Drug crystals are identified in two thirds of drug-induced stones. Other stones have an apparent metabolic origin (1). Ambroxol (ABX) is one of the most often prescribed drugs (2, 3). It was a chance discovery that ambroxol parenteral administration led to stone formation within a period of two months in half of treated rats. Stones were composed of 67% of xanthine and 33% of calcium oxalate (CaOx) (4, 5). Previous studies had suggested that high doses of ambroxol reduced the plasma uric acid concentration by increasing its clearance (6).

Stone formation is a complex multifactor phenomenon. It is often difficult to find factors responsible for lithogenesis. This study was undertaken to examine the serum uric acid levels and urine pH in rats after ambroxol parenteral treatment.

EXPERIMENTAL

Wild rats (Rattus sp.) were used in the experiment. 1 month before and during the whole experiment animals had free access to chow (Murigran) and tap water. Chow was obtained from Agropol and BASF (Poland). Murigran was composed of protein (23%), raw fat (3%), raw ash (7.5%), raw fibers (5%), lysine (1.5%), methionine and cysteine (0.8%), tryptophan (0.15%), calcium (1.1%), phosphorus (0.7%), sodium (0.2%), vitamins A, D, and E and cuprum. The 6 a.m. – 6 p.m. day and night cycle was maintained artificially.

To establish the ambroxol influence on the purine metabolism the uric acid level was measured. 5 rats were treated with commercially available ambroxol (Mucosolvan, Boehringer Ingelheim) subcutaneously (60 mg/kg sc, daily) during 2 weeks. Ambroxol withdrawal resulted in sequential urine pH decrease. 11 days after interruption of ambroxol therapy pH reached the starting value. Urine pH changes and possible disturbances in uric acid metabolic pathway may influence on the stone formation in rats after ambroxol parenteral treatment. The influence of ambroxol on urinary tract GAG layer and the balance between xanthine and CaOx in the urine should be checked.

Keywords: drug-induced stones, ambroxol, hypoxanthine, calcium oxalate

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performed, using commercially available UA plus kit (Roche Diagnostics GmbH, Germany). The results were presented as the means ± standard deviation. The Student t test was used to compare the mean values, p < 0.05 was considered significant.

To examine the ambroxol influence on urine pH 45 rats were divided into 3 equal groups. Rats from the 1st and 2nd group received ambroxol during 2 weeks in daily doses of 30 mg/kg and 60 mg/kg sc every morning, respectively. During the ambroxol therapy rat urine was collected once daily. Urine pH was measured with laboratory strip kit (SWW1331-28, POCh, Poland). All values were presented as the means with standard deviations. The Student t test was used to compare the mean values of pH in tested group. p < 0.05 was considered significant.

To observe dynamics of pH changes after ambroxol administration 4 rats were treated parenterally 10 days with a dose of 60 mg/kg/24h. Then, the rats were observed during the next two weeks, until the urine pH recovered to starting level. Every day in the morning urine pH was measured using strip kit (SWW1331-28, POCh, Poland).

RESULTS AND DISCUSSION

We have previously shown that ambroxol parenteral treatment induced lithogenesis in rats (5). In humans, ambroxol is metabolized to dibromoanthranilic acid (DBAA) and 6,8-dibromo-3-(trans-4-hydroxycyclohexyl)-1,2,3,4-tetrahydroquinazoline (DHTQ). The ambroxol metabolism is similar in human and rat (7). Previous studies had suggested that high doses of ambroxol could reduce the plasma uric acid concentration (6). In our study serum uric acid concentration was elevated above the values of control group (Fig. 1). Oosterhuis et al. had shown that plasma hypoxanthine levels were not affected by ambroxol (6). On the other side, it was observed that some drugs administered in cardiovascular diseases elevated serum uric acid (8). In this study was found that xanthine was one of the components of urinary stones after ambroxol treatment. Xanthine comprised about 67% of the stones weight. Xanthine urinary stones are rare in humans. In the control group serum uric acid level was 5.7 ± 1.0 mg/dL. Serum uric acid level increased up to 8.7 ± 1.0 mg/dL after two weeks of ambroxol parenteral treatment in a dose of 60 mg/kg/24h. The difference of serum uric acid level between control and treated group was statistically significant, p < 0.002 (Fig. 1). The authors observed that xanthine urinary stones in rats after ambroxol treatment had coexisted with elevated serum uric acid level, which is possible side effect of ambroxol treatment in rats.

Xanthine stones occurred in hypoxanthine-guanine phosphoribosyltransferase deficiency, because of accumulation of guanine (9). It was proved that partial deficiency of hypoxanthine-guanine phosphoribosyltransferase (HPRT) led to hypoxanthine and serum uric acid accumulation (10, 11). It was shown on in vitro model that uric acid metabolites accumulate in the case of HPRT deficiency (12). It can be speculated that ambroxol treatment can affect the uric acid metabolism. This pathway should be examined in the future.

The urine pH is another important factor responsible for lithogenesis. The H⁺ ions influence the solubility of many substances in the urine. The urine pH in the control group was 6.7 ± 0.4. It was noticed that average urine pH was higher than in control after two weeks of ambroxol treatment. In the 1st and 2nd group the urine pH increased up to 7.5 ± 0.5 and 7.6 ± 0.5, respectively. There were significant differences between experimental groups and the control. No difference was observed between pH values of two experimental groups (p = 0.28, Fig. 2).

Ten days of the continuous ambroxol treatment at higher dose (60 mg/kg/24h) resulted in the pH increase up to 7.6. Ambroxol withdrawal resulted in sequential urine pH decrease. 11 days after interruption of the therapy pH reached the previous value.
Uric acid plasma level and urine dH in rats treated with ambroxol

(Fig 3). It was observed in this study that pH increased in a short period of time after ambroxol administration. The next two weeks after ambroxol withdrawal pH decreased to original value (Fig. 3). It should be emphasized that both administrated doses increased pH within similar ranges (Fig. 2). Xanthine is a purine base. The solubility of xanthine decreases concomitantly with an increase of pH. The relationship between pH changes and purine bases solubility is strong. The pH also effects the solubility of calcium oxalate. Uric acid (UA) and sodium urate (NaU) crystals could induce the precipitation of calcium oxalate (CaOx) from its inorganic metastable solutions (13, 14). It was shown that cystine adding to undiluted human urine resulted in the marked enhancement of calcium oxalate precipitation (15). When the concentration of the components increases beyond the saturation level, a state of super saturation exists in the urine, which is thermodynamically unstable. Xanthine nucleation may provoke precipitation of the calcium oxalate from its solution (16).

It can be resumed that ambroxol parenteral treatment leads to formation of stone comprised of xanthine and calcium oxalate in a very short period. The urine pH changes and possible disturbances in uric acid metabolic pathway may influence on the stone formation in rats after ambroxol parenteral treatment. Thus, the influence of ambroxol on the balance between xanthine and CaOx in the rat urine should be checked.

REFERENCES


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Erratum
In the paper of S. Ray, K. Roy and Ch. Sengupta – Acta Pol. Pharm. 64, issue 4 pp. 335-344 in the title and in the text instead of *Spirulina plantesis* should be *Spirulina platensis*.