THE PHARMACOLOGY AND METABOLISM OF TESTOSTERONE UNDECANOATE (TU), A NEW ORALLY ACTIVE ANDROGEN

By
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ABSTRACT
Testosterone undecanoate (TU), either dissolved in arachis oil or as micro-crystal suspension, displays androgenic activity on oral administration to rats in the Hershberger test. The solution in arachis oil is more active than the crystal suspension. When [3H]TU is administered orally to rats, most of it is metabolized in the intestinal wall. The majority of the metabolites probably lose their undecanoate group and are absorbed via the portal vein. The non-metabolized [3H]TU and at least two metabolites are absorbed exclusively via the lymphatic system. One of these metabolites has been identified as 5α-dihydrotestosterone undecanoate (5α-DHTU). TU has no harmful effects on the liver of the rabbits on oral administration.

It is well known that testosterone is inactive in man on oral administration (Foss & Camb 1939) while the widely used orally active androgen methyltestosterone may cause hepatic dysfunction in man: BSP retention (deLorimier et al. 1965) and jaundice (Werner et al. 1950; Foss & Simpson 1959). Foss & Simpson (1959) showed that testosterone propionate had no effect on liver function in man on parenteral administration. The present study describes the androgenic activity in rats, the effect on liver function in rabbits and the metabolism in rats of a new orally active androgen, testosterone undecanoate.
The following abbreviations are used:

- TU – testosterone undecanoate
- DHTU – dihydrotestosterone undecanoate
- GLC – gas-liquid chromatography
- MS – mass spectrometry
- mla – levator ani muscle
- SGOT – serum glutamate oxalate transaminase
- SGPT – serum glutamate pyruvate transaminase
- BSP – sulphobromophthalein
- TLC – thin layer chromatography

The chemicals used were of A. R. quality. Male Wistar rats, S. P. F.-bred, were supplied by TNO*. Male Chinchilla rabbits were used. TLC was performed on Silica Gel HF 254–366 plates (Merck, Darmstadt, West Germany). Radioactivity was measured with a Packard Tricarb (3375) liquid scintillation spectrometer, using Instagel (Packard) as scintillation liquid. Autoradiography of the thin layer plates was performed by spraying the plates with Omnifluor (New England Nuclear) and storing them with Kodak Royal Blue film at −80°C for several days. GLC was performed with a Hewlett Packard gas chromatograph model F & M 402. Six feet all glass columns were used. MS was performed with a Varian MAT CH7 mass spectrometer. Ultracentrifugation was carried out with an IEC International preparative ultracentrifuge model B 60 with rotor 488. [³H]TU was labelled by the Radiochemical Centre, Amersham, England. The specific activity was 130 mCi/mg.

**Hershberger test in rats**

The androgenic properties of TU were studied by observing the effect of an oral administration twice daily for 7 days on mla, ventral prostate and seminal vesicle weights in castrated rats of approximately 50 g (Hershberger et al. 1953).

**Liver function test in rabbits**

The effect on liver function was investigated according to a modification of the method of Carmichael et al. (1963). Rabbits of 2–2.5 kg were treated orally with 10 mg/kg/day for 10 days. The preparations were administered as tablets. Control animals received placebo tablets. Twenty-four hours after the last administration blood was taken for the determination of SGOT and SGPT and the rabbits were injected iv with 15 mg/kg BSP into the marginal ear vein. Five, 10 and 15 min later blood was collected from this vein. The BSP concentration was determined by adding 4.5 ml of 0.1 n NaOH in saline to 0.5 ml plasma. After mixing, the optical density was read in a colorimeter at 580 nm. SGOT and SGPT were measured with a Technicon AutoAnalyzer according to the method of Fingerhut et al. (1962).

**In vitro incubation of TU with rat gastric juice and intestinal juice and its determination in intestinal and gastric contents after oral administration to rats**

Rats weighing 200–250 g were fasted overnight and anaesthetized with pentobarbital

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(6 mg/100 g ip). The small intestine was ligated at the pylorus and just proximal to the caecum. Two hours later the rats were sacrificed and the contents of the stomach were collected. The intestine was isolated as far as the caecum and rinsed with 2 ml of saline.

To 1 ml of gastric or intestinal juice 10 μg ([3H]TU dissolved in 15 μl ethanol was added. After incubation at 37°C for 21 h the mixture was extracted with ethyl acetate. The extract was evaporated to dryness under a stream of nitrogen at 50°C and the residue dissolved in 100 μl ethyl acetate. Twenty-five μl were submitted to TLC, system toluene:ethyl acetate (3 + 2, v/v). The spots were visualized by autoradiography, scraped off and the radioactivity counted. The contents of the intestine and the stomach 90 min after oral administration of 4 mg [3H]TU (1 mCi) in 1 ml arachis oil were analyzed for the presence of metabolites. The contents were extracted and the extracts submitted to TLC following the procedure described above. The TLC plate was submitted to autoradiography.

Absorption of TU from the rat intestinal tract in vivo

At various times after the oral administration of 4 mg TU, either dissolved in arachis oil or suspended in 0.5% gelatin solution, to rats of 200–250 g which were fasted overnight, the gastro-intestinal tract was opened and the contents extracted 3 times with acetone. The extract was evaporated to dryness under a stream of nitrogen at 50°C. The residue was dissolved in 10 ml ethyl acetate and washed with 2 ml distilled water. The washed ethyl acetate extract was evaporated to dryness. The residue was dissolved in 4 ml ethyl acetate and 50 μl of this was applied to a thin layer plate. System: chloroform:n-hexane:ethyl acetate (5 + 4 + 1, by vol.). The spots were located under UV at 254 nm, scraped off and eluted with 2.5 ml ethanol. After centrifugation 2 ml of the clear supernatant was mixed with 0.5 ml isonicotinic acid hydrazide reagent. Extinctions were measured spectrophotometrically at 385 nm.

Determination of TU and its metabolites in rat portal vein plasma, aorta plasma and lymph

Ninety min after administration of 4 mg [3H]TU (1 mCi), either dissolved in 1 ml arachis oil or suspended in a 0.5% gelatin solution, to rats weighing 200–250 g 3 ml of blood was collected simultaneously from the abdominal aorta and the portal vein.

In some cases the thoracic duct was cannulated according to the method of Bollman et al. [1948]. In these cases the lymph was collected from the time of administration until the time of blood collection. The plasma and lymph samples were extracted with ethanol:diethyl ether (3 + 1, v/v). After evaporation of the greater part of the extract under a stream of nitrogen at 50°C the remaining residue was diluted with distilled water and extracted with ethyl acetate. The extracts were evaporated to dryness and the residue was dissolved in a small volume of ethyl acetate. (The results of this extraction procedure will henceforth be referred to as the ethyl acetate extract). Ten μl was taken for measuring radioactivity and the remainder submitted to TLC, system n-hexane:ethyl acetate (4 + 1, v/v) followed by autoradiography.

Purification and identification of the lymph metabolite

After oral treatment of cannulated rats with [3H]TU, lymph was collected and extracted according to the procedure described above. The resulting solution was submitted to TLC (system: n-hexane) using 5α-DHTU as reference. After the run the plate was developed another time in the same direction (system: n-hexane:ethyl
acetate (4 + 1, v/v). 5α-DHTU and 5β-DHTU were not separated in these systems. The spot with the same \( R_f \) value as 5α-DHTU was scraped off and eluted with acetone. The eluate was evaporated to dryness under a stream of nitrogen at 50°C and the residue was dissolved in a small volume of ethanol. Identification was performed with TLC and GLC.

Three different TLC systems were used: I. n-hexane, II. n-hexane:ethyl acetate (4 + 1, v/v) and III. n-hexane:ethyl acetate:chloroform (4 + 1 + 5, by vol.). GLC was performed with a 1% SE 30 phase. The oven temperature was 283°C.

For MS identification the substance was further purified by GLC. The fraction containing the peak with the same retention time as the 5α-DHTU standard was collected in a glass tube with the aid of an effluent stream splitter. The glass tube was rinsed with ethyl acetate and the eluate was evaporated to dryness and the residue dissolved in a small volume of n-hexane.

MS was performed by direct probe insertion.

**The binding of TU to rat plasma protein and chylomicrons**

A dose of 4 mg [3H]TU in 1 ml arachis oil (1 mCi) was given orally to rats of 250–300 g body weight. Portal vein blood was taken 90 min after drug administration and plasma prepared. Chylomicrons were separated by centrifugation at 25 000 g for 20 min. TLC system: n-hexane:ethyl acetate (4 + 1, v/v) followed by autoradiography was performed with the ethyl acetate extracts of the chylomicrons collected and also with a portion of the chylomicron-free plasma. In the other portion of the plasma, lipoproteins (supernatant) were separated from the remainder of the plasma (infra-natant) by ultracentrifugation, the density of the plasma being adjusted with KBr to 1.21. The plasma was submitted to ultracentrifugation at 100 000 g at 4°C for 20 h. The ethyl acetate extracts of the supernatant and infranatant were also submitted to TLC, followed by autoradiography (system: n-hexane:ethyl acetate (4 + 1, v/v)). To study the distribution of radioactivity over the lipoproteins, agar-agarose gel electrophoresis was performed with a sample of the supernatant, according to the method of Noble (1968) as modified by Dyerberg & Hjorne (1970), although in our experiments no albumin was added to the buffer.

**RESULTS**

A. **Androgenic activity of TU in the Hershberger test in rats**

From Table 1 it is obvious that testosterone, either dissolved in arachis oil or in tablet form, has only a very weak androgenic activity upon oral administration. Esterification of testosterone to TU leads to a great improvement in the androgenic properties. The differences in the effects between testosterone and the corresponding dose levels of TU are highly significant (\( P < 0.001 \) for seminal vesicles, ventral prostate and mla). This comparison is made on a weight base. On a molecular base (TU contains 63% of testosterone) the results are still more in favour of TU. Administration of TU in arachis oil instead of the crystalline form gives a further increase in the androgenic activity of the compound. The potency ratios (with 95% confidence limits) between the
Table 1.
Influence of the vehicle on the androgenic activity of testosterone and TU in the Hershberger test in rats after oral administration. The organ weights are given as the geometric mean, together with the SEM. The dose was calculated with regard to the initial body weight of 50 g.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Number of rats</th>
<th>Seminal vesicle weight (mg)</th>
<th>Ventral prostate weight (mg)</th>
<th>mla weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (arachis oil)</td>
<td>6</td>
<td>5.9 ± 0.4</td>
<td>10.8 ± 0.9</td>
<td>20.0 ± 2.8</td>
</tr>
<tr>
<td>Control (suspension fluid)</td>
<td>6</td>
<td>6.4 ± 1.4</td>
<td>10.1 ± 0.7</td>
<td>23.0 ± 2.3</td>
</tr>
<tr>
<td>Testosterone (arachis oil)</td>
<td>11</td>
<td>13.8 ± 1.1</td>
<td>42.8 ± 2.7</td>
<td>22.3 ± 1.9</td>
</tr>
<tr>
<td>2 x 40</td>
<td>30</td>
<td>11.7 ± 0.3</td>
<td>36.9 ± 1.9</td>
<td>24.0 ± 1.1</td>
</tr>
<tr>
<td>Testosterone (tablets)</td>
<td>6</td>
<td>16.3 ± 0.7</td>
<td>37.2 ± 2.5</td>
<td>39.8 ± 2.1</td>
</tr>
<tr>
<td>2 x 40</td>
<td>6</td>
<td>30.1 ± 1.8</td>
<td>60.2 ± 5.6</td>
<td>47.4 ± 2.6</td>
</tr>
<tr>
<td>TU (arachis oil)</td>
<td>6</td>
<td>13.4 ± 0.4</td>
<td>30.4 ± 0.8</td>
<td>31.7 ± 3.1</td>
</tr>
<tr>
<td>2 x 10</td>
<td>6</td>
<td>22.4 ± 1.2</td>
<td>56.2 ± 4.8</td>
<td>42.1 ± 0.6</td>
</tr>
<tr>
<td>2 x 40</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

two pharmaceutical formulations of TU are as follows: with regard to the seminal vesicle the arachis oil solution is 1.85 (1.37–2.63) times as active as the crystalline form. For the ventral prostate and the mla the ratios are 1.39 (0.97–2.08) and 1.45 (0.85–2.56) respectively.

B. Influence of TU on the liver function in rabbits

It is well known that 17α-alkyl steroids can cause hepatic dysfunction. In the liver function test used here (see Table 2) effects of 17α-methyltestosterone on SGOT, SGPT and BSP clearance are very clear on oral administration. TU showed no effect on these parameters.
Table 2.
The effect of TU and 17α-methyltestosterone on the liver function in rabbits after a 10 day oral treatment with 10 mg/kg a day. Each group consisted of 5 rabbits. Values are expressed as the mean together with the SEM. Statistical significances were calculated using Student's t-test with regard to the control.

<table>
<thead>
<tr>
<th></th>
<th>BSP (μg/ml)</th>
<th>SGOT (Karmen U/ml)</th>
<th>SGPT (Karmen U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min</td>
<td>10 min</td>
<td>20 min</td>
</tr>
<tr>
<td>Control</td>
<td>81 ± 12</td>
<td>33 ± 5</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>TU</td>
<td>106 ± 9</td>
<td>35 ± 5</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Methyl testosterone</td>
<td>161 ± 25*</td>
<td>76 ± 13*</td>
<td>19 ± 4*</td>
</tr>
</tbody>
</table>

* P < 0.05  ** P < 0.01.

C. Metabolic studies
1. Stability of TU in rat gastric and intestinal juice in vitro and in vivo. – After in vitro incubation of [3H]TU for 21 h in gastric juice, no hydrolysis took place. Incubation in intestinal juice caused some hydrolysis, i.e. approximately 30%. This is rather low in view of the long incubation time. Gastric and intestinal contents 90 min after oral administration of [3H]TU in oil were

Table 3.
The disappearance of TU from the gastro-intestinal tract after oral administration to rats. Values are expressed as mean percentage (together with the SEM) of TU recovered from the rat gastro-intestinal tract at various times after a single oral dose of 4 mg/rat. Each group consisted of 12 rats, last column 6 rats. Time 0 = 100%.

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>Small intestine and stomach</th>
<th>Colon and caecum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hours after administration</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Arachis oil</td>
<td>81 ± 3</td>
<td>56 ± 3</td>
</tr>
<tr>
<td>Gelatin suspension (0.5%)</td>
<td>80 ± 3</td>
<td>76 ± 4</td>
</tr>
</tbody>
</table>
extracted with ethyl acetate, submitted to TLC followed by autoradiography. The amount of metabolites present in the intestinal contents was found to represent less than 1% of the administered dose of radioactivity. No metabolites were present in the gastric contents. Hence TU is fairly stable in the gastro-intestinal tract.

2. Disappearance of TU from the gastro-intestinal tract after oral administration to rats. – Five hours after administration of a single dose of TU to rats, most of the TU administered had disappeared from the intestinal lumen (Table 3). Absorption was slower after administration of TU as a suspension

![Autoradiogram of the TLC patterns of the ethyl acetate extracts of plasma from portal vein and aorta collected from a rat 90 min after oral administration of [3H]TU in 1 ml suspension fluid (1 mCi).](image)

Fig. 1.
than after administration of an oily solution. After 5 h only small amounts could be recovered from the lower part of the intestinal tract. This indicates that the disappearance from the intestine is probably due to absorption of the steroid.

3. Presence of [3H]TU and its metabolites in plasma from portal vein and aorta, and in lymph after oral administration of [3H]TU to rats. – In Figs. 1 and 2 the autoradiograms of the TLC patterns of the ethyl acetate extracts of plasma from portal vein and aorta as well as of the lymph of intact and cannulated rats respectively after oral administration of [3H]TU as a crystal
suspension are presented. Both Figs. 1 and 2 show a marked difference between the patterns derived from plasma from the portal vein and aorta.

The polar metabolites present in the plasma from portal vein are not present in that of the aorta. This shows that the metabolism of TU starts already in the intestinal wall. Another remarkable phenomenon is that TU and one of its metabolites are only present in the plasma from portal vein and aorta of the intact rats. After drainage of the thoracic duct these compounds are present in the lymph and not in the plasma from the portal vein and the aorta. Hence TU and this lymph metabolite are absorbed exclusively via the lymphatic system. The amount of radioactivity absorbed via the lymphatic system in a 5 h period is approximately 2% of the administered dose. We found no differences between crystal suspension and arachis oil with regard to the distribution of the radioactivity in the lymph, plasma from aorta and plasma from portal vein (see Table 4). Moreover the TLC patterns were identical.

4. Identification of the lymph metabolite. – In the thin layer systems I, II and III the metabolite has the same \( R_F \) value as 5\( \alpha \)-DHTU. 5\( \alpha \)-DHTU and 5\( \beta \)-DHTU were not separated in these systems. On GLC the retention times of 5\( \alpha \)-DHTU and 5\( \beta \)-DHTU were different: 3 min and 45 seconds and 3 min and 20 seconds respectively.

The retention time of the metabolite was 3 min and 45 seconds, i.e. the same as for 5\( \alpha \)-DHTU.

After further purification the metabolite was compared with 5\( \alpha \)-DHTU on

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>Intact rats</th>
<th>Cannulated rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>portal vein</td>
<td>aorta</td>
</tr>
<tr>
<td>Arachis oil</td>
<td>749</td>
<td>518</td>
</tr>
<tr>
<td></td>
<td>773</td>
<td>644</td>
</tr>
<tr>
<td>Gelatin suspension</td>
<td>626</td>
<td>395</td>
</tr>
<tr>
<td>(0.5%( \alpha ))</td>
<td>379</td>
<td>n. d.*</td>
</tr>
</tbody>
</table>

* n. d. = not determined.

Table 4.

Radioactivity (nCi/ml) in plasma from portal vein and aorta and in lymph from intact and thoracic duct-cannulated rats after a single oral dose of 4 mg [\(^3\)H]TU (1 mCi). The lymph was collected for 90 min after drug administration. Blood from portal vein and aorta was collected simultaneously 90 min after drug administration.
MS. No significant differences between the MS spectra of both compounds were found. From these results it is clear that the lymph metabolite is 5α-DHTU.

5. The binding of TU and its metabolites to rat plasma proteins and chylomicrons. – TU and 5α-DHTU appearing in the circulation after oral administration of [3H]TU to rats are mainly incorporated into chylomicrons. The remainder of the TU and 5α-DHTU is bound to the lipoproteins, as is shown by ultracentrifugation of chylomicron-free plasma (supernatant). The polar metabolites are bound to the proteins (infranatant) (Fig. 3). In Fig. 4 an electropherogram of the lipoproteins (supernatant) and the distribution of the radioactivity over the electropherogram is shown. The lipoprotein bands coincide with the areas of high radioactivity.
Fig. 4.
Electrophoretic pattern of the lipoprotein fraction (supernatant) obtained by ultracentrifugation of plasma from portal vein of a rat after separation of chylomicrons, together with the distribution of radioactivity over the electropherogram. Blood was collected 90 min after administration of a single oral dose of 4 mg [³H]TU in 1 ml arachis oil (1 mCi).

DISCUSSION

It is generally accepted that the lack of activity of orally administered testosterone in man is caused by inactivation of the compound in the liver, which occurs after absorption via the portal vein system. However, it has been demonstrated that the biotransformation of orally administered steroids starts already in the intestinal wall (testosterone in dogs, Harri et al. (1970) and progesterone in dogs, Nienstedt & Hartiala (1969)).

Our study shows that TU also appears to be metabolized in the rat intestinal wall. Only a small amount of the administered TU is absorbed unmetabolized via the lymphatic system. This emphasizes the importance of the intestinal
wall in the metabolism of orally administered substances. This still leaves the question unanswered of how some TU remains unmetabolized. In our view the following mechanism is involved: because of its lipophilic character TU is incorporated in the intestinal wall into newly produced chylomicrons. Once incorporated, TU is protected from being metabolized by the intestinal wall enzymes and is transported via the lymph to the peripheral circulation. The fact that TU is more active in the mla test as an arachis oil solution than in crystalline form fits very well with this hypothesis, since arachis oil leads to chylomicron production.

However, the increased androgenic activity is not reflected in the amounts of radioactivity absorbed by the lymphatic system after administration of TU in the two forms of administration (Table 4). On the other hand, the experiment in which the disappearance of TU from the intestinal lumen was studied and in which it was found that an oily solution of TU disappeared more rapidly than a crystal suspension (Table 3) gives some support to the above mentioned hypothesis. The polar metabolites are totally absorbed by the portal vein system. This finding is in agreement with the results of Martin et al. (1965) who demonstrated the portal vein route for the absorption of testosterone and its metabolites from the intestine of the dog.

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