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Treatment of Infected Urinary Stones in Rats by a New Hydroxamic Acid, "N-(Pivaroyl)glycinohydroxamic Acid"

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Summary. The effectiveness of a new urease inhibitor, N-(pivaroyl)glycinohydroxamic acid, in the treatment of infected urinary stones was investigated. The hydroxamic acid markedly inhibited the alkalinisation of urine and stone formation when it was administered orally to rats with urinary tract infection caused by *Proteus mirabilis;* its inhibitory effect was potentiated by concomitant treatment with Cephalexin. This compound may become a useful medicine for the treatment of struvite stones.

Key words: Urease inhibitor, N-(Pivaroyl)glycinohydroxamic acid, Urolithiasis, Urinary tract infection, Urea-splitting bacteria.

Introduction

Struvite stones ($MgNH_4PO_4 \cdot 6H_2O$) are called *"malignant stones"* because of the poor prognosis for renal function. These stones are thought to develop in urinary tracts infected with urea-splitting bacteria such as *Proteus* species. Since the hyperammonuria and alkalinisation of urine by bacterial urease are important factors in the formation of struvite stones, control of urinary tract infection in necessary for the prevention of stone formation. However, the antibiotics presently available are not always effective in the treatment of infection by urea splitting bacteria.

Hydroxamic acid has been found to be a specific and powerful inhibitor of urease of plant and bacterial origins [4, 5]. Griffith et al. reported that acetohydroxamic acid had some effect against stone formation in rats and humans with urinary tract infection [2, 3]. Our previous study revealed that *m*-methoxyhippurohydroxamic acid (UCD II) also effectively prevented stone formation in rats with urinary tract infection [11]. But both compounds were considered to be of little clinical use because of their mutagenicity [8]. N-(Pivaroyl)-glycinohydroxamic acid (P-GHA), newly synthesised by Munakata et al. [9], was thought to be promising because of its potent inhibitory power, high renal excretion rate and absence of mutagenic acitivity [8]. Therefore, we studied its ability to counteract alkalinisation of urine and stone formation in rats with urinary tract infection caused by *Proteus mirabilis*, including the combined effect with Cefalexin.

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Materials and Methods

1 Chemical

N-(Pivaroyl)glycinohydroxamic acid was kindly provided by Dr. K. Munakata of Eisai Co.

2. Bacterium and antibacterial activity

P. mirabilis was isolated from the urine of a patient with urinary tract infection associated with staghorn calculi and cultured in bouillon broth. Minimum inhibitory concentrations (MIC) of P-HGA and Cephalexin (CEX) against the growth of *P. mirabilis* were determined by the agar dilution method using heart infusion agar (Difco, USA). MICs of P-GHA and CEX were >500 μg/ml and 25.0 μg/ml, respectively.

3. Formation of infected bladder stones in rats

Urinary infection was induced in male rats (Wistar strain, 200 g body weight) by the method of Vermeulen and Goetz [12]. Zinc discs, dipped into a saline suspension containing 10^8 *P. mirabilis* per ml, were implanted in the bladder. Rats were housed individually in metabolic cages and maintained on water and chow *ad libitum*.

The effect of P-GHA and alkalinisation of infected urine was studied as follows. P-GHA in a single dose of 20 mg was administered to rats by gastric tube 5 days after implantation of the discs. Every two hours urine specimens were collected for the determination of urinary pH and P-GHA concentration. The pH of each sample was measured with a pH meter (Tohwa Dempa, Model HM-20E). Urinary P-GHA concentration was determined enzymatically according to the method of Kobashi et al. [6].

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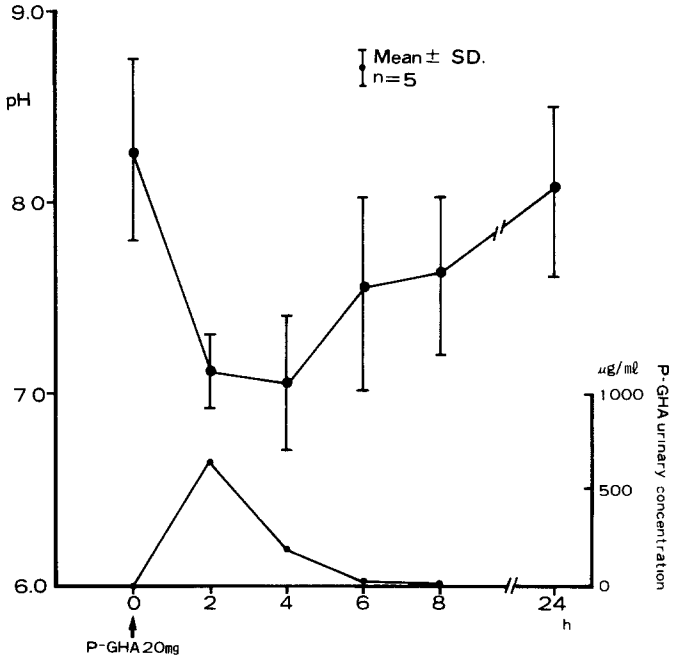

Fig. 1. Effect of P-GHA on alkalinisation of infected urine

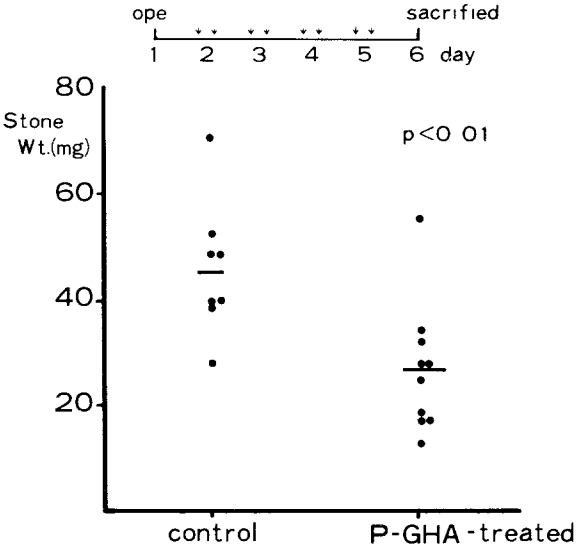

Fig. 2. Effect of P-GHA on stone formation. The horizontal bars represent means of stone weight. Statistical significance between the control and P-GHA treated group is indicated by *P* value

The preventive effect of P-GHA on the formation of bladder stones was studied as follows. A dose of 20 mg of P-GHA was administered orally twice daily for 4 days beginning the day after infection. On the 5th day, 4 h urine specimens were collected for pH determination and 24 h urine specimens were collected for the determination of P-GHA concentration. Rats were sacrificed on the 6th day and urine aspirated from the bladder was inoculated on MacConkey and CLED plates (Dip slide method, Uricult, Daiichi Chemical Ind. Ltd.). The approximate number of bacteria was calculated. Bladder stones were dried overnight at 37 °C in an incubator and were weighed and analysed by an infra-red spectrometer (Hitachi, Model 215).

The combined effect of P-GHA and CEX on the formation of bladder stones was studied as follows. Infected rats were divided into four groups and treated: control, P-GHA 20 mg/day, CEX 1 mg/day, P-GHA 20 mg/day plus CEX 1 mg/day. Drugs were administered orally twice daily for 5 days beginning 5 days after the surgical procedure. Urinary pH and P-GHA concentration were measured as described above. Rats were sacrificed on the 10th day and bladder urine was cultured and bladder stones were weighed as described above. The gross appearance of the kidneys was noted.

The rats which died before the completion of the study of failed to be infected were excluded. Comparisons were made by Student's *t* test.

Results

1. Effect of P-GHA on Alkalinisation of Infected Urine

As shown in Fig. 1, urinary pH decreased remarkably for several hours after the oral administration of a single dose of 20 mg of P-GHA and gradually increased up to the initial value. Urinary concentrations of P-GHA were high for 4 h after administration. The cumulative amounts of unchanged P-GHA reached about 7% of the dose within 24 h.

2. Effect of P-GHA on Formation of Infected Bladder Stones

Urine volume was not significantly different between the P-GHA treated group and the controls. Urine pH was lower in the P-GHA treated group than in the controls. The mean concentration of P-GHA was 87 μg per ml of urine. As shown in Fig. 2, the mean weights of stones in the control and P-GHA treated groups were 46.1 ± 12.7 mg (mean ± S.D.) and 27.2 ± 12.3, respectively; this difference is statistically significant ($P < 0.01$). Evidence of pyelonephritis with cortical abscess or pyonephrosis was incidental in the control group.

3. Effect of combination of P-GHA and CEX on Infected Bladde Stone Formation

The combined effect of P-GHA and CEX on bladder stone formation is summarised, with other related observations, in Table 1.

In all the infected rats, more than 10^5 *P. mirabilis* per ml was found in the bladder urine at the time of autopsy. The number of viable cells was not affected by treatment with CEX. Urine volume was not significantly different among the four groups. Separate administration of P-GHA (20 mg/day) and CEX (1 mg/day) was ineffective in the inhibition of stone formation, but the combined administration of P-GHA and CEX was remarkably effective. Furthermore the combination therapy also inhibited alkalinisation of infected urine and lowered the incidence of pyelonephritis.

IR analysis showed that nearly all the bladder stones were struvite stones.

Table 1. Combined effect of P-GHA and CEX on infected bladder stone formation

	No. of rat	Urine volume (ml)	pH	P-GHA conc. (μg/ml)	Stone weight (mg)	No. of rats with pyelonephritis
Control	10	20 ± 6 (Mean ± S.D.)	8.60 ± 0.29	–	74.2 ± 19.0	9/10
P-GHA 20 mg treated	11	13 ± 6 (n = 10)	8.22 ± 0.62	27.3 ± 15.6	61.0 ± 19.4	9/11
CEX 1 mg	9	17 ± 4	8.07 ± 0.62[a]	–	79.1 ± 37.7	7/9
P-GHA 20 mg, CEX 1 mg treated	12	15 ± 5	7.46 ± 0.79[b]	21.7 ± 12.2	38.0 ± 25.3[b]	4/12

Values represented as mean ± standard deviation. Significant difference from the control: [a] $p < 0.05$, [b] $p < 0.01$

Discussion

N-(Pivaroyl)glycinohydroxamic acid (P-GHA) powerfully inhibits the ureolytic activity of *P. mirabilis* (I_{50} = 7.1 μg/ml) [7]. About 11% of the total dose of the hydroxamic acid is excreted in the urine unchanged 24 h after oral administration to rats [7].

In our experiments oral administration of P-GHA inhibited alkalinisation of infected urine and stone formation in rats with urinary tract infection caused by *P. mirabilis*. Furthermore, combination therapy with lower doses of P-GHA and Cephalexin prevented stone formation, while separate therapy was ineffective against stone formation and bacterial growth. These observations suggest that CEX enhances the inhibitory action of P-GHA on bacterial urease by potentiation of the permeability of the bacterial cell membrane. It is very interesting that some small stones seem to be dissolved by combination therapy. The combination of a hydroxamic acid and an antibiotic may dissolve struvite stones by normalisation of infected urine.

It has been reported that some hydroxamic acids potentiate the antibacterial activity of several antibiotics [1, 10]. In our experiments P-GHA did not potentiate the antibacterial activity of CEX at the dosage used, but a combination of P-GHA and CEX reduced the severity and incidence of pyelonephritis. Therefore, P-GHA may also have a potential usefulness in the chemotherapy of urinary tract infections. Further investigation of this effect of P-GHA is necessary.

The present study suggests that P-GHA may become a useful medicine in the treatment as well as the prevention of struvite stones associated with infection by urea-splitting bacteria. Further pharmacological and toxicological evaluations are necessary before the clinical application of P-GHA.

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