High Steady-State Levels of Uric Acid Produced in Rats by Dietary Training and Potassium Oxonate

JEROLD NEWBURGER, TING-FENG HSU, and ALAN B. COMBS ˣ

Received August 17, 1978, from the *College of Pharmacy, University of Texas, Austin, TX 78712.* Accepted for publication October 18, 1978.

Abstract □ To reduce the inherent variability in serum uric acid levels of animals allowed *ad libitum* exposure to food containing potassium oxonate and uric acid, male Sprague–Dawley rats were trained to eat their daily food allotment in a 1.25-hr period each morning. After training, the rats were fed a food mixture containing 5% potassium oxonate and 2% uric acid (w/w each). Serum blood levels of uric acid reached a steady state within 2 hr; these levels were maintained for an additional 4 hr. It is believed that the stomach emptying rate is a zero-order process under these experimental conditions.

Keyphrases □ Uric acid—steady-state serum levels, rats, dietary training, potassium oxonate □ Hyperuricemia—animal models, rats □ Potassium oxonate—used to produce hyperuricemia in rats

Hyperuricemia can result in clinical gout (1) and can complicate the treatment of certain neoplastic diseases (2). The drugs presently used in the treatment of hyperuricemia all have distinct disadvantages and can cause adverse effects (3, 4).

A potential alternative therapy may be indicated by recent reports characterizing the *in vitro* decomposition of uric acid in the presence of light and riboflavin (5, 6). The selection of an animal model for *in vivo* testing of phototherapy is difficult, because most naturally hyperuricemic animals are typically exotic, protected, expensive, or nonmammalian (7–10). However, a murine model for hyperuricemia was reported recently. Rats given the uricase inhibitor oxonic acid can become hyperuricemic (11, 12). Moreover, these animals will develop gouty renal pathology after chronic oral feeding of oxonate and uric acid (13, 14).

We recently demonstrated that serum uric acid levels of rats fed *ad libitum* a ground laboratory ration containing potassium oxonate and uric acid are subject to a diurnal variation with higher morning levels (15). The variability produced by this method complicated the evaluation of phototherapy effects on serum uric acid. This paper reports the effects on serum uric acid levels of feeding potassium oxonate and uric acid to rats trained to eat their total daily food allotment within a short period.

EXPERIMENTAL

Male Sprague–Dawley rats[1], 150–200-g initial weights, were housed individually in hanging cages and were maintained on 12-hr light–dark cycles. They were allowed water *ad libitum* throughout the study. The animals were fed a standard pelleted laboratory ration[2] *ad libitum* for 1 week before dietary training. Following a previously reported procedure for dietary training and synchronization (16, 17), the rats were fed their laboratory ration as a ground powder given for only 1.25 hr each morning. By the end of 1 week, most animals had learned to eat their total daily ration during this brief feeding period. Animal body weights stabilized and generally began to increase again after 2 weeks. The animals were maintained on this feeding schedule for 4 weeks prior to use in the study.

On the morning of the study, animals were fed the ground laboratory ration mixed with 5% potassium oxonate[3] and 2% uric acid[4] (w/w each). The duration of feeding was the usual 1.25 hr. Animals were sacrificed for serum uric acid determinations immediately prior to drug administration and at 2, 4, 6, and 24 hr afterward. The animals were anesthetized with ether, and 3–5 ml of blood was drawn from each into heparinized syringes *via* the inferior vena cava. Analysis of serum uric acid was performed by high-pressure liquid chromatography as previously described (6). Statistical comparisons were made using analysis of variance followed by Scheffé's method of multiple contrasts (18).

RESULTS

The animal weights and the amounts of food, potassium oxonate, and uric acid ingested are listed in Table I. Serum uric acid levels as a function of time after the mixture was presented to the animals are shown in Fig. 1. The rats became hyperuricemic within 2 hr. The blood levels remained high and essentially constant for a minimum of another 4 hr. Levels returned to control values by 24 hr.

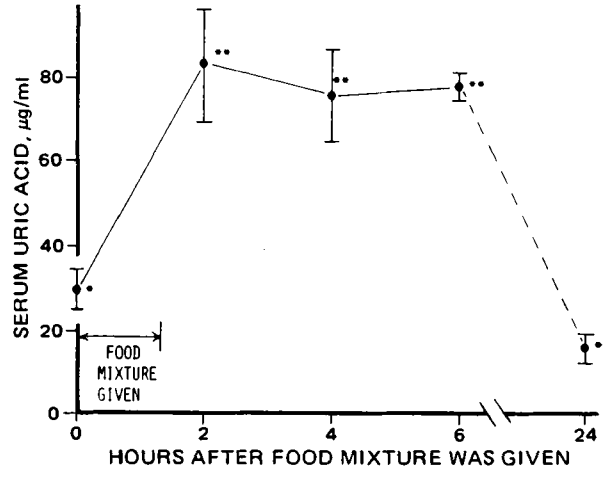

Figure 1—*Serum uric acid levels as a function of time after exposure of rats to a food mixture containing 5% potassium oxonate and 2% uric acid (w/w each). The animals were trained to eat their full daily food rations during a 1.25-hr period each morning. Each data point represents the mean ± SEM for three animals; for * versus **, $p < 0.05$ by analysis of variance followed by Scheffé's method of multiple contrasts.*

[1] Tempco Breeding Laboratories, Houston, Tex.
[2] Wayne Lab Blox, Allied Mills.
[3] Calbiochem, San Diego, Calif.
[4] Fisher Scientific Co., Fair Lawn, N.J.

0022-3549/79/0500-0651$01.00/0

Table I—Values Observed on Day of Experiment [a]

Parameter	Value
Animal weight, g	225 ± 38
Amount of mixture eaten, g/kg[b]	57.1 ± 7.2
Potassium oxonate ingested, g/kg	2.86 ± 0.36
Uric acid ingested, g/kg	1.14 ± 0.14

[a] Values are reported as mean ± *SD*. [b] Mixture consisted of ground laboratory ration containing 5% potassium oxonate and 2% uric acid (w/w each).

Accumulation to a plateau would be expected to occur with a zero-order administration process (19). The absorption of uric acid from the rat intestine, however, has been reported to be first order (20). Therefore, gastric emptying rather than intestinal absorption may be rate limiting. Reported data (16, 17) for untreated control animals trained by this technique are consistent with a zero-order stomach emptying rate for the first 10–12 hr following the daily feeding. This animal model will be used to test the efficacy of light and riboflavin in the reduction of serum uric acid.

REFERENCES

(1) J. R. Klinenberg, R. Bluestone, L. Schlosstein, J. Waisman, and M. W. Whitehouse, *Ann. Intern. Med.*, **78**, 99 (1973).

(2) I. H. Krakoff and R. L. Meyer, *J. Am. Med. Assoc.*, **193**, 1 (1965).

(3) W. N. Kelley and J. B. Wyngaarden, *Semin. Drug Treat.*, **1**, 119 (1971).

(4) "The Pharmacological Basis of Therapeutics," 5th ed., L. S. Goodman and A. Gilman, Eds. Macmillan, New York, N.Y., 1975, pp. 340, 342, 351, 863, 864, 1496.

(5) J. Newburger and A. B. Combs, *Life Sci.*, **17**, 443 (1975).

(6) J. Newburger, A. B. Combs, and T. F. Hsu, *J. Pharm. Sci.*, **66**, 1561 (1977).

(7) W. E. C. Wacker, *N. Engl. J. Med.*, **283**, 151 (1970).

(8) P. Christen, W. C. Peacock, A. E. Christen, and W. E. C. Wacker, *Eur. J. Biochem.*, **12**, 3 (1970).

(9) M. D. Skeith and L. A. Healey, *Am. J. Physiol.*, **214**, 582 (1968).

(10) T. K. Shnitka and M. M. Youngman, *J. Ultrastructure Res.*, **16**, 598 (1966).

(11) B. Stavric, W. J. Johnson, and H. C. Grice, *Proc. Soc. Exp. Biol. Med.*, **131**, 512 (1969).

(12) W. J. Johnson, B. Stavric, and A. Chartrand, *ibid.*, **131**, 8 (1969).

(13) B. Stavric, E. A. Neva, W. J. Johnson, and F. A. Salem, *Invest. Urol.*, **11**, 3 (1973).

(14) R. Bluestone, J. Waisman, and J. R. Klinenberg, *Lab. Invest.*, **33**, 273 (1975).

(15) J. Newburger, A. B. Combs, and T. F. Hsu, *Drug Chem. Toxicol.*, in press.

(16) S. H. Dieke, *Proc. Soc. Exp. Biol. Med.*, **76**, 788 (1951).

(17) S. N. Giri and S. Rice, *Life Sci.*, **9**, 1109 (1970).

(18) W. L. Hays, "Statistics," Holt, Rinehart and Winston, New York, N.Y., 1963, pp. 485–487.

(19) "Principles of Drug Action," 2nd ed., A. Goldstein, L. Aronow, and S. M. Kalman, Eds., Wiley, New York, N.Y., 1974, p. 311.

(20) J. H. Oh, J. B. Dossetor, and I. T. Beck, *Can. J. Physiol. Pharmacol.*, **45**, 121 (1967).

ACKNOWLEDGMENTS

Supported in part by Biomedical Sciences Support Grant 26-16994-0650 from the University of Texas at Austin.

The authors thank Mr. Paul Godley and Mr. Joseph Lew for their assistance.

High-Performance Liquid Chromatographic Determination of Cephacetrile

ALBERTO MANGIA ˣ, SILVESTRO SILINGARDI, FULVIO BORTESI, GIAMPIERO GRISANTI, and MAURO DI BITETTO

Received June 6, 1978, from the *Research Laboratories, Pierrel SpA, Via Degli Artigianelli 10, 20159 Milan, Italy* Accepted for publication October 19, 1978.

Abstract □ A rapid and accurate quantitative determination of cephacetrile in finished bulk and dosage forms is reported. The high-performance liquid chromatographic method is free of interference by acetyl hydrolysis products and synthesis by-products. The assay can be performed in about 15 min, affording <0.7% coefficients of variation within and between days. The chromatographic results are in good agreement with the microbiological assay requested by the "Code of Federal Regulations" for certification of cephacetrile sodium.

Keyphrases □ High-performance liquid chromatography—analysis, cephacetrile in various dosage forms □ Cephacetrile—high-performance liquid chromatographic analysis in various dosage forms □ Antibacterials—cephacetrile, high-performance liquid chromatographic analysis in various dosage forms

Cephacetrile (I) sodium [7-cyanoacetamido-3-acetoxymethyl-3-cephem-4-carboxylic acid, sodium salt] is a new cephalosporin antibacterial for parenteral use (1). The approved methods of analysis for certification are the microbiological agar diffusion assay and the hydroxylamine colorimetric assay (2).

This report outlines a more selective, rapid, and accurate method for the quantitative determination of cephacetrile in bulk drugs and pharmaceutical formulations using high-performance liquid chromatography (HPLC). The method is free of interference by acetyl hydrolysis products and synthesis by-products. Furthermore, the desacetyl derivative (II) of cephacetrile and lactone (III) can be detected accurately up to 0.1%.

EXPERIMENTAL

Materials—Cephacetrile FDA reference standard (microbiological potency of 885 μg/mg, lot 9/72) was used as received. Desacetyl derivative (II) of cephacetrile was prepared by enzymatic hydrolysis with acetyl esterase (3), while lactone (III) was synthesized by reacting trifluoroacetic acid with cephacetrile. Cephalosporins (IV*a* and IV*b*) were obtained by reaction of V with the proper acid chloride in acetone in the presence of urea (4), while IV*c* was prepared by reaction of methanesulfonyl chloride with the silyl derivative of V in dichloroethane (Scheme I). Cefazolin was Pierrel working standard. Methanol[1], acetic acid[2], and 1 *N* NaOH[2] were analytical reagent grade.

Apparatus—A liquid chromatograph[3], equipped with a 20-μl valve

[1] E. Merck, Darmstadt, West Germany.
[2] C. Erba, Milan, Italy.
[3] Model 1084 A, Hewlett-Packard, Böblingen, West Germany.

0022-3549/79/0500-0652$01.00/0