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PENTAVALENT ANTIMONIAL NEPHROTOXICITY IN THE RAT

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SUMMARY

Aspects of the renal function were assessed in rats treated with the pentavalent antimonials Glucantime (Meglumine Antimoniate, Rhodia) or Pentostam (Sodium Stibogluconate, Wellcome). In dose of 30 mg of Sb^{v} (Glucantime or Pentostam) by 100 mg of weight by day for 30 days, renal functional changes were observed consisting of disturbances in urine concentrating capacity. Such disturbances were expressed by significantly low values of urine osmolality as compared to the basal values previous to the drugs. The decrease in urine osmolality was associated to a significant increase in urinary flow and in negative free-water clearance. There was no alteration in osmolar clearance and in fractional excretion of sodium. These observations suggest an interference of the drugs in the action of the antidiuretic hormone. The disturbance in urine concentration was reversible after a seven days period without the drugs administration. No significant histopathological alterations were observed in the kidneys of the rats treated with the drugs. On the other hand, the rats treated with a high dose of Pentostam (200 mg/100 grams of weight/day) showed the functional and the histopathological alterations of the acute tubular necrosis.

KEY WORDS: Pentavalent antimonial; Renal function; Urinary concentrating capacity; Acute tubular necrosis; Rat.

INTRODUCTION

Pentavalent antimonials (Sb^{v}) are the drugs of choice for parenteral treatment of leishmaniasis[8]. Urinary excretion of these drugs is rapid and a higher dose of antimony base for longer than the usual period of treatment is recommended for the resistant cases, with a good response and few side effects[3, 9, 12]. Some side effects were reported with a higher dose than 20 mg of Sb^{v}/kg of weight/day, such as electrocardiographic changes, alterations in the levels of the transaminases and alkaline phosphatase, and proteinuria[4, 14, 18].

The occurrence of a defect in renal concentrating capacity in patients treated with pentavalent antimonials drugs had been described[16, 17]. "In vitro" studies showed that the pentavalent antimonials induce inhibition of the osmotically stimulated water flow in the isolated toad bladder[5, 6].

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The present study was carried out in order to evaluate the effect of pentavalent antimonials drugs commonly used in treatment of leishmaniasis, on the renal function of normal rats.

MATERIAL AND METHODS

1. Animals and Drugs

Forty-eight male Wistar rats weighing 130-160 grams were used in the study. Twelve animals constituted the control group and the remainders the group that received the drugs. The treated group was divided in three subgroups according the treatment received: a) 12 animals treated with 30 mg of Glucantime/100 grams of weight/day (G-30 sub-group); b) 12 animals treated with 30 mg of Pentostam/100 grams of weight/day (P-30 sub-group); c) 12 animals treated with 200 mg of Pentostam/100 grams of weight/day (P-200 sub-group). Glucantime is the Meglumine Antimoniate derivate (Rhodia) and the Pentostam is the Sodium Stibogluconate derivate (Wellcome), the two pentavalent antimonials drugs commonly used in leishmaniasis.

To determine the optimal dose for to induce renal toxicity, increasing doses of Sb^{V} was given by intraperitoneal route to a different group of twenty-four rats. The dose of 200 mg of Sb^{V}/100 grams of weight/day was selected considering that at this dosage the drug induced renal functional changes in the most of the animals without significant mortality. The calculated mean lethal dose of the pentavalent antimonials was aproximately 350 mg/100 grams of weight. The dose of 30 mg/100 grams of weight was alternatively chosen since the dose frequently used in the mice and hamster for treatment of experimental leishmaniasis is between 20-60 mg of Sb^{V}/100 grams of body weight[10], NEAL, R.A.*)

2. Experimental Procedure

The rats were kept in individual metabolic cages under controlled conditions of light, humity and temperature, with food and water "ad libitum". After a period of three days for adaptation of the animals, the experiment began with a basal renal functional test performed in all the rats before administration of the drugs. Following the basal tests the animals received the pentavalent antimonials (treated group) or 0.9% NaCl solution (control group), by intraperitoneal daily injections for a thirty days period.

The renal function was again assessed in all the rats at the 15th and 30th days during the continuated administration of the drugs since the first day. A new functional test was repeated in thirty-two rats (treated and controls) after a seven days period without administration of the drugs. The glomerular function was evaluated by the standard methods of clearance studies, and the renal concentrating ability by a test consisting of eight hours of water deprivation as described by NETO[11].

Blood samples (1.0 ml) for plasmatic determinations were collected by sectioning the tip of the tail. The urine voided during the tests was collected without fecal contamination for urinary flow measurement and separation of samples for analysis. From the urine and blood samples the following functional parameters were determined: a) the creatinine clearance, C_{cr} (ml . min^{-1} . 100 g^{-1}), to estimate the glomerular filtration rate; b) the osmolar clearance, C_{osm} (ml. min^{-1}); c) the water transportation in collecting ducts, $T^{c}H_2O$ (ml . min^{-1}); d) the fractional sodium excretion, FE_{Na} (%); e) the urinary osmolality, U_{osm} (mOsm/kg water), and the plasma osmolality, P_{osm} (mOsm/kg water); f) the urinary flow, V (ml . min^{-1}); g) the urinary protein excretion as albuminuria (mg. h^{-1}). The creatinine and sodium levels were also determined.

3. Analytical Methods

The plasma and urine creatinine levels were measured by the Jaffe reaction[15]. The sodium was measured by flame photometry (Micronal Photometer B262). The urine albumin by colorimetric reaction with sulphosalisilic acid[1]. Urine and plasma osmolality were determined by the freezing point depression method (Fiske Osmometer).

4. Histopathological study

At the termination of the thirty days period of drugs administration (30th day), four rats of

*) Personal communication, 1986.

the control group and two of the each treated sub-group were killed by ether asphyxia. After systemic perfusion with PBS (pH 7.2), the kidneys were removed and small cortical fragments were obtained and fixed in Bouin's solution for histological study; the fragments were stained with hematoxylin-eosin and PAS.

5. Statistical Analysis

The results were statistically analysed by the paired Student's t-test. Differences were taken as significant at the probability level of 5% ($p < 0.05$). All the values are expressed as mean ± sd.

RESULTS

There was a significant increase ($p < 0.05$) in urinary flow (V, ml . min^{-1} . 10^{-3}) from 4.3 ± 0.9 (0th day) to 6.6 ± 1.2 (15th day) and to 7.6 ± 1.9 (30th day) for the G-30 sub-group, and from 4.5 ± 1.3 to 6.8 ± 2.4 and to 8.9 ± 2.4 for the P-30 sub-group of rats (Figure 1). A significant decrease ($p < 0.05$) was observed in urinary osmolality (U_{osm}, mOsm/kg water) from 2688 ± 290 (0th day) to 1727 ± 372 (15th day) and to 1557 ± 486 (30th day) for the G-30 sub-group, and from 2664 ± 464 to 1574 ± 303 and to 1314 ± 210 for the P-30 sub-group (Figure 2); and, also

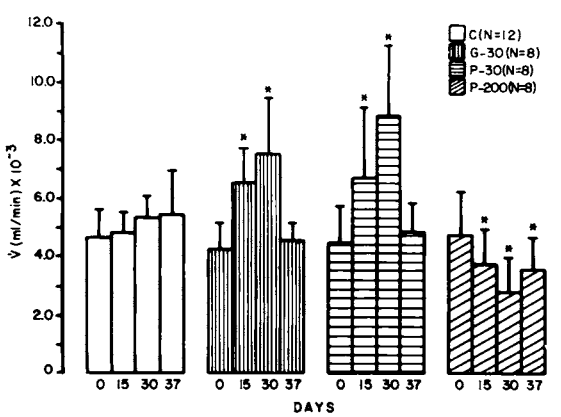

Fig. 1 — Urinary flow (mean + sd) in control rats (C) and in rats treated for 30 days with 30 mg of Glucantime/100 g of weight/day (G-30 sub-group), with 30 mg of Pentostam/100 g of weight/day (P-30 sub-group), and with 200 mg of Pentostam/100 g of weight/day (p-200 sub-group).
The days indicated are the basal day (0th), the 15th and 30th days during the drug treatment, and the 7th (37th) day after the drug suspension.
* $p < 0.05$ (paired t-tested).

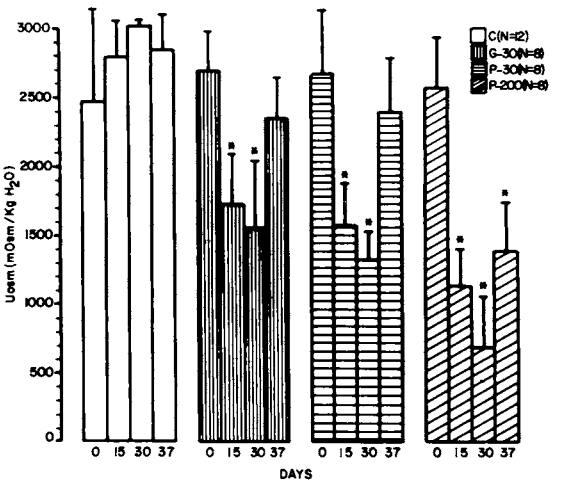

Fig. 2 — Urinary osmolality (mean + sd) in control rats and in the rats treated with the pentavalent antimonials. Notations and abbreviations as in Figure 1.

in water ductal transportation (T^cH_2O, ml . min^{-1} . 10^{-3}) from 34 ± 5 (0th day) to 27 ± 4 (30th) for the G-30 sub-group, and from 36 ± 14 to 26 ± 7 for the P-30 sub-group (Figure 3). None statistically significant change ($p > 0.05$) in osmolar and creatinine clearances and in fractional sodium excretion was observed in both G-30 and P-30 sub-groups of rats (Figures 4 to 6).

In the rats treated with a high dose of Sb^v (P-200 sub-group) a significant decrease ($p < 0.05$) was observed in the V, U_{osm}, C_{cr}, C_{osm} and T^cH_2O, and a significant increase ($p < 0.05$) in the FE_{Na}. The urinary flow (ml . min^{-1} . 10^{-3}) changed from 4.8 ± 1.5 (0th day) to 3.8 ± 1.2 (15th dav) and to 2.8 ± 1.2 (30th day), attaining 3.6 ± 1.1 at the 7th day after the suspension of the drug (37th day) (Figure 1). The urinary osmolality (mOsm/kg water) changed from 2560 ± 356 (0th day) to 1130 ± 270 (15th day) and to 678 ± 370 (30th day), showing a value of 1380 ±350 at the 37th day (Figure 2). For the creatinine clearance (ml . min^{-1} . 100 g^{-1}) was observed a decrease from 0.62 ±0.12 (0th day) to 0.18 ±0.09 (15th) and to 0.16 ±0.08 (30th day) and 0.38 ±0.11 (37th day) (Figure 5); and from 42 ±11 to 14 ±8 and to 6 ±3 and 16 ±7, respectively for the different periods, for the osmolar clearance (ml . min^{-1} . 10^{-3}) (Figure 4). The water transportation in collecting ducts (ml . min^{-1} . 10^{-3}) decreased from 37 ±11 (Oth day) to 10 ±3 (15th day) and to 9 ±4 (30th day) and 12 ±6 (37th day) (Figure 3). The fractional

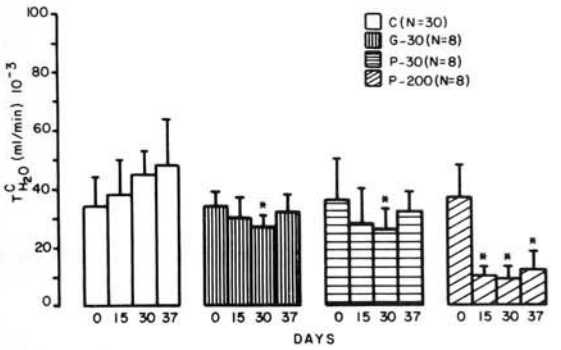

Fig. 3 — Water transportation in collecting ducts (mean + sd) measured in control rats and in the rats treated with the pentavalent antimonials. Notations and abbreviations as in Figure 1.

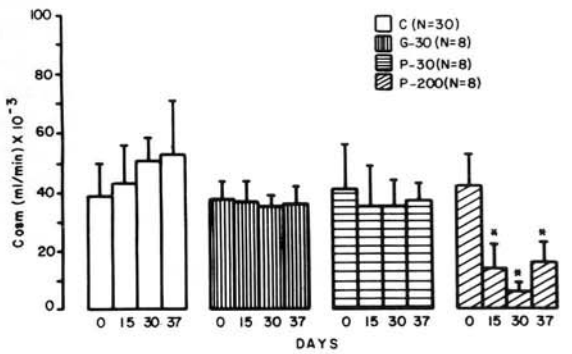

Fig. 4 — Osmolar clearance (mean + sd) in control rats and in the rats treated with the pentavalent antimonials. Notations and abbreviations as in Figure 1.

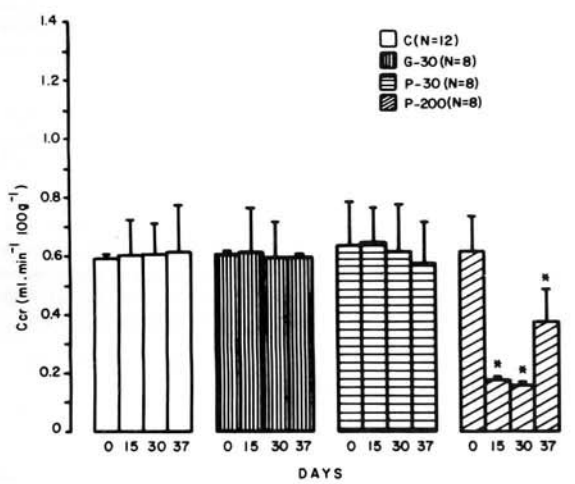

Fig. 5 — Creatinine clearance (mean + sd) in control rats and in the rats treated with the pentavalent antimonials. Notations and abbreviations as in Figure 1.

sodium excretion increased from 0.62 ±0.14% (0th day) to 1.28 ±0.02% (15th day) and to 1.13 ±0.04% (30 th day) (Figure 6). Also, the mean basal urinary protein excretion (mg . h^{-1}) presented for this sub-group of rats, increased significantly ($p < 0.05$) from 0.16 ±0.03 to 0.65 ±0.04 (15th day) and to 0.97 ±0.03 (30th day).

With respect to the histological study of the kidneys at optical microscopy, none alteration it was observed in the G-30 and P-30 sub-groups of rats in comparison to the control group. In the rats of the P-200 sub-group, histopathological changes were observed consisting of cellular necrosis and degeneration mainly in proximal convoluted tubule, loop of Henle and cortical collecting tubules (Figure 7).

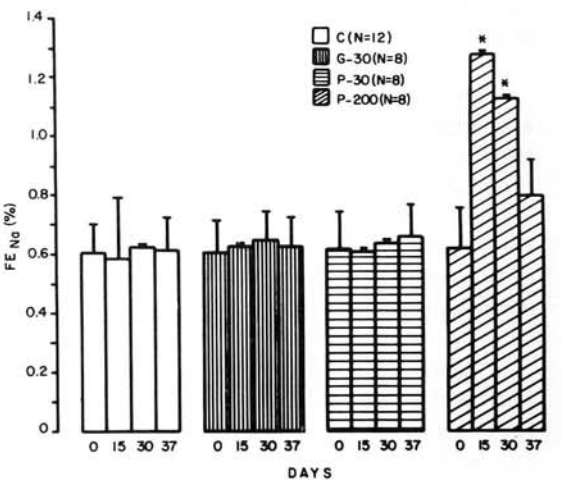

Fig. 6 — Fractional sodium excretion (mean + sd) in control rats and in the rats treated with the pentavalent antimonials. Notations and abbreviations as in Figure 1.

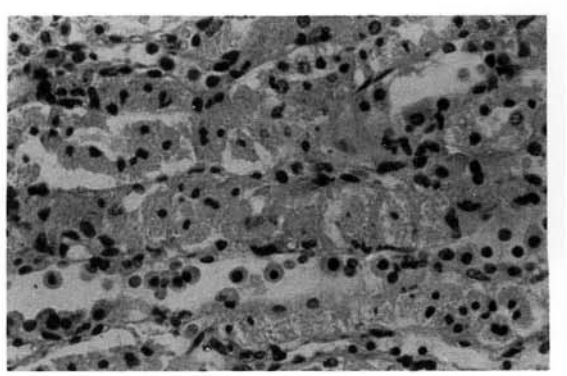

Fig. 7 — Photomicrograph showing the kidney cortex of a rat treated with 200 g of Pentostam/100 g of weight/day. Most of the tubular cells have died and sloughed off into the lumen of the tubules. (H & E x 320).

DISCUSSION

The present results showed that intraperitoneal injection of pentavalent antimonials (Glucantime and Pentostam) in normal rats in a daily dose of 30 mg of Sb^v/100 g of weight during a 30 days period caused renal tubular dysfunction. This was characterized by a decreased U_{osm} and T^cH_2O without alterations in the C_{osm} and C_{cr}. None histopathological lesions were observed in these groups of rats. On the other hand, the injection of a high dose of the antimonials induced renal function changes compatible with acute renal failure, as demonstrated by a decrease in the C_{cr} and C_{osm} and an increase in the FE_{Na} and proteinuria. Microscopic examination of the kidneys of this group of rats showed necrosis and degeneration of the tubular cells mainly in proximal convoluted tubule.

The action of the Sb^v in the nephron that explains the inhibition of the urinary concentration mechanism is not known. Patients treated with 20 mg of Sb^v/kg of body weight/day during a period of 30 or more days also exhibited such tubular defect[16, 17]. If a lower dose of the antimony is used for a shorter time this renal disturbance is uncommon[7].

The tubular defect appears to be based upon two mechanisms: interference with the antidiuretic hormone action and a direct toxic effect on the tubular cells. Similar results have been obtained from experiments in isolated toad bladder, which is considered to be functionally analogous to the distal mammalian nephron[5, 6].

High dose of the pentavalent antimonials caused decrease in glomerular filtration rate with consequent acute renal failure in the rats. Thus, these antimonials in high dose appears to be toxic for the proximal tubular cells. This effect probably was secondary to a direct action of the metal molecule on the tubular cells. This functional disturbance is rarely described in patients treated with the usual doses of the pentavalent antimonials[2, 13].

In conclusion, the treatment of rats with a low dose of pentavalent antimonials induced renal tubular dysfunction characterized by diminished urinary concentration capacity. This alteration appears to be reversible after suspension of the drugs. With a higher dose the antimony induced changes characteristics of the acute renal failure.

RESUMO

Disfunção tubular renal em ratos tratados com antimoniais pentavalentes.

Estudou-se a função renal de ratos tratados com Glucantime (Antimoniato de Meglumine, Rhodia) e Pentostam (Estibogluconato de Sódio, Wellcome) na dose de 30 mg de Sb^v por 100 g de peso por dia, durante 30 dias. Observou-se um distúrio na concentração urinária, que foi reversível 7 dias após cessada a administração das drogas. O estudo histopatológico do rim, por meio da microscopia óptica, não evidenciou alterações significativas. Por outro lado, ratos tratados com altas doses dos antimoniais (200 mg de Sb^v por 100 g de peso por dia) mostraram alterações funcionais e histopatológicas renais compatíveis com necrose tubular aguda.

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