methods to identify antidepressant-insensitive animals and electrophysiological, neurochemical, biochemical and molecular studies should be performed in these animals. *In-vitro* studies should also be performed by using cells from "altered" animals. In this way, the concept of predictive validity cannot be applied for future research anymore.

The definition of "antidepressant-insensitive" should depend on scientifically-based evidence. Thus, one should be sure that the insensitivity does not depend on pharmacokinetic/metabolic profile of the drug or particular seasonal effects. This implies replication of a particular test throughout the year with concomitant plasma level assay. However, nobody has the golden recipe to discover original antidepressants, but after 50 years, where only me-too antidepressants in the therapeutical sense were introduced in the market, it is time to change. The first

change should be to not use any more normal animals or normal cells. For example, there is a wonderful review on the effects on brain dopamine after antidepressant and drug treatment in normal animals[38]. However, whether such a review may increase the insight in the therapeutical effects of antidepressants is questionable, even if the hypothesis that the authors put forward on dopamine D_1 receptors is fascinating. In fact, almost all data refer to normal animals. Thus, the hypothesis that antidepressants may enhance dopaminergic D_1 sensitivity should be supported by data originated in "altered" animals.

Finally, the problem is how to screen for new antidepressants. Of course, the experiments should be randomized and the observations performed by observers who are unaware of the treatment. The question is whether it is worth spending such a long time for such a process. It is personal opinion of the author of this editorial that it is necessary, if we want to embark a new era in the field of antidepressants.

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2nd Annual Pediatric Pharmacology Conference
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6th Asian Pacific Congress of Heart Failure
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4th International Conference on Drug Discovery & Therapy
Dubai, United Arab Emirates

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Keystone Symposia on Molecular and Cellular Biology
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May 2-4, 2012
8th Annual Pediatric Clinical Trials Conference
Philadelphia, PA, United States

May 13-18, 2012
Keystone Symposia on Molecular and Cellular Biology
Drug Resistance and Persistence in Tuberculosis
Kampala, Uganda

May 16-19, 2012
International Stress and Behavior Society
17th International "Stress and Behavior" Conference
St. Petersburg, Russia

June 7-9, 2012
British Pharmacological Society
Focused Meeting on Neuropeptides
London, United Kingdom

June 9-12, 2012
The Neutrophil in Immunity
Quebec City, PQ, Canada

June 10-15, 2012
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Snowmass Village, CO, United States

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39th Annual Meeting and Exposition
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Protein Phosphatases
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July 17-20, 2012
6th European Congress of Pharmacology
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July 22-27, 2012
FASEB Summer Research Conferences
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Snowmass Village, CO, United States

July 27-30, 2012
International Academy of Cardiology
17th World Congress on Heart Disease
Toronto, ON, Canada

July 29 - August 3, 2012
FASEB Summer Research Conferences
Integration of Genomic and Non-Genomic Steroid Receptor Actions
Snowmass Village, CO, United States

August 2-5, 2012
American Psychological Association
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Orlando, FL, United States

August 5-9, 2012
26th Symposium of The Protein Society
San Diego, CA, United States

September 9-13, 2012
10th International Catecholamine Symposium
Pacific Grove, CA, United States

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American College of Clinical Pharmacology
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Chicago, IL, United States

October 13-17, 2012
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World Journal of Pharmacology

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Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

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Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

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Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
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