Ultrastructural changes induced by anabolic steroids in liver of trained rats

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Summary. The effects of anabolic steroid treatment in association with endurance training on biochemical serum parameters and liver ultrastructure have been investigated in male rats. Values of serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase were not significantly affected by administration of high doses of fluoxymesterone or methylandrostanolone. Electron microscopic examination of hepatic tissue from treated animals revealed ultrastructural alterations of hepatocytes. The most prominent changes were swelling of mitochondria, which presented electron-lucent matrix and slightly defined cristae, and a marked increase in the number of lysosomes. These changes were evident in both sedentary and trained treated rats, indicating that liver cell damage is produced by anabolic-androgenic steroids despite the simultaneous realization of physical exercise. The alterations observed were not detected by means of conventional biochemical liver tests.

Key words: Fluoxymesterone, Methylandrostanolone, Hepatotoxic effects, Serum enzymes, Exercise training

Introduction

Anabolic-androgenic steroids, a group of synthetic compounds structurally related to testosterone, are used in medical practice in status of muscle wasting or debilitation, to stimulate erythropoiesis in some anaemias and in the treatment of hypogonadal status (Rahwan, 1988). They are also taken in high doses by athletes who wish to improve physical performance (Wilson, 1988; Lamb, 1989). Although adverse effects have been reported in patients treated with these compounds (Ishak and Zimmerman, 1987; See et al., 1992), knowledge about the side effects of suprapharmacological doses of anabolic steroids in athletes is limited. Reduced levels of serum high density lipoproteins and testicular atrophy have been shown in top-level and amateur athletes using anabolic steroids (Wilson, 1988; Hickson et al., 1989; Lamb, 1989; Graham and Kennedy, 1990). In the case of oral anabolicizing androgens, the presence of the 17α-alkyl group introduced to retard hepatic degradation, seems to be associated with hepatotoxic effects and, in fact, three cases of fatal liver tumours have been reported in otherwise healthy athletes who had been taking anabolic steroids for several years (Hickson et al., 1989). Levels of enzymes and metabolites related to hepatic function have been reported to be slightly elevated (Hakkinen and Alen, 1986; Lenders et al., 1988) or not substantially modified (Alen, 1985; Ballarin et al., 1986; Thompson et al., 1989) in the serum of athletes using different anabolic steroids. However, the possibility of liver lesions not being detected by conventional liver function tests exists.

It is well-known that androgens modulate several hepatic functions such as metabolism of lipoproteins, steroids, drugs,... (Rahwan, 1988). Likewise, morphometric analysis of rat liver after castration shows a significant reduction in the number of hepatocytes (Tanganelli et al., 1988). The anabolic-androgenic steroids, although exhibiting some of the characteristics and actions of the endogenous androgens, could be regarded in the high doses taken by athletes as a xenobiotic load for the liver, probably related to functional and structural alterations of this organ. On the other hand, exercise is known to influence a large number of physiological factors (haemodynamics, blood pH, body temperature, etc.) which may affect the pharmacokinetics of numerous drugs. Thus, the concurrence of training and anabolic steroid ingestion could be expected to modify the potential hepatotoxicity of these compounds. Nevertheless, to date information about the effects of anabolic-androgenic steroid treatment and simultaneous exercise training on liver structure is scarce. Pathological changes indicative of cholestasis have been observed in the liver of motor-active mice (Mus wagneri rotans) after 4 weeks of methandrostenolone treatment (Stang-Voss and Appell, 1981), but the relevance of this model with respect to the...
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The purpose of the present work was to study the effects of a prolonged treatment with high doses of oral anabolizing androgens, in association with endurance training, on rat liver ultrastructure and levels of serum parameters related to hepatic function. The results show that anabolic-androgenic steroids induce ultrastructural changes in liver cells although hepatic function indicators in serum are not modified. Exercise training does not prevent the morphological alterations observed.

Materials and methods

Anabolic-androgenic steroids

Fluoxymesterone (11β, 17β-dihydroxy-9α-fluoro-17α-methyl-4-androsten-3-one) and methyl-17α-methyl-5α-androstan-3-one (17β-hydroxy-17α-methyl-5α-androstan-3-one) were obtained from Sigma Chemical Co (St. Louis, MO, USA).

Training programme and anabolic-androgenic steroid treatment

Male Wistar rats (initial body weight 115 ± 5 g) were obtained from Charles River (Barcelona, Spain). Animals had free access to laboratory chow and tap water. They were maintained on a 12:12 h light-dark cycle and housed in an animal room where temperature (22-24 °C) and humidity (65-75%) were controlled.

Thirty-six rats were randomly divided into sedentary and exercise training groups. The animals of the trained group were exercised by running on a motor-driven treadmill 5 days/week for 12 weeks. During the first 4 weeks, the speed and duration of the daily exercise sessions were progressively increased until the rats were capable of running continuously for 45 min at 25 m/min. At the beginning of the fifth training week, when maximal exercise intensity was reached, each group was arbitrarily subdivided into three groups: control; fluoxymesterone-treated; and methylandrostanolone-treated. The animals selected for anabolic-androgenic steroid treatment received by gastric intubation 2 mg steroid/kg body weight, as a homogeneous suspension in 1 ml of water, 5 days per week for 8 weeks. The high level of anabolizing androgens was chosen in an attempt to simulate the massive doses of anabolic-androgenic steroids reported to be used in athletics (Wilson, 1988). An additional group of six sedentary rats received 5 mg fluoxymesterone/kg body weight for 8 weeks, following the same protocol described above.

Serum analyses

After completion of the 12-week exercise programme, rats were not exercised for 36-44 h and received the last dose 14-18 h before they were sacrificed (between 8:00 and 12:00 a.m.). Animals were fasted overnight, weighed, and under ether anaesthesia blood was collected by cardiac puncture. Serum was obtained by centrifugation at 3000g for 15 min, aliquoted and stored at -40 °C for enzyme assays. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were analyzed by using commercial kits from Böhringer (Böhringer Biochemica GmbH, Mannheim, Germany). Total and direct bilirubin were determined in fresh serum aliquots by a colorimetric procedure (Ames, Miles Italiana S.p.A., Milano, Italy).

Electron microscopy

For electron microscopy, livers of the animals from the different groups were perfused with 2.5% glutaraldehyde in Millonig's buffer, pH 7.3, and quickly removed. Small tissue specimens were post-fixed in buffered 2% osmium tetroxide, dehydrated in a graded series of acetone and embedded in Araldite. Ultrathin sections were cut by a diamond knife with a Reichert UM-2 ultra-microtome and stained with uranyl acetate and lead citrate. Observations were carried out on a Philips EM-201 electron microscope.

Statistical analysis

In these experiments, values of serum parameters were analyzed using two-way analysis of variance (ANOVA: factor 1 = exercise training; factor 2 = steroid treatment). If an overall significant F value was obtained, a Scheffé post hoc analysis was performed. A level of p < 0.05 was selected to indicate statistical significance.

Results

The training programme used in these experiments induced a significant decrease in the body weight of the exercised animals when compared to the sedentary controls (Table 1). On the other hand, body weight of the rats was not affected by anabolic steroid treatment, as shown in Table 1. This fact suggests that abnormal retention of fluids was not produced as a consequence of the anabolizing androgen administration.

The determination of metabolites and enzyme activities in the serum can be of great value for the detection of toxic effects on the liver. In this respect, mean values of serum bilirubin as well as transaminases and alkaline phosphatase activities remained within normal range in all the groups studied (Table 1). These results suggest that neither exercise training nor the administration of anabolic-androgenic steroids induced modifications in the liver function, being consistent with those obtained with power athletes who self-administered very high doses of testosterone and anabolic steroids (Alen, 1985; Ballarin et al., 1986; Thompson et al., 1989).

The principal features of the hepatic cells in trained control rats were similar to those of sedentary control
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Table 1. Effect of anabolic-androgenic steroid administration and exercise training on body weight and serum levels of transaminases, alkaline phosphatase and bilirubin.

<table>
<thead>
<tr>
<th>EXPERIMENTAL GROUP</th>
<th>BODY WEIGHT (g)</th>
<th>AST (UI/l)</th>
<th>ALT (UI/l)</th>
<th>ALP (UI/l)</th>
<th>BILIRUBIN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total (µg/dl)</td>
</tr>
<tr>
<td>SEDENTARY</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>390±16</td>
<td>62±8</td>
<td>29±5</td>
<td>27±5</td>
<td>222±14</td>
</tr>
<tr>
<td>F</td>
<td>394±26</td>
<td>53±18</td>
<td>26±4</td>
<td>36±5</td>
<td>262±62</td>
</tr>
<tr>
<td>M</td>
<td>398±28</td>
<td>58±10</td>
<td>26±4</td>
<td>28±7</td>
<td>150±40</td>
</tr>
<tr>
<td>TRAINED</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>331±22**</td>
<td>61±5</td>
<td>27±7</td>
<td>29±4</td>
<td>232±19</td>
</tr>
<tr>
<td>F</td>
<td>345±26*</td>
<td>48±8</td>
<td>21±4</td>
<td>33±8</td>
<td>266±80</td>
</tr>
<tr>
<td>M</td>
<td>344±21*</td>
<td>52±5</td>
<td>23±5</td>
<td>29±5</td>
<td>190±98</td>
</tr>
</tbody>
</table>

AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; C: untreated control group; F: fluoxymesterone-treated group; M: methylandrostanolone-treated group. Results are mean±SD (n= 6 animals). *= P<0.05 and **= P<0.01 (trained group vs corresponding sedentary group).

Another prominent change in the hepatocytes of animals treated with anabolic steroids was an increase in the number of lysosomes dispersed throughout the cytoplasm. They appeared as single-membrane-bound bodies with variable electron density, morphology and size. The lysosomes contained heterogeneous material, i.e., substrates in the process of being digested and undigested residues, which appeared as irregular electron-dense masses in a clear matrix (Fig. 6). When higher doses of fluoxymesterone (5 mg/kg body weight for 8 weeks) were administered to the sedentary animals, different forms of lysosomes with respect to their morphology were observed in the hepatocytes. Thus, lysosomes appeared as irregular electron-dense masses in a less dense matrix (Figs. 7A,B) and as irregular and very compact electron-dense masses, containing inside low electron-density vesicle-like zones (Figs. 7A, 8). The latter cellular structures were observed associated...
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with lipid droplets and they seemed to be expelled into the sinusoids (Fig. 8). Multivesicular bodies were seen in the hepatic cells of the anabolic steroid-treated rats (Fig. 7A). They were spherical structures surrounded by a single membrane and containing membranous vesicles in a low electron-dense matrix.

Discussion

The use of anabolic-androgenic steroids as ergogenic aids is accompanied by exercise training. Since exercise is known to affect a large number of physiological factors, it was necessary to control the possible influence of training on the effects of anabolizing androgens on the liver. Thus, in this investigation we have studied the effect individually and in combination of both variables, exercise training and steroid treatment.

The exercise programme employed can be considered as of moderate-intensity for normal rodent standards. The 12 weeks of endurance training induced the well-

Fig. 3. Hepatic cell from a trained rat treated for 8 weeks with fluoxymesterone (2 mg/kg body weight). Mitochondria are swollen and present reduced matrical density. x 29,500

Fig. 4. Mitochondria of a hepatocyte from a sedentary rat treated with methylandrostanolone (2 mg/kg body weight). Mitochondria appear swollen and cristae are embedded in a matrix of low electron-density. Rough endoplasmic reticulum (RER) appear surrounding mitochondria. N, nucleus. 84,000

Fig. 5. Autophagic vacuole including a mitochondrion in a hepatic cell from a sedentary rat treated with fluoxymesterone (2 mg/kg body weight). 300,000

Fig. 6. Electron micrograph of a hepatocyte from a trained rat treated with fluoxymesterone (2 mg/kg body weight). Lysosomes (L1) containing heterogeneous material appear as irregular electron-dense masses in a clear matrix. 127,000
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Fig. 7. A, B. Hepatic cell from a sedentary rat treated for 8 weeks with fluoxymesterone (5 mg/kg body weight). Lysosomes (L2) appear as irregular electron-dense masses in a less dense matrix or as irregular and very compact electron-dense masses containing low electron density vesicle-like zones (L3). MB, multivesicular bodies. A) x 85,500. B) x 94,500

Fig. 8. Hepatic cell from a sedentary rat treated with fluoxymesterone (5 mg/kg body weight). Electron-dense masses containing low electron-density vesicle-like zones (L4) appear associated with lipid droplets. x 88,000

characterized increase in oxidative capacity as indicated by the elevation of the activity of some skeletal muscle mitochondrial enzymes, such as carnitine palmitoyltransferase I (73% in soleus and 48% in extensor digitorum longus) and succinate dehydrogenase (38% and 48%, respectively) (Guzmán et al., 1991; Saborido et al., 1991a). On the other hand, serum aspartate aminotransferase activity was not increased in trained animals (Table 1) indicating the absence of muscle damage as expected for a mild endurance training.

Training was also without apparent effects on liver ultrastructure since the characteristics of the hepatic cells in trained rats were similar to those of sedentary animals. This result is consistent with that of previous studies showing absence of changes in liver ultrastructure even after exhaustive exercise (King and Gollnick, 1970). However, treatment with fluoxymesterone or methylandrostanolone induced morphological alterations of the liver cells both in sedentary and trained rats. Main abnormalities observed were changes in mitochondria and lysosomal proliferation.

Mitochondrial alterations similar to those described in this work have been previously observed in mouse kidney proximal tubules after testosterone propionate administration (Koenig et al., 1980a), in cardiac and skeletal muscle of rats, Guinea pigs and mice following methandrostenolone and nandrolone treatment (Behrendt and Boffin, 1977; Appell et al., 1983; Soares and Duarte, 1991) and in perivenous hepatocytes from rats treated with the synthetic sexual steroid levonorgestrel (Kretzschmar et al., 1989). Besides, studies in vitro with isolated liver mitochondria have shown that some steroids exert a direct effect on mitochondrial membranes and inhibit mitochondrial respiration (Mohan and Cleary, 1989). The effects that mitochondrial swelling may have had on the metabolic capacity of the liver are unclear but it is well known that in isolated mitochondria, disruption of the basic structural configuration can loosen or completely uncouple oxidative phosphorylation. Thus, it is possible that the functional capacity of the liver and post-exercise oxygen uptake have been adversely affected, if such loss of respiratory control were to occur in vivo in the treated animals.

An interesting finding in this study was the presence of numerous lysosomes in the hepatic cells after anabolic steroid administration. We could observe four morphological types of lysosomes, which probably correspond to different phases of lysosomal activity. A similar response has been observed in fibroblasts of mouse tendon after 10 weeks of treatment with the anabolic steroid methandrostenolone (Michna, 1989). The characteristic changes consisted of the appearance
of numerous intracellular lysosomes with different shape, size and electron density and the emergence of matrix vesicles, some of which were identified as extracellular lysosomes. On the other hand, methandrostenolone in combination with exercise training has been shown to cause an increase in lysosomal hydrolytic activities in the right ventricular wall of the dog heart muscle (Takala et al., 1992). Testosterone has also been reported to induce significant increases in the specific and total activities of several lysosomal hydrolases in mouse kidney (Koenig et al., 1980a; Wilson et al., 1988), skeletal muscle (Koenig et al., 1980b), aorta (Goldstone et al., 1981), brain (Koenig and Lu, 1980) and myocardium (Koenig et al., 1982). In mouse kidney and myocardium, the modification of these biochemical parameters is associated with alterations in mechanical stability of the lysosomal membrane and ultrastructural changes of the lysosomal-vacuolar system, including an increase in autophagy and accumulation of enlarged lysosomes filled with myeline-like membranes. These results suggest that an acceleration of lysosome-mediated protein degradation may be a significant feature of the liver response to endogenous androgens. To our knowledge, increases in lysosomal activities have not been reported as a response of the liver to anabolic-androgenic steroid treatment and this subject is currently being investigated in our laboratory. The possibility that the increase in the lysosomal content observed in the hepatic cells of treated rats reflects, at least partly, a disordered metabolism in liver because of the changes in mitochondria, cannot be dismissed.

It is interesting to notice that the treadmill exercise performed by the rats does not appear to prevent the effects that the anabolic steroid treatment induced on liver ultrastructure. Accordingly, we have previously demonstrated that the capacity of the liver to metabolize drugs can be modified by prolonged ingestion of these compounds in sedentary as well as trained male and female rats (Saborido et al., 1991b, 1993).

This study also shows that serum parameters commonly evaluated in athletes to test hepatic function are not substantially modified by administration of the anabolic steroids employed. Likewise, in previous studies showing functional liver changes in anabolic steroid-treated rats, these biochemical markers remained unchanged (Saborido et al., 1991b, 1993). Even with higher doses of fluoxymesterone (5 mg/kg body weight), the hepatic serum parameters of the treated rats were not significantly modified (data not shown). Thus, it appears that the structural and functional liver alterations induced by anabolic-androgenic steroids are not adequately reflected in routine biochemical tests, at least in their initial phases. Although the molecular mechanism underlying these alterations remains to be determined and interspecies differences must be borne in mind, our results indicate that a real health risk exists in the use of anabolic steroids by athletes.

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References

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