Intravenous Infusion of Diltiazem Causes Uricosuria with Concomitant Hypouricemia in Rats

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The effects of the continuous intravenous infusion of diltiazem on plasma urate levels and the urinary excretion of urate were examined in urethane anesthetized oxonate-loaded rats. The intravenous infusion of diltiazem (4 or 10 μg/rat/min) caused a gradual decrease in blood pressure, a gradual increase in renal blood flow and a transient increase in glomerular filtration rate. This infusion also caused diuresis, natriuresis, uricosuria and definite hypouricemia. Our results show that diltiazem infused i.v. has uricosuric and hypouricemic effects.

Key words: diltiazem; urate excretion; hypouricemia; continuous infusion

Calcium channel blockers appear to be useful in the treatment of hypertension complicated by hyperuricemia, because these agents have diuretic and natriuretic effects in the absence, probably, of any aggravating effects on hyperuricemia. Nifedipine and manidipine, two derivatives of dihydropyridine, have been found to be uricosuric and hypouricemic in patients with hypertension. Moreover, verapamil, a phenylalkylamine derivative, affects the transport of urate in slices of rat renal cortex. We previously reported that an i.v. bolus injection of diltiazem, a benzothiazepine derivative, caused uricosuria without concomitant hypouricemia in rats. However, if the drug were to be administered continuously, a hypouricemic effect might be anticipated. Thus, in this study, we administered diltiazem by continuous i.v. infusion into oxonate-loaded rats, and we examined the uricosuric and hypouricemic effects of this treatment.

MATERIALS AND METHODS

Male Wistar rats weighing 270–280 g were used. In a series of experiments, we examined the time dependent effects of diltiazem on urine volume (Uvol), and on the rates of urinary excretion of urate (Uua) and sodium (UNa). The animals were anesthetized with urethane (1.2 g/kg, s.c.) and placed on a warm plate (34°C). The left femoral vein, left jugular vein and urinary bladder were catheterized for the infusion of the loading solution, the administration of vehicle and drug solutions and the collection of urine, respectively. After catheterization, the loading solution, containing 0.1% oxonate, 4% mannitol, 1.5% inulin, 0.03% sodium bicarbonate and 0.85% sodium chloride (w/v), was infused at a rate of 2.6 ml/rat/h, because the animals were all roughly of the same weight. The saline vehicle solution was infused at 3.0 ml/rat/h. After equilibration for 90 min, a 10 min urine sample was collected five times: once 20 to 10 min prior to diltiazem administration, and then at four consecutive 10 min periods after the administration of the drug. Diltiazem (dissolved in saline; Sigma, St. Louis, MO, U.S.A.) was administered by changing the infusing solution from the vehicle solution to the drug solution at time 0. The drug was infused throughout the experiment. The control animals received only the vehicle. Our preliminary study showed that diltiazem infused at a rate of 2 μg/rat/min was not uricosuric; and at doses of 4 and 10 μg/min, the drug was uricosuric. However, it was no longer uricosuric at a dose of 20 μg/min. Diltiazem, therefore, was infused at the rate of 4 or 10 μg/rat/min in the present study; and in this series, a total of twelve rats in the control and two treatment groups (n=4 for each group) were used.

In a separate series of experiments, a clearance study was carried out with a total of fifteen rats in the control and two treatment groups (n=5 for each group). Animal preparation, infusion of the loading solution, drug administration and urine sampling were performed as described above, and the animals received the same doses of diltiazem. A sample of blood was taken from the right jugular vein at the mid-point of each urine-collection period, namely, 15 min before, 15 min after and 35 min after the start of administration of the drug, and plasma was obtained by centrifugation. Mean systemic blood pressure (BP), heart rate (HR) and renal blood flow (RBF) were monitored throughout the experiment. BP was measured from the right femoral artery with a pressure transducer (P23XL; Ohmeda, Liberty Corner, NJ, U.S.A.) and HR was measured with a heart rate counter (AT601G; Nihon Kohden, Tokyo). RBF was measured from the right renal artery with an ultrasonic pulsed Doppler flow meter (model VF-I; Crystal Biotech, Holliston, MA, U.S.A.).

Urate was quantified by phosphotungsticstate colorimetric assay with a Uric Acid-Test Wako kit (Wako Pure Chemical Co., Osaka). Inulin was quantified by the fluorometric method. Sodium was quantified with an atomic absorption spectrophotometer (AA640; Shimadzu, Tokyo). The glomerular filtration rate (GFR) was calculated via the rate of inulin clearance (Cin).

Data were expressed as means ± S.E. and were analyzed by a two-way analysis of variance followed by Dunnett’s Multiple Range test.

RESULTS AND DISCUSSION

The time dependent effects of i.v. infusion of diltiazem on Uvol, Uua and UNa are shown in Fig. 1. Mean pre-treatment values for all rats (n=12) were 23.5±

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1.3 µl/min for Uvol, 38.7 ± 2.7 µg/min for Uua and 74.4 ± 12.0 µg/min for UNa. When infused at a dose of 4 or 10 µg/min, diltiazem increased Uvol, Uua and UNa within 10 min of administration. The increases in these three parameters at the higher dose of 10 µg/min were larger than those at the lower dose. At the higher dose, maximum increases, namely, 28.5 ± 11.3% in Uvol, 18.6 ± 3.0% in Uua and 124.4 ± 30.6% in UNa were observed during a 20 min period starting from 10 min after administration of the drug.

The excretion of urate in patients with hypertension treated with nifedipine and manidipine, as well as in normotensive subjects, was reported to be positively correlated with the excretion of sodium. We also obtained positive correlations between Uua and Uvol or UNa during the period 20 to 30 min after the administration of diltiazem, during which marked increases were observed: Uua versus Uvol (r = 0.85, p < 0.05, n = 12) and Uua versus UNa (r = 0.60, p < 0.05, n = 12).

At the higher dose, Uvol, Uua and UNa increased time dependently until 30 min after administration of the drug. The degree of increase in Uua and UNa tended to be reduced somewhat during the later period. In contrast, Uua fell more quickly to the pre-treatment level than Uvol and UNa. There are two possible explanations for the different patterns of change in the three parameters. Firstly, diltiazem might have different effects on the transport of water, urate and sodium, or secondly, the hypouricemic effect of diltiazem might modify the excretion of urate, as described above.

The results of the clearance study are shown in Fig. 2. Mean pre-treatment values of all rats (n = 15) were 29.1 ± 2.5 µg/min for Uua, 2.17 ± 0.11 mg/dl for plasma urate (Pua), 1.50 ± 0.10 ml/min for Cua, 2.11 ± 0.12 ml/min for GFR and 0.682 ± 0.023 for Cua/Cin. Pua levels decreased with doses of 4 and 10 µg/min from the early period to the late period of diltiazem infusion. The decreases with 4 and 10 µg/min during the early period, from 10 to 20 min after administration of DIL (B): Changes during the late period, from 30 to 40 min after administration of DIL. Data are expressed as percentage changes relative to pre-treatment values and are given as means ± S.E. (n = 3 for each group). *p < 0.05 vs. the control group.

GFR increased slightly, about 10%, and dose-independently during the early period of diltiazem infusion. In contrast, increases in urate clearance (Cua) and in the ratio of Cua to Cin (Cua/Cin) during infusion of the drug were larger; for example, at the higher dose the maximum increases in Cua and in Cua/Cin were 27.4 ± 4.5% and 28.6 ± 5.4%, respectively. The increases in Cua and in Cua/Cin during the early period of infusion reflected the stimulation of urate excretion, but those during the late period probably resulted from a decrease in Pua.

The diuretic and natriuretic action of Ca-channel blockers involves direct inhibition of the tubular reabsorption of water and sodium, and possibly site of the natriuretic action of diltiazem is at the distal part of the nephron. Our observations also suggest a tubular uricosuric action of diltiazem, but the site of the uricosuric action of this drug is unknown. Further studies are required to determine this site.

The effects of diltiazem on hemodynamics are shown in
between Uua and GFR ($r = 0.63$, $p < 0.05$, $n = 15$) during the early period of diltiazem infusion and a weak positive correlation between Uua and RBF during the same period, but the latter relation was not significant ($r = 0.48$, $n = 15$). Pua was not correlated with RBF during the early period. However, these parameters showed very weak negative correlation during the late period, although this relation too was not significant ($r = -0.38$, $n = 15$). This result is similar to previous reports that Pua in hypertensive patients is negatively correlated with RBF, and that Uua levels after the administration of nifedipine are positively correlated with GFR and renal plasma flow.

Our results support our previous finding that the mechanisms of the uricosuric action of diltiazem may be related not only to alterations in the tubular transport of urate, but also to changes in GFR and RBF. They also show that i.v. infused diltiazem has uricosuric and hypouricemic effects, and that decreased plasma levels of urate might influence the uricosuric effect.

REFERENCES