7. SUMMARY

Metabolism and analysis of selected orally-active 17α-alkyl Steroids with anabolic potential in the horse

This study tried to investigate the metabolism of the anabolic steroids dehydroepiandrosterone (DHA), methandriol (MAD) and 17α-methyltestosterone (17α-MT) in the Equine and to subsequently develop a gas-chromatographic / mass-spectrometric (gc/ms) screening method for the detection of the two latter orally-active anabolic agents.

For each experiment 200 mg of the respective steroid were administered to two thoroughbred horses in training. The administered substances contained a mixture of the same amount of deuterated and non-deuterated steroid. Additionally, a radioactive isotop was administered in the DHA (^{3}H-DHA and ^{14}C-DHA) and MAD (^{3}H-MAD) studies. In the case of 17α-MT no radioactive isotop was administered.

Blood samples were taken at regular intervals for a three- up to a five-day period. Freely-voided urine was collected for the same period of time. The absorption and elimination of the administered steroids were determined by scintigraphy of the ß-emission in all radio labelled samples collected. Only the urine samples were subsequently extracted for the detection of possible steroid metabolites by gc/ms-analysis.

The half-life of intramuscularly administered ^{3}H-DHA ranged from between 9 to 12 h. The half-life of orally administered ^{14}C-DHA was around 3 h in both horses and hence about ¼ to ⅓ that of ^{3}H-DHA. This can mainly be explained by the first-pass effect to which orally administered ^{14}C-DHA had been exposed in the liver. The half-life of orally administered ^{3}H-MAD was 11.99 h for one of the two horses. This value is similar to the one for intramuscularly administered ^{3}H-DHA. This would indicate that the 17α-alkylation has protected the orally administered MAD from the first-pass effect of the liver. For the second horse the half-life was only 3.75 h, which is similar to the one for orally administered ^{14}C-DHA being exposed to the first-pass effect of the liver. This large difference in half-life could not be explained.

Overall, about 60% of the total intramuscularly administered ^{3}H-DHA was excreted via the urine. For orally administered ^{14}C-DHA and ^{3}H-MAD this value was about 50%. In ^{3}H-DHA and ^{3}H-MAD ≥95% of the radioactive dose excreted via the urine had been excreted only after 50 h. For ^{14}C-DHA this value had already been reached between 30-35 h after administration of the steroid.

The urinalysis of the radioactively labelled steroids showed great losses of radioactivity, i.e., of steroid metabolites in the urine after extraction as well as in the solutions used for

washing the extracts. Two main reasons were assumed for these losses: (1) it was thought that losses which occurred into the urine after extraction were at least partly caused by capacity problems of the cartridges used; whereas (2) losses into solutions used for washing the different extracts might have their origin in the existence of a number of very polar metabolite(s), which were hence “washed out” of the lipophilic extracts by the hydrophilic wash solutions used.

Due to these losses the gc/ms analysis of DHA and MAD partly showed rather unsatisfactory results; most of the detected metabolites were only present in very low concentrations. This resulted in difficulties to assign the appropriate chemical structure to a number of the detected metabolites.

In general, DHA and its metabolites were excreted only to a very low percentage as free steroids (< 2%). About 10-15% were excreted as conjugates with glucuronic acid while around 60% of the metabolites showed a conjugation with sulphuric acid. The excretion pattern of MAD and its metabolites showed similar results; where only around 4% were excreted as free steroid, while 10-15% were excreted as conjugates of glucuronic acid and around 50% as conjugates of sulphuric acid.

The following three metabolites have been determined for DHA:

1. 3ß-Hydroxyandrost-5-en-17-one (I = parent drug),
2. 5-Androsten-3,17-diol (II/4 isomers), and
3. 5-Androstane-3,17-diol (III/2 isomers).

The following five metabolites have been determined for MAD:

1. 17α-Methyl-5-androsten-3ß,17ß-diol (I/2 isomers = parent drug),
2. 17-Hydroxy-17α-Methyl-5-androstane-3ß-ol (II),
3. 17-Hydroxy-17α-Methyl-5-androstane-3,(15/16)-diol (IV),
4. 17-Hydroxy-17α-Methyl-5-androstane-3,(6/7),16-triol (V/2 isomers),
5. 17-Hydroxy-17α-Methyl-androst-4-en-(6/7),16-diol-3-one (VI), as well as

Since in the case of 17α-MT no radioactively labelled isotope had been administered no information could be obtained about the absorption or excretion profile, the plasma half-life of the substance or the extent of its phase-II metabolism in the horse.

The gc/ms analysis of this steroid though showed a large number of metabolites present in the analysed extracts in viable concentrations. It was therefore assumed that the losses during urinalysis as described for DHA and MAD did not occur at all or only to a much lesser extend in the case of 17α-MT.

The following 12 metabolites have been determined for 17α-MT:

1. 17α-Methyl-17ß-Hydroxy-4-androsten-3-one
2. 17-Hydroxy-17α-methyl-5-androstane-3-ol

3. 17-Hydroxy-17α-methyl-5-androstane-(6/7)-ol-3-one
4. 17-Hydroxy-17α-methyl-androst-4-en-(6/7)-ol-3-one
5. 17-Hydroxy-17α-methyl-5-androstane-3,(6/7 or 15/16)-diol
6. 17-Hydroxy-17α-methyl-5-androst-3,(6/7)-diol
7. 17-Hydroxy-17α-methyl-5-androstane-3,16-diol
8. 17-Hydroxy-17α-methyl-5-androstane-3,(6/7),(15/16)-triol
9. 17-Hydroxy-17α-methyl-5-androstane-3,(6/7),16-triol
10. 17-Hydroxy-17α-methyl-androst-4-en-3,(6/7),(15/16)-triol
11. 17-Hydroxy-17α-methyl-androst-4-en-(6/7),16-diol-3-one
12. 17-Hydroxy-17α-methyl-5-androstane-(6/7),(15/16)-diol-3-one

For MAD the prerequisite for the establishment of a routine detection method is the optimization of the method used, which in its current form does not yet yield sufficient results viable to be included into a screening-analysis procedure.

For 17α-MT instead the search for metabolites 2, 6, 7 and 9 during a routine gc/ms screening analysis is considered sufficient to prove the administration of 17α-MT to the horse for doping purposes. Using 'single ion monitoring (SIM)' it should be screened for the fragment ions with the mass-to-charge (m/z) ratios m/z 143, m/z 218 and m/z 231 since they are characteristic for all 17α-methylated steroids. This method allows the detection of a 17α-MT application to the horse for a period of at least 50 h after administration. Since in the course of this study no later urine samples had been collected, additional studies will be needed to determine the maximum time of detection after administration of 17α-MT to a horse for doping purposes when using this method of extraction and analysis.