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The effect of the oral administration of propyphenazone on the urinary concentration of testosterone and 19-nortestosterone metabolites

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INTRODUCTION

Propyphenazone belongs structurally to the group of pyrazolone derivatives and therapeutically to non-steroidal anti-inflammatory drugs (NSAID); it has also analgesic effects. It is generically used for the treatment of fever, pain during flu or cold and after vaccination [1-2]. The drug is not prescribed often nowadays because of the many significant side effects; the normal dose is 150-300 mg 1 to 3 times per day. Some athletes use pharmaceutical formulations containing propyphenazone during multi-days events to suppress pain.

This study originates from the observation of some unknown peaks detected in a considerable number of samples by the screening procedure for the anabolic steroids. The spectrum of these peaks corresponded to the propyphenazone metabolites (Figure 1). The same samples showed an unusually altered steroid profile (Table 1)

Table 1 Steroid profile of some representative real samples, found to contain propyphenazone metabolites

Substance	Sample 1 (ng/mL)	Sample 2 (ng/mL)	Sample 3 (ng/mL)	Normal range(ng/mL)
Testosterone	168.2	21.8	45.5	20-150
Epitestosterone	111.6	17.6	24.1	20-150
Androsterone	**152.7**	**162.3**	**15.8**	**900-3000**
Etiocholanolone	**317.2**	**124.7**	**431**	**900-3000**
11β-OH-androsterone	**2.8**	**12.2**	**2.3**	**50-300**
11β-OH-etiocholanolone	**6.2**	**7.7**	**2.4**	**50-300**
5α-androstane-3α,17β-diol	47.4	15.2	8.5	30-200
5β-androstane-3α,17β-diol	212.4	73.7	109.2	30-200

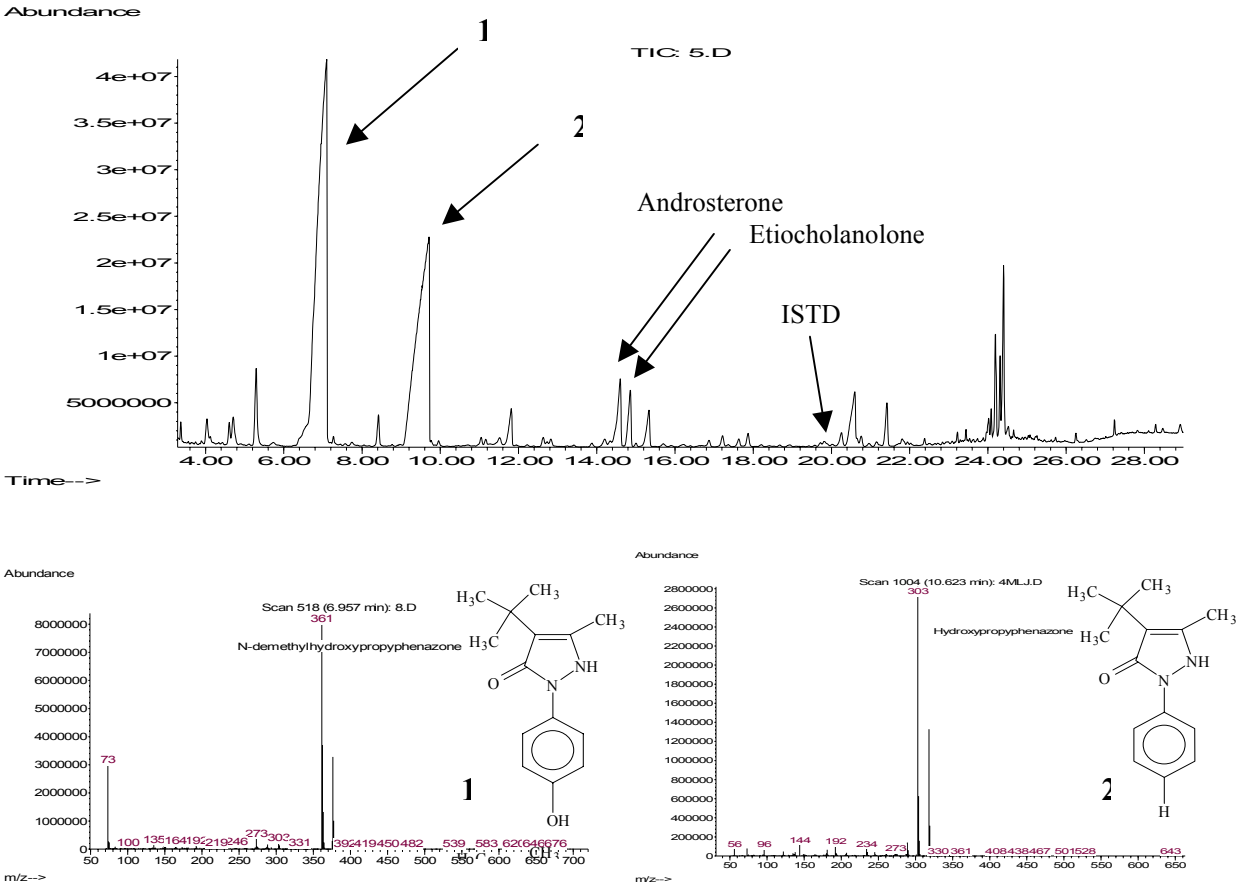

Figure 1. Total ion chromatogram of the GC-MS screening for the steroid total fraction (TMS-derivatives) (above) and spectrum and molecular formula of peaks **1** and **2** (below), corresponding respectively to the molecular structures of the propyphenazone metabolites N-demethylhydroxypropyphenazone and hydroxypropyphenazone.

This observation prompted us to study the effect of the oral administration of propyphenazone on the excretion/metabolism of testosterone and its synthetic analogues (e. g. 19-norsteroids). A single dose (a tablet containing 400 mg of propyphenazone) was taken orally by 10 healthy male volunteers, alone or concurrently with 19-norsteroids administration. Urine samples were collected every two hours, before and after drug administration.

EXPERIMENTAL SECTION

Propyphenazone excretion

The experiments have been carried out on male subjects (age 35±5 years), engaged in normal physical activity, undergoing treatment with propyphenazone. Circadian variability of the endogenous androgens profile was assessed before and after treatment with a single dose of 400 mg of propyphenazone, by collecting urine samples for three days every two hours

(from the first urine in the morning to the last urine in the night). The following endogenous hormones (glucuronate plus free fraction) were considered: androsterone, etiocholanolone, 11β-OH-androsterone, 11β-OH-etiocholanolone, 5α-androstane-3α,17β-diol and 5β-androstane-3α,17β-diol. The urine samples were stored on ice packs after collection to prevent changes in the concentrations of steroids caused by bacterial contamination or thermal degradation [3-5]. All concentration values were corrected for a value of the specific gravity of 1.020 g/L.

Concurrent administration of propyphenazone, norandrostenediol and norandrostenedione

The experiments have been carried out on five male subjects (age 35±5 years), engaged in normal physical activity, administered with 400 mg of propyphenazone, 2 mg of 19-norandrostenedione and 2 mg of 19-norandrostenediol. The norandrosterone and noretiocholanolone urinary profile was assessed before and after treatment with a single dose of 400 mg of propyphenazone, by collecting urine samples for three days every two hours (from the first urine in the morning to the last urine in the night). The urine samples were stored as described above.

Analytical procedure

To 3 mL of urine, 50 µL of internal standard (17α-methyltestosterone), 1 mL of phosphate buffer pH=7.4. and 30 µL of beta-glucuronidase from E. coli were added and hydrolysis was performed for 1 h at 50 °C. The buffered solution was then alkalinized with 1 mL of carbonate buffer and the steroids were extracted with 10 mL of tert-butylmethyl ether on a mechanical shaker for 5 minutes. After centrifugation, the etheral layer was transferred and evaporated to dryness under vacuum; the residue was derivatized by 50 µL of N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA):NH_4I:Dithioerythrytol (1000:2:4 v/w/w) and 1 µL of the derivatized extract was injected directly into the injection port.

The gaschromatograph-mass spectrometer (GC-MS) system used was a Hewlett Packard 6890N GC and 5973N MSD (Agilent technologies SpA, Cernusco sul Naviglio, MI, Italy) equipped with a 17 m × 0.2 mm (i.d.), 0.11 µm film fused silica capillary column HP1 (cross linked methylsilicone). The oven temperature was set at 180 °C for 4.5 min, then programmed from 180 °C to 230 °C at 3 °C/min and then programmed from 230 °C to 290 °C at 20 °C/min and held at 290 °C for 2min finally from 290 °C to 320 °C at 30 °C/min and held at 320 °C for 0.80 min; the temperature of the injection port and the detector was set at 280 °C, split (1:10) injection, the mass selective detector was operated in the electron impact

(EI) mode at 70 eV. Acquisition was carried out in selected ion monitoring (SIM) of the following fragments: m/z 405 and m/z 420 for norandrosterone and noretiocholanolone, m/z 432 and m/z 417 for testosterone and epitestosterone, m/z 434 for androsterone and etiocholanolone, m/z 241 for 5α-androstane-3α,17β-diol and 5β-androstane-3α,17β-diol, m/z 522 for 11β-OH-androsterone and 11β-OH-etiocholanolone, m/z 318, 303, 361, 376 for propyphenazone metabolites. Helium gas with a flow rate of 0.8 mL/min was used.

All the values of urine concentration were calculated by the peak area of the detected signal relative to the internal standard methyltestosterone (m/z 301). Samples with pH > 7 were not included in the study. For calibration of the GC/MS instrument, the following reference mixture was used:

Reference substance	Working solution (μg/mL)	Concentration in urine (ng/mL)
Testosterone	4	40
Epitestosterone	4	40
Androsterone	200	2000
Etiocholanolone	200	2000
11β-hydroxyandrosterone	75	750
11β-hydroxyetiocholanolone	75	750
5α-androstane-3α,17β-diol	22.5	225
5β-androstane-3α,17β-diol	22.5	225

RESULTS AND DISCUSSION

TREATMENT WITH PROPYPHENAZONE

Baseline study

In the preliminary circadian study, single concentration data for all hormones in each urine collection (every two hours starting from 8:30 am) of each volunteer were compared with the corresponding concentration value of each hormone in the urine sample collected at 8:30 am.

The variability of the urinary concentration values of the urine samples collected from the same volunteer at the same time was very low: on the contrary, the absolute concentration values of each steroids showed marked interindividual differences. Nonetheless, the circadian trend was qualitatively the same for all volunteers: the excretion of the endogenous hormones did not show any significant variation along the day especially for testosterone and epitestosterone. (Figure 2).

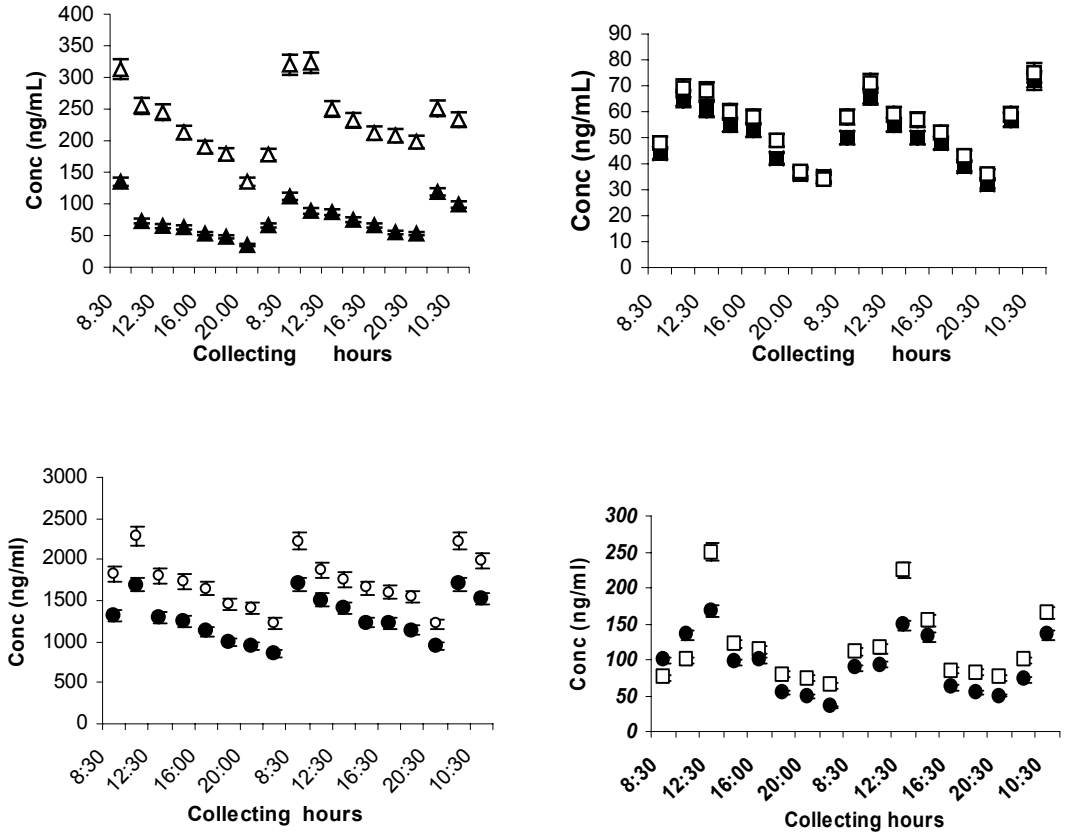

Figure 2. Circadian variability of the urinary concentration of △=11β-OH-androsterone, ▲=11β-OH-etiocholanolone (above left); □=epitestosterone, ■=testosterone (above right); ●=androsterone, ○=etiocholanolone (below left); ●=5β-androstane-3α,17β-diol, □=5α-androstane-3α,17β-diol (below right).

In-treatment profile

Figure 3 shows the mean urinary concentration values of androgens after oral administration of propyphenazone. As it can be seen, a statistically significant variation of the urinary concentration of 11β-OH-androsterone, 11β-OH-etiocholanolone, androsterone and etiocholanolone, as well as of the androsterone/etiocholanolone ratio, was detected after two hours from propyphenazone administration. On the contrary, testosterone, epitestosterone, 5α-androstane-3α,17β-diol and 5β-androstane-3α,17β-diol did not show any significant variation from the baseline profile.

Post-treatment profile

The follow up studies were carried out to verify whether the effect of propyphenazone was maintained even after the suspension of the administration. A reduced urinary

concentration of 11β-OH-androsterone, 11β-OH-etiocholanolone, androsterone and etiocholanolone was indeed recorded for one day: this effect was recorded in all subjects (Figure 4). The same qualitative trend was recorded for the androsterone/etiocholanolone concentration ratio (Fig. 5).

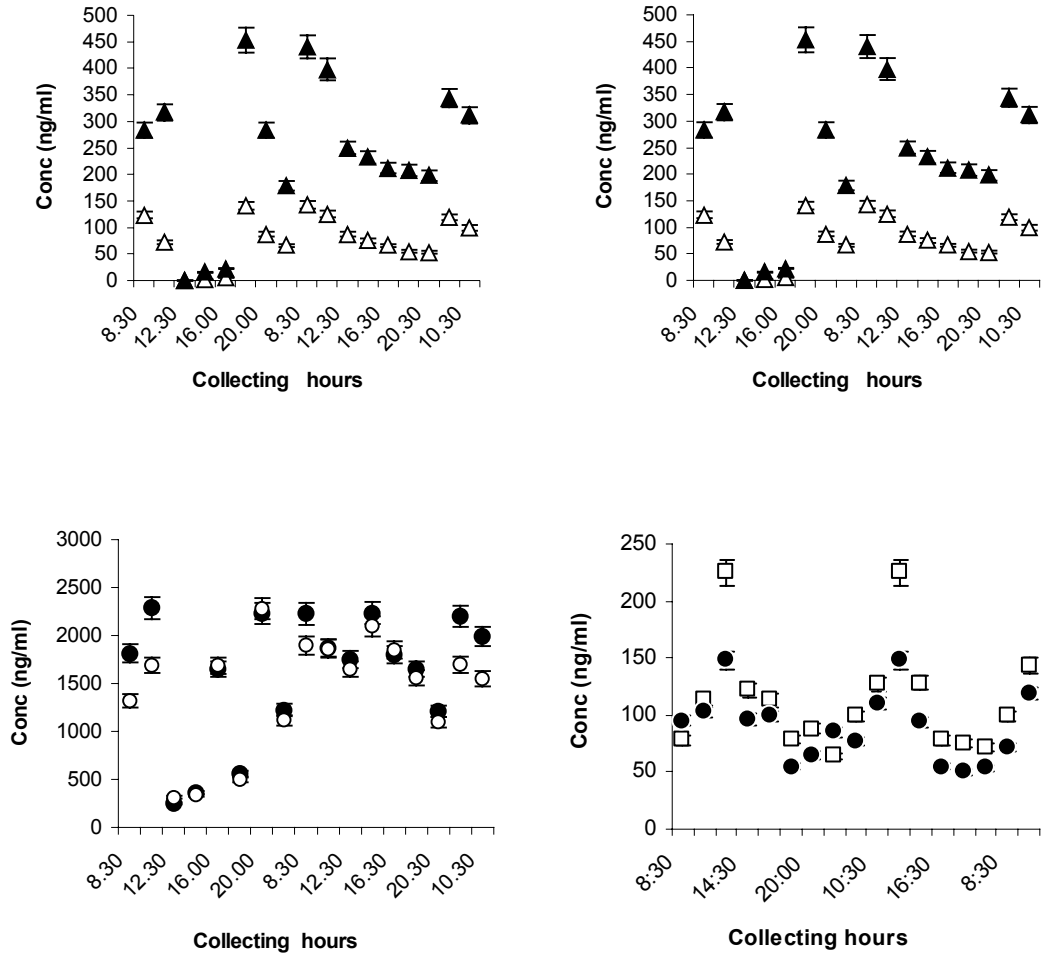

Figure 3. Effect of the administration of propyphenazone on the circadian variability of the urinary concentration of △=11β-OH-androsterone, ▲=11β-OH-etiocholanolone (above left); □=epitestosterone, ■=testosterone (above right); ●=androsterone, ○=etiocholanolone (below left); ●=5β-androstane-3α,17β-diol, □=5α-androstane-3α,17β-diol (below right).

Concurrent administration of norandrostenedione/norandrostenediol and propyphenazone

Urinary concentration of 19-norandrosterone and 19-noretiocholanolone

Figures 6 and 7 show the mean excretion values of both 19-norandrosterone and 19-noretiocholanolone before and after propyphenazone administration. The 19-norsteroids

excretion study indicated that, after two hours from propyphenazone administration, there was a statistical significant decrease of the measured urinary concentration of both 19-norandrosterone and 19-noretiocholanolone from baseline profile.

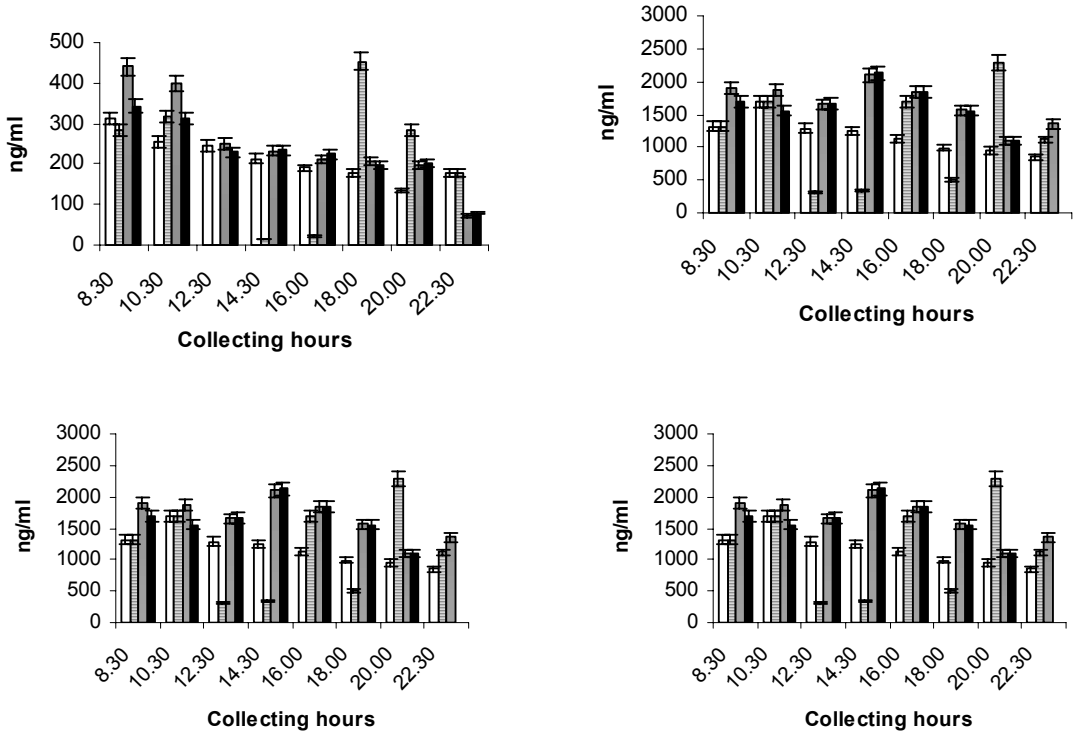

Figure 4. Circadian variability of the urinary concentration of androsterone (left A), etiocholanolone (right A), 11β-OH-androsterone (left B) and 11β-OH-etiocholanolone (right B), recorded before (open bar) and after 1 (dashed), 2 (grey), and 3 (black) days from the suspension of the treatment with propyphenazone.

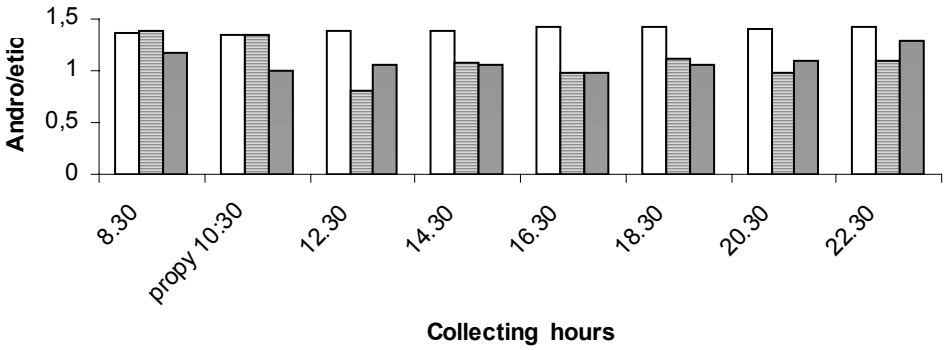

Figure 5. Circadian variability of the urinary androsterone/etiocholanolone ratio recorded before (open bar), and after (dashed: day 1; gray: day 2) administration of propyphenazone.

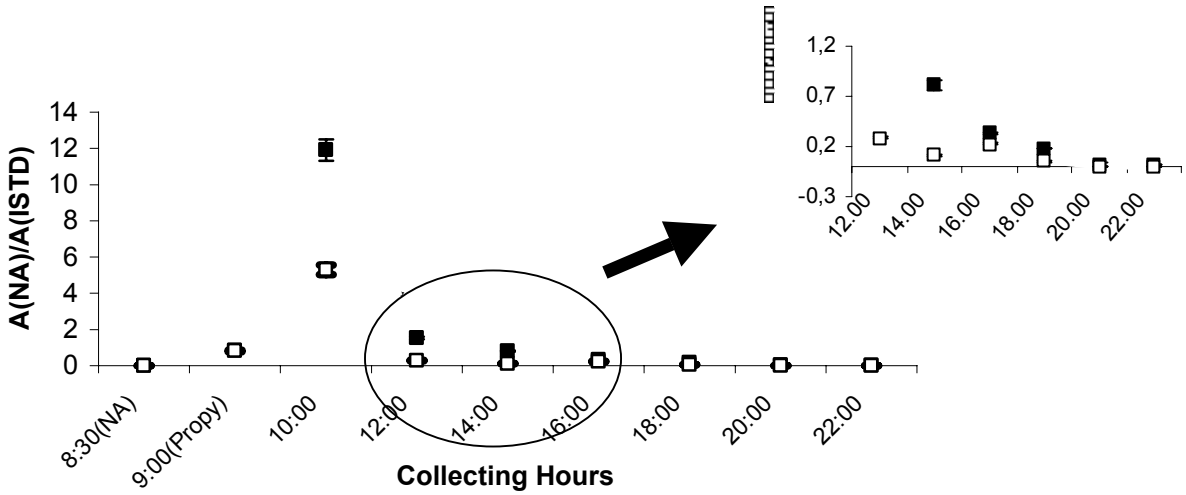

Figure 6. Excretion study of 19-norandrosterone before (■) and after (□) propyphenazone administration.

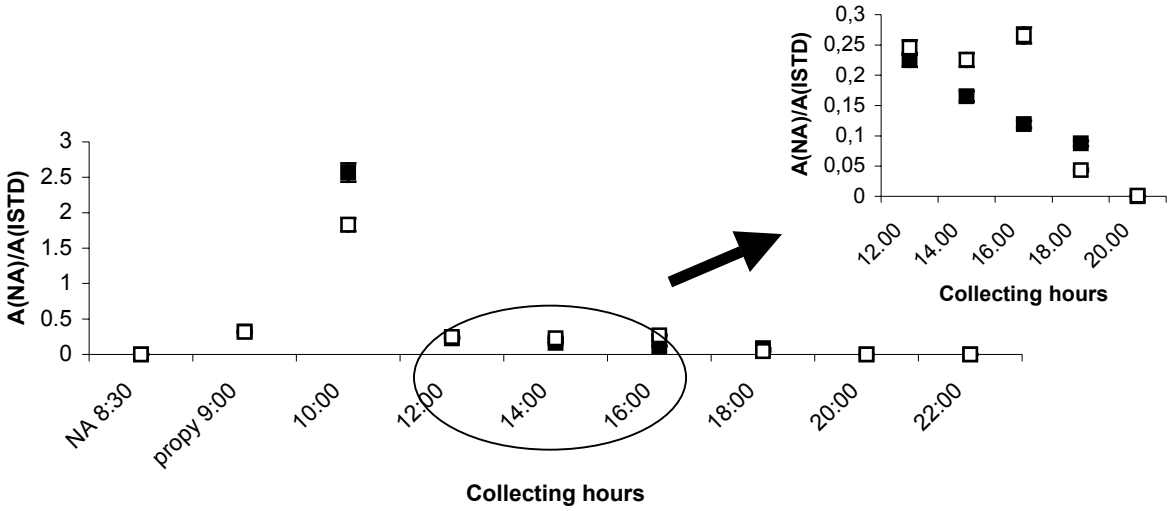

Figure 7. Excretion study of 19-noretiocholanolone before (■) and after (□) propyphenazone administration.

Urinary concentration of propyphenazone metabolites

Figure 8 shows the urinary excretion profile of propyphenazone metabolites with and without 19-norsteroids administration.

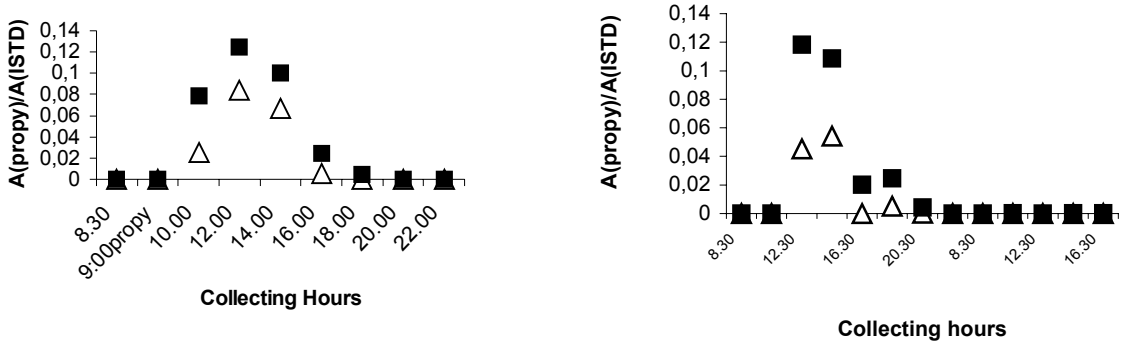

Figure 8 Urinary excretion profile of the two propyphenazone metabolites (■: N-demethylhydroxypropyphenazone; and □: hydroxypropyphenazone) with (left) and without (right) the concurrent administration of 19-norandrostenedione and 19-norandrostenediol.

CONCLUSIONS

- Propyphenazone administration caused in all volunteers a transient, but statistically significant, suppression of the absolute urinary concentration of 11β-OH-androsterone, 11β-OH-etiocholanolone, androsterone, etiocholanolone, 19-norandrosterone and 19-noretiocholanolone. No effect on the T/E ratio was recorded.
- The maximum value of the urinary concentration of the propyphenazone metabolites overlaps, in all subjects studied, with the suppression of 11β-OH-androsterone, 11β-OH-etiocholanolone, androsterone, etiocholanolone, 19-norandrosterone and 19-noretiocholanolone.
- The excretion of testosterone metabolites returns within the normal range of urinary concentration values after elimination of propyphenazone metabolites.
- The excretion of both 19-norandrosterone and 19-noretiocholanolone has a second maximum corresponding with the elimination of propyphenazone metabolites.
- Additional data are necessary to further clarify the mechanism leading to the observed effects, especially at higher doses, and also to consider other similar drugs.
- Our suggestion is to monitor propyphenazone, in all cases where an abnormal steroid profile or the presence of 19-nortestosterone metabolites is detected.

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