EDITORIAL

This special issue is dedicated to the effects of Androgenic Anabolic Steroids (AAS), what AAS are, the mechanisms of action as well as the untoward effects on health status in athletes.

Androgenic anabolic steroids (AAS) are used worldwide to help athletes gain muscle mass and strength. They are beneficial in athletic competition and are particularly beneficial for power lifters, bodybuilders, student athletes, and fitness enthusiasts. The real incidence is difficult to evaluate, but a recent study indicated that more than more 1 million Americans are current or former users. The true incidence of AAS related medical problems is not known, due to several drawbacks in human studies. The entity of AAS side effects, in fact, depends on the sex, the dose, the duration of treatment, whether they are taken during exercise training or under sedentary conditions, and the susceptibility of the individuals themselves to androgen exposure partly depending on genetic factors. Both the acute and the chronic effects can lead to toxicity, but generally the serious and even fatal effects depend on the time and the duration of AAS administration, the most serious being observed when AAS are used in high dose and over prolonged time and the milder and more frequently seen side effects disappearing upon discontinuation of use. A limitation of human studies is represented by the fact that information about the intake of steroids are, generally, self reported and it is hardly possible to assess the exact dosage in an objective way. Four of all available AAS seemed to be more used than others; testosterone, nandrolone, methandrostenolone and stanozolol. AAS can be bought legally in some parts of the world, whereas in other countries AAS are classified as illegal narcotic substances. Furthermore AAS are often used in combination with other dugs or substances at high dosage, so it is extremely difficult to separate the toxic effects of AAS from those caused by the other drugs abused.

The fact remains, as Maravelias said, that the abuse of androgenic anabolic steroids (AAS) is a remarkably prevalent problem in competitive and non-competitive athletes. The goal of this special issue is to summarize the clinically relevant data regarding AAS abuse, including mechanism of action, efficacy and adverse effects.

Although there are three typical forms of AASs intake (i.e., oral pills, injection, and skin patches), oral administration is by far the most common and convenient. Oral testosterone is rapidly absorbed but it is rapidly converted into inactive metabolites, so that nearly 15% of it persists in active form. As such, testosterone derivatives are alkylated at the position 17 (e.g., methyltestosterone and fluoxymesterone) to reduces liver catabolism and ultimately enhance bioavailability. No differential effects in increasing sport performances have however been reported according to the different patter of administration. The activity of androgens is mediated by a specific receptor, which belongs to the nuclear receptor superfamily, as Lippi and collaborators explain in detail. It is composed by a DNA binding domain and two transcriptional activation domains, AF-1 and AF-2. Androgen receptor transcriptional activity is mainly mediated by the N-terminal AF-1 domain. When the hormone reach the target cells, it binds to the receptor ligand-binding domain. Then, the receptor is dissociated from protein chaperones and becomes active, moving from cytoplasm to nucleus. Activated receptors interact as homodimers with the androgen response element on the chromatin, which triggers the formation of a transcription complex. Co-activator and co-repressor complexes for nuclear-receptor-mediated transcriptional regulation are present in cells, generally inducing gene activation, transcription of the gene, translation and a resultant alteration in cell function, growth or differentiation.

Lavandero and collaborators focalized their paper about the numerous studies demonstrated increases in intracellular Ca2+ in response to AAS. These Ca2+ mediated responses have been seen in a diversity of cell types, including osteoblasts, platelets, skeletal muscle cells, cardiac myocytes and neurons. The versatility of Ca2+ as a second messenger provides these responses with a vast number of pathophysiological implications. Classically, anabolic androgenic steroids (AAS) act through binding to androgen receptors (AR), which once bound by their ligands, function as nuclear transcription factors promoting the expression of genes under the control of steroid-response elements (SRE). This programmed gene expression is achieved within a time course of hours after AAS binding to ARs. More recently, however, it has been described that steroidal hormones including AAS can also provoke faster responses, which do not involve gene expression. These effects have been termed as 'nongenomic', and they cover a wide range of intracellular processes such as the activation of membrane bound receptors, triggering of downstream pathways that involve protein kinases and phosphatases, mobilization of intracellular Ca2+, as well SRE-independent changes in transcription. The origin of these responses has been attributed to AR-AAS complexes present in caveolin-enriched zones of the plasma membrane, however, recent studies identify orphan candidates for membrane-bound AR that after binding to AAS, trigger activation of intracellular second messengers.

The Impact of AAS on Neuropeptide Systems is the targeted review treated by Hallberg.

Although the impact of AAS on neuropeptide systems has been the main focus for this review it should be emphasized that it is known that AAS administration to rats also effect other systems with high relevance for the altered behaviors attributed to AAS abuse. These include AAS impact on e.g. the serotonin, dopamine and glutamate systems. It should also be emphasized that the high doses and accumulated levels of nandrolone could lead to activation also of other related steroid receptors such as

estrogen, progesterone and mineralcorticoid, as well as glucocorticoid receptors. Furthermore, activation of membrane bound steroid receptors or neurosteroid receptors, e.g. GABAA and NMDA receptors, could all contribute to the observed alterations of the neuropeptide systems. AAS or their sulfate conjugates could also interact with neurosteroid receptors or alternatively AAS could indirectly modulate levels of endogenous neurosteroids.

The conclusions drawn from Riezzo and others, based on data about experimental animal studies, support the hypothesis that the combined effects of vigorous weight training, anabolic steroids abuse and stimulation of the sympathetic nervous system, may predispose to myocardial injury (myocardial disarray, contraction band necrosis, interstitial fibrosis, apoptosis) and subsequent cardiac failure (colliquative myocytolysis) mediated by oxidative stress. These cardiovascular effects of AAS are mediated by genomic (intracellular androgen receptors – nuclear transcription – gene expression) and non-genomic mechanisms.

Cardiac hypertrophy is a leading predictor of progressive heart disease which often leads to heart failure and to a loss of cardiac contractile performance associated with profound alterations in intracellular calcium handling.

The pathophysiology on sudden cardiac death phenomena during AAS abuse and the underlying pathologic mechanisms are reviewed by Nascimento and collaborator. They focused on the direct cardiotoxic effects of AAS and discussed the evidences of the potential mechanisms involved in these phenomena. The results of heart rate variability analysis in human and animal model showing an AAS-induced cardiac autonomic imbalance, with reduction of parasympathetic cardiac modulation and increase of sympathetic cardiac modulation, are in accord with post-mortem histopathological data from AAS users, since the presence of contraction band necrosis in myocardium is associated with adrenergic overstimulation. Thus, the scientific works published at the time, at least in our knowledge, taken together strongly sustain the hypothesis that AAS abuse increases the sudden cardiac death risk inducing autonomic, mechanical, electrical and morphological cardiac remodeling.

Neri, Marshall and Pomara, with their collaborators, focused on sectorial organ dysfunction induced by AAS-abuse; liver damage, immune system dysfunction and renal alteration are reviewed and discussed, respectively.

Stanozolol, and other orally active AAS, has been shown to cause inflammatory or degenerative lesions in centrilobular hepatocytes, ultrastructural alterations in the canaliculi and degenerative changes in mitochondria and lysosomes. Furthermore a prolonged AAS administration provokes an increase in the activities of liver lysosomal hydrolases and a decrease in some components of the microsomal drug-metabolizing system and in the activity of the mitochondrial respiratory chain complexes without modifying classical serum indicators of hepatic function.

The administration of AAS may have severe consequences on immune function as they may either over activate immune cell function or significantly dampen immune related activities. Although little is known about the effects of AAS on the immune system, there are suggestions that when they are administered at supraphysiological concentrations, AAS can decrease immune related cellular functions. Differing concentrations of testosterone for example, have been shown to affect neutrophil functions. In particular, testosterone at a dose of 10nM can reduce extra- and intracellular superoxide, increase phagocytosis, suggesting a decrease in the overall oxidative capacity of the neutrophils.

Abusing high doses of the anabolic androgenic drug for long periods can cause wide peculiar histological modifications of the normal kidneys structure which may lead to produce serious renal disorders such as renal failure in late stages. Despite this, the molecular mechanism of AAS direct toxic effect on kidney is still far to be completely understood a great contribute could be provided in the future explaining the meaning of HSPs overexpression after acute and chronic administration of AAS.

Last but not least, Chiarotti and others dedicated the review to antidoping control analysis. They conclude that androgen anabolic steroid analysis has become an indispensable and well-established procedure routinely carried out in antidoping laboratories. The tools available for the identification of these substances are now several, as well as the analytical methods developed by the laboratories, allowing a very high diagnostic power for the determination of AASs. In the last years a new phenomenon closely connected to the superior efficiency of doping control to detect these substances has been arisen. Chemically modified steroids that are not used in clinical practice and either have been synthesized in the past or have been specifically developed to circumvent doping control are detected from doping authorities.

Various kinds of mass spectrometers, mainly interfaced to gas chromatography, are powerful analytical tools for the identification of steroids and their metabolites, at sub-nanogram levels, in complex matrices such as biological fluids and tissues. Tandem mass spectrometry (MS/MS), coupled to gas chromatography or to liquid chromatography, can provide an additional analytical dimension in case of trace analysis or confirmatory purposes. High Resolution-MS allows the identification of substances at very low concentrations, by increasing the signal to noise ratios of the accurate masses characteristic of each analyte. Liquid chromatography, coupled to different kinds of mass spectrometers, is becoming a routinely used technique both in antidoping and forensic toxicology laboratories for the analysis of AASs. Finally, abundance ratios of stable isotopes, as measured

by gas chromatographic combustion - mass spectrometry, has been used as unsurpassed pillar to characterize and authenticate the synthetic origin of exogenous steroids in biological samples.

This special issue want to contribute for a better understanding on AAS (androgen anabolic steroid) related chemical structures, metabolism, cellular responses, physiological and pathological effects.

From the data presented in these reviews, we can realize that considerable research to date has led to the identification of a growing number of AAS-adverse effects due to abuse in healthy athletes. However, we should keep in mind that, although substantial progress has been made in identifying novel mechanism of damage of AAS-abuse, great efforts are still need to advance to their pathophysiological mechanisms. Therefore, I hope that this special issue of Mini-Reviews in Medicinal Chemistry can be useful to those readers already working on sports medicine and substances abuse, stimulating discussion and further studies, but principally serving to attract new researchers from other fields interested to clarify the cellular responses and to understand the pathological effects of these substances.

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