

To implement this goal peripheral blood was used, the lymphocytes of which were typed by the reaction of direct immunofluorescence for expression of CD45RA and CD45RO molecules.

The study of the content of naive lymphocytes and memory lymphocytes among the representatives of the three groups of examinees has revealed a number of important differences. Clinically healthy people (a control group 1) are different from the comparison group for higher presence of naive lymphocytes, reflecting the sufficiency of lymphopoiesis (the comparison is significant ($p < 0.005$)). In the other two groups (HIV-infected individuals and people older than 75 years), this index is lower. But the mechanisms of lymphopoiesis limitation are different: in the presence of HIV-infection they are provided with the influence of HIV on hematopoietic cells. This observation is consistent with the other researchers' findings who conducted the study as part of the features of HIV infection and changes in the immune system during human life.

The study leads to the following conclusions:

1. Summary immunologic experience, manifested by the presence of memory lymphocytes accumulates over time.
2. The presence of chronic lymphotropic infection (HIV) does not show the formation of an immunological total experience, and leads to lymphopoiesis violation, i. e., the reduction of the number of "naive" lymphocytes.
3. Summary immunological experience is formed by opposite parity changes – a decrease in the number of "naive" lymphocytes and an increase in the number of memory cells.

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STUDY OF ANABOLIC AGENTS METABOLISM USING IN VITRO SYSTEM OF HUMAN HEPATOCYTE CELL LINE

Abuse of anabolic steroids is one of the most important issues in sports. In doping control the detection of steroids is performed on the basis of urinary steroid profile, and the knowledge of it is very important to provide accurate control. Metabolism studies are usually performed by collecting urine samples after administration by volunteers of the steroid (excretion study). During last years human hepatocyte cell line has become a widely used system for metabolic studies since in vitro drug metabolism studies serve as a convenient screening mechanism to investigate drug metabolites.

The aim of this study is to clarify the suitability and possibility of using the in vitro system (human hepatocyte cell line Hep G2) for identification and separation new anabolic drug metabolites and compare the results with in vivo investigations.

For the detection of known metabolites present in a routine doping analysis target GC-MS/MS was used. In order to identify the maximum range of possible products of hepatocytes metabolism we applied the high resolution LC-MS (orbitrap) with full scan mode and LC-MSn for structure clarification.

Initially, to test the metabolic activity of the cell lines the study is concentrated on the phase I metabolites of the endogenous anabolic steroid – testosterone. We have identified both major metabolites (androstenedione, androsterone, etiocholanolone etc.) and rare minor metabolites. Subsequently, metabolic profiles of two exogenous anabolic steroids - oxymetholone and methandienone – have obtained.

Comparison of the results with studies in vivo showed the presence of the majority of known metabolites. At the same time number of metabolites were revealed which are the intermediate forms, as well as rare isomeric forms.

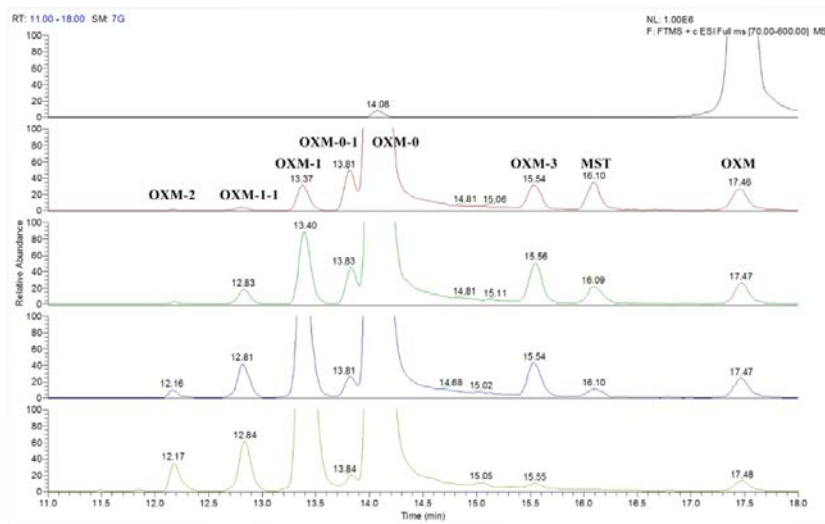


Figure 1. – High resolution LC-MS analysis of the hepatocyte culture incubated with oxymetholone for 15 min, 2, 4, 8 and 24 hours

Further identification of these forms in the frame of in vivo studies may provide additional mechanisms to enhance long-term drug control of prohibited substances in sports.