

Project Review

“Are liposomes masking agents? An investigation on the interaction between liposomes and anabolic steroids”

F. Botrè, X. de la Torre, A. Cadwallader, A. Tieri, (Federazione Medico Sportiva Italiana, Italy),

The project was developed using the observation that “Liposom Forte®”, a pharmaceutical-grade product containing “empty” liposomes, was reportedly used by athletes. Liposom Forte® was found stored together with banned and non-banned drugs during investigations carried out by Italian legal authorities.

Due to the negligible direct effect on the enhancement of performance of “Liposom Forte®” and similar pharmaceutical products containing empty liposomes, we postulate these products could be used as masking agents to make detection of other forbidden drugs more difficult. Liposomes can be used as masking agents by following one or more of the following hypothesized strategies:

- i) Injection immediately after being mixed with steroids to produce “home-made” slow/sustained release preparations;
- ii) Injection as such (“empty”), before an “expected” anti-doping test to promote interaction with steroids/metabolites circulating in the organism and altering the excretion profile;
- iii) Direct addition to the sample collected for anti-doping tests to reduce the concentration of “free” (i.e. not bound to liposomes) steroids/metabolites and reducing the efficacy of the laboratory analytical procedures used for detection.

We have preliminarily shown that an interaction between liposomes and androgenic anabolic steroids can cause a reduced efficacy of the analytical procedures (normally based on GC/MS analysis of the corresponding TMS-derivatives after enzymatic hydrolysis) followed by anti-doping laboratories. We have also preliminarily demonstrated that direct addition of liposomes to urine samples containing steroids causes a masking effect: the amount of steroid detected in the samples was significantly reduced after liposome addition.

We plan to continue the present research considering the following aspects:

- i) More thorough evaluation of the binding ability and masking potential of liposomes in vitro.
- ii) Assessment of the masking potential of liposomes in vivo.
- iii) Characterize the physico-chemical properties of liposomes and improve analytical methods used to detect the presence of liposomes in biological samples

“Are liposomes masking agents? An investigation on the interaction between liposomes and anabolic steroids”

F. Botrè, X. de la Torre, A. Cadwallader, A. Tieri, (Federazione Medico Sportiva Italiana, Italy)

Results and Conclusion:

One of the hottest topics in antidoping research is the study of the so called masking agents, i. e. substances or methods capable of “hiding” other forbidden substances, thus reducing the efficacy of the experimental strategies used to detect the abuse of doping agents by the analysis of biological fluids. The class of masking agents was originally limited to substances with diuretic effects, but it has been progressively expanding, now comprising also agents that can interfere with either the pharmacokinetics of other banned substances and/or with the analytical procedures normally applied by the WADA-accredited anti-doping laboratories for the detection and, where required, the quantitative determination of the concentration, in biological fluids, of other banned substances.

To the best of our knowledge, this research project is the first one to specifically consider the possible relevance, as masking agents in sport doping, of liposomes, a class of supramolecular structures constituted by phospholipids extensively studied in the pharmaceutical field for their properties as drug delivery systems. More specifically, we have focused our attention on the potential masking effects of liposomes on anabolic androgenic steroid (AAS), to date the most widely abused class of prohibited substances in sports doping.

The results we have obtained can be summarized as follows:

- I. Liposomes have been shown to interact with anabolic androgenic steroids, leading to a reduced analytical recovery of both the parent compound and the glucuronide metabolites.
- II. The interaction occurs in a relatively wide range of experimental conditions, and it has been verified for several representative pseudo-endogenous anabolic androgenic steroids and for various liposomes, differing in charge, size and chemical composition.
- III. The effect is particularly noteworthy whenever a quantitative determination of the target steroid(s) is required, as it is the case of threshold substances (i.e. 19-norandrosterone) and/or of the evaluation of the urinary steroid profile in the framework of longitudinal testing and/or of the Athlete Biological Passport.
- IV. Specific countermeasures to ideally annul the observed “masking effect” include the strict monitoring and control of all quality parameters of the analytical

procedure(s) followed for the analysis of AAS, with special emphasis, in the case of GC-MS based methods, on the efficacy of the derivatization step.

V. A novel analytical procedure has been designed, developed and validated, to detect and quantitate a wide variety of liposome constituents (phospholipids and sphingomyelins) in biological matrices (blood and/or urine) and in pharmaceutical products, with the aim to identify suitable markers for the detection of liposome intake by the analysis of blood and/or urine samples.

The results of the present project unearthed a new and effective class of masking agents, that go beyond those with a direct pharmacodynamic effect, that act as true and effective "doping delivery systems", altering the pharmacokinetic properties (i.e. transport, distribution and elimination) of doping drugs.