"*GEORGE EMIL PALADE*" *UNIVERSITY OF MEDICINE, PHARMACY, SCIENCES AND TECHNOLOGY FROM TÂRGU MUREȘ*
Doctoral School of Medicine and Pharmacy

Androgenic manipulation of muscle strength and aesthetics - "in vivo" animal model. A pharmacological and analytical perspective.

PhD Thesis Summary

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The population's interest in substances designed to improve physical appearance is increasing, a consequence of the clichés imposed by mass-media, omitting the possible risks caused by exposure to these substances.

Selective androgen receptor modulators (SARMs) are compounds with anabolic effect, acting selectively on androgen receptors located in the muscle and bone tissue, but with minimal influence on those located in androgen-dependent tissues, a huge difference from classical androgenic derivatives mechanism of action.

SARMs are substances of interest intended to manipulate strength in athletes, but mostly muscle aesthetics among younger people with narcissistic personality. Despite these compounds are developed for over two decades, the use is not yet approved in therapy, while young, healthy individuals administer them in supra-pharmacological doses without assessing the possible risks.

The doctoral thesis combines analytical, pharmacological and biochemical aspects of compounds currently in phase II and III clinical trials, abusively administered for other than medical use, the developed studies being at the interface between analytical and experimental medical research.

The aim of the thesis is to evaluate, from an analytical point of view (qualitative/ quantitative), two SARM compounds (andarine and ostarine) in dietary supplements, *a priori* investigating their pharmaco-toxicological tropism on androgen-dependent tissues (testis, prostate), on drug elimination pathways (liver, kidneys) and the potential influence on the hypothalamic-pituitary-gonadal axis by determining the plasma level of testosterone and pituitary gonadotropins, in animal doping models in male rats.

The purpose of the first study was a critical, qualitative, quantitative, but also pharmaco-technical evaluation of the quality of the dietary supplements with andarine and ostarine, by two validated UHPLC methods. These methods were used to accurately determine the active substance in the supplements administered in the experimental animal doping groups. Andarine supplements were underdosed, the content of active substance being approximately 5 times less than the manufacturer's stated content, but the dosage was consistent in the mass uniformity test. The quality ostarine supplements was good, regarding the uniformity of mass and the content of active substance, very close to that stated by the manufacturer (92.91%).

The second study describes the development and validation of two LC-MS/MS methods for profiling the concentration of active substances (andarine and ostarine) in the serum of rats treated with previously evaluated supplements, in order to test the "in vivo" absorption of the active substance. The developed and validated LC-MS/MS methods are accurate and precise in the determination of the two SARMs in rat serum, demonstrating the favorable oral bioavailability of these substances. The described methods for SAMRs determination in serum samples are fast and simple, using also an affordable and fast method for sample preparation (protein precipitation), a volume of only 2 µl of serum and a short analysis time (2 minutes/analyzed sample).

The purpose of the third study was to assess the possible influence of SARMs on the hypothalamic-pituitary-gonadal axis, compared to classical androgens by quantifying the plasma concentration of hormones (testosterone, FSH and LH), relevant for reproductive capacity, in animal doping models. An LC-MS/MS method appliable on a wide range of concentrations (50-50000 pg/mL), with an isotope-labeled internal standard was developed to determine testosterone, in plasma samples obtained from animal doping models with andarine, ostarine and testosterone (classic steroid). The animals in testosterone group presented a marked increase in plasma testosterone levels, with a consecutive and statistically significant decrease in LH concentration, which demonstrates that anabolic steroids inhibit the hypothalamic-pituitary-testicular axis, unlike the results obtained in animals from andarine and ostarine dopping groups

In the fourth study, the pharmaco-toxicological tropism of the compounds on drug elimination pathways (liver, kidneys) and on androgen-dependent tissues (testis, prostate) in animal doping models was investigated. In the histological analysis of the hematoxylin-eosin (HE) stained tissues of the study groups, the presence/absence of morphological changes was compared to the control group. In the study of reproductive toxicity, spermogram test was also

performed to assess the number and morphology of sperm. It was concluded that in the liver, testosterone, andarine and ostarine can induce protein dystrophy, but karyolysis is more evident in testosterone. Testosterone and ostarine can induce renal hyperemia, more pronounced in ostarine, while andarine causes glomerular morphological changes. In androgen-dependent tissues, testosterone can induce prostatic glandular hyperplasia, visible especially in the peripheral zone of the prostate, while andarine and ostarine induce diffuse prostatic hyperplasia. There were no morphological changes in the testis, proving SARMs' selectivity and the lack of effect on fertility, while the spermogram test in testosterone group indicated oligospermia compared to andarine and ostarine.

In conclusion, the thesis should be considered an alarm signal given the growing interest of young people to improve their physical appearance, imposed by the aggressive online advertising and the ease of purchasing for these highly active pharmacologic substances with variable doses, used as "body image enhancing drugs", comercialised as dietary supplements of questionable quality.

The developed LC-MS/MS analytical methods are fast, affordable and reliable and can be used both in pharmacokinetic studies and for doping tests from various biological matrices in sport.

The doctoral thesis is a synthesis of sophisticated analytical determinations, developed with the main purpose of quantifying the direct relationship between abuse and organ and reproductive toxicity, in an animal model that can be extrapolated to humans.

These are the aspects in which the doctoral thesis brings important scientific data, representing a useful material for the analyst (development of methods), researcher (animal models), sport and exercise physicians (aspects of toxicity/legal status), authorities (compounds on WADA prohibited list).