ELSEVIER

European Journal of Pharmacology 406 (2000) 45–48

ejp

www.elsevier.nl/locate/ejphar

Short communication

Effect of anti-inflammatory bowel disease drug, E3040, on urate transport in rat renal brush border membrane vesicles

Harumi Yamada [a,*], Hajime Kotaki [a,1], Tomoo Itoh [a,b], Yasufumi Sawada [c], Tatsuji Iga [b]

[a] *Department of Pharmacy, The University of Tokyo Hospital, Faculty of Medicine, The University of Tokyo, Tokyo, Japan*
[b] *School of Pharmaceutical Sciences, Kitasato University, Japan*
[c] *Faculty of Pharmaceutical Science, Kyusyu University, Japan*

Received 26 May 2000; received in revised form 11 August 2000; accepted 15 August 2000

Abstract

To confirm the assumption that 6-hydroxy-5,7-dimethyl-2-methylamino-4-(3-pyridylmethyl)benzothiazole (E3040) acts on urate reabsorption by inhibiting urate–anion exchange at the luminal membrane of renal tubules, we investigated the inhibitory effect of E3040 and its two conjugated metabolites on hydroxyl ion (OH^-) gradient-dependent urate uptake into brush border membrane vesicles from rat renal cortex and compared it with other uricosuric agents. The order of potency was AA193 (5-chloro-7,8-dihydro-3-phenylfuro[2,3-*g*]-1,2-benzisoxazole-7-carboxylic acid) > benzbromarone > E3040 > probenecid > E3040 sulfate > E3040 glucuronide. Furthermore, kinetic analysis revealed that E3040 may be a competitive inhibitor of the OH^- gradient-dependent uptake of urate into brush border membrane vesicles.

Keywords: E3040; Urate uptake; Brush border membrane vesicle; (Rat)

1. Introduction

6-Hydroxy-5,7-dimethyl-2-methylamino-4-(3-pyridylmethyl)benzothiazole (E3040), developed by Eisai (Tokyo, Japan), is a novel anti-inflammatory drug used in the treatment of bowel disease. We have reported that, in rats and mice, E3040 also has a uricosuric effect and that its site of action may be in the proximal tubules of the kidney (Yamada et al., 1999a,b).

Recent studies have demonstrated that an active transport process (Frankfurt and Weinman, 1977; Kramp and Lenoir, 1975; Weiman et al., 1976) drives the transepithelial reabsorption of urate in the proximal tubules of rats. Furthermore, Kahn and Weinman (1985) have reported that the urate–anion exchanger in luminal membranes, which mediates hydroxyl ion (OH^-) gradient-dependent urate uptake into brush border membrane vesicles, acts as the initial step in the reabsorption of urate in the renal proximal tubules. A hydroxyl ion or chloride gradient provided a driving force for the uptake of urate into brush border membrane vesicles, and trans-stimulation of urate and other anionic agents was observed. Thus, it seems that the transport of urate into the brush border membrane involves a urate–anion exchanger (Blomstedt and Aronson, 1980; Guggino et al., 1983; Kahn and Aronson, 1983). Furthermore, 4,4′-diisothiocyano-2,2′-disulfonic stilbene (DIDS), an inhibitor of the reabsorption of urate in rat proximal tubules, inhibited the transport of urate into cells by blocking this urate–anion exchanger (Kahn and Aronson, 1983; Kahn et al., 1983; Roch-Ramel et al., 1994). It has been reported that clinically important uricosuric drugs act on carrier-mediated urate transport at the luminal membrane of the renal proximal tubules (Diamond, 1978). Brush border membrane vesicles from rat renal cortex are sensitive to anion exchanger inhibitors such as probenecid, salicylate, furosemide, DIDS, and uricosuric agents such as benzbromarone and 5-chloro-

* Corresponding author. Department of Pharmacy, The Research Hospital, Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 1080071, Japan. Tel.: +81-3-5449-5353; fax: +81-3-5449-5440.

E-mail address: hami-tky@umin.ac.jp (H. Yamada).

[1] Present address: Department of Pharmacy, The Research Hospital, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 1080071, Japan.

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00626-9

7,8-dihydro-3-phenylfuro[2,3-*g*]-1,2-benzisoxazole-7-carboxylic acid (AA193) (Dan and Koga, 1990).

In the present study, we examined the inhibitory effect of E3040 and its two conjugated metabolites on the OH^- gradient-dependent uptake of urate into brush border membrane vesicles, and this effect was compared with that of other uricosuric agents. In addition, we investigated the mechanism of inhibition produced by E3040.

2. Materials and methods

2.1. Chemicals

E3040, E3040 sulfate and glucuronide were kindly provided by Eisai (Tokyo, Japan) and AA193 was supplied by Chugai Pharmaceutical (Tokyo). Probenecid and benzbromarone were purchased from Sigma (USA). Micro TP test WAKO® and Alkaliphospha B test WAKO® were obtained from Wako (Osaka, Japan). [8-^{14}C] Uric acid (50 mCi/mmol) was purchased from Moravek Biochemical (USA). All other reagents used were of analytical grade.

2.2. Preparation of brush border membrane vesicles

Brush border membrane vesicles from the renal cortex of male Wistar rats (Japan Laboratory Animals, Tokyo), 300–350 g body weight, were prepared by a Ca^{2+}-aggregation method (Itoh et al., 1998). The brush border membrane vesicles were suspended in the urate uptake buffer consisting of 150 mM mannitol, 2 mM $MgSO_4$ and 50 mM potassium phosphate buffer (pH 7.5) to give a final protein concentration of 8–10 mg/ml and were stored at −80°C and used within a week. Purification of the brush border membrane vesicles was monitored by assaying alkaline phosphatase as a marker enzyme in the brush border membrane (Bessey et al., 1964) and (Na^+–K^+) ATPase in the basolateral membrane (Jorgensen, 1974). The alkaline phosphatase activity in brush border membrane vesicles was 10- to 15-folds greater than that in renal cortex homogenate, while the activity of (Na^+–K^+) ATPase was no greater than 2-fold.

The protein concentrations in the membrane suspensions and whole cortical homogenate were determined according to the pyrogaroll-red method using the Micro TP test WAKO. The degree of purification of the brush border membrane vesicle preparation was determined by comparing the activity of alkaline phosphatase and (Na^+–K^+) ATPase in brush border membrane vesicles and in whole cortical homogenate. Alkaline phosphatase activity was determined by using *p*-nitrophosphoric acid as a substrate with Alkalicphospha B test Wako, and (Na^+–K^+) ATPase activity was determined by the method of Quigley and Gotterer (1969).

2.3. Uptake studies

The uptake of [8-^{14}C]uric acid was assayed by a modification of the rapid filtration methods reported previously (Dan and Koga, 1990; Kahn et al., 1983).

Brush border membrane vesicles were thawed on ice and then resuspended and allowed to equilibrate at 25°C for 10 min. Uptake was initiated by mixing 20 μl of the membrane suspension with 180 μl incubation buffer (150 mM mannitol, 2 mM $MgSO_4$, 50 mM potassium phosphate buffer; pH 6.0) containing 20 μM [8-^{14}C]uric acid, and the uptake inhibitors. Incubation was performed for 10 s at 25°C. In each experiment at least three different membrane preparations were used. All uptake measurements were corrected for the nonspecific binding of radiolabeled solute to the vesicles and filters. The effect of drug on the initial velocity of uptake of the radiolabeled solutes was evaluated using the following equation: % of inhibition = (uptake with drug/uptake without drug) × 100.

2.4. Data analysis

The inhibition curves were fit simultaneously according to the full nonlinear regression analysis for inhibition as expressed in Eq. (1). Eq. (1) was derived from the sigmoid E_{max} model as expressed in Eq. (2). The IC_{50} values of the various inhibitors were obtained from this by nonlinear regression analysis. Fitting was accomplished by using "Multi" described by Yamaoka and Tanaka (1985) with a weighting function of $1/Y^2$.

$$\text{uptake}\,(\%) = 100 - \frac{100}{1 + \exp\left(\gamma\left(\text{IC}_{50} - \log[I]\right)\right)} \qquad (1)$$

where γ is the slope constant of the inhibition curves, IC_{50} is the concentration that gives a half-maximum inhibitory effect, and $[I]$ is the concentration of urate uptake inhibitors.

$$E = \frac{E_{max}}{1 + \exp\{\gamma(\ln\text{EC}_{50} - \ln[C]\}} \qquad (2)$$

where E_{max} is the maximum effect of the drug, γ is the slope constant of the effect curve, EC_{50} is the concentration that gives a half-maximum effect, and $[C]$ is the concentration of drug.

K_i (inhibitor constant) was analyzed by the "Simultaneous Nonlinear Regression" method described by Kakkar et al. (1999). The average observed rate vs. substrate concentration data were fitted simultaneously according to the full nonlinear expression for competitive inhibition as expressed in Eq. (3).

$$v = \frac{V_{max}[S]}{K_m\left(1 + \frac{[I]}{K_i}\right) + [S]} \qquad (3)$$

where v is the rate of metabolite formation, V_{max} is the maximal rate of uptake, K_m is Michaelis constant, K_i is the inhibitor constant, $[S]$ is the substrate concentration, and $[I]$ is the inhibitor concentration.

Values are expressed as means ± S.D.

3. Results

3.1. Effect of E3040 and uricosuric agents on OH^- gradient-dependent urate uptake into brush border membrane vesicles

Fig. 1, panel A shows the effect of various uricosuric agents and E3040 on OH^- gradient-dependent urate uptake into brush border membrane vesicles. All agents inhibited the OH^- gradient-dependent uptake of urate in a concentration-dependent manner. The IC_{50} value for AA193, benzbromarone, E3040 and probenecid was 0.3 ± 1.8, 19.4 ± 5.2, 89.6 ± 11.2 and 170.2 ± 34.2 μM, respectively. The slope constant of inhibition curves for AA193, benzbromarone, E3040 and probenecid was 0.5 ± 0.8, 1.0 ± 0.7, 1.0 ± 0.6 and 1.7 ± 1.0 μM, respectively.

3.2. Effect of E3040 and its conjugates on OH^- gradient-dependent urate uptake into brush border membrane vesicles

E3040 and its two conjugates showed a concentration-dependent inhibition of urate uptake (Fig. 1, panel B). The smallest IC_{50} value was found for E3040, followed by E3040 sulfate and glucuronide. The IC_{50} for E3040, E3040 sulfate and glucuronide was 89.6 ± 11.2, 1948.9 ± 226.1 and 10 265.7 ± 2555.4 μM, respectively. The slope constant of inhibition curves for E3040, E3040 sulfate and glucuronide was 1.0 ± 0.6, 0.6 ± 0.6 and 0.6 ± 0.9 μM, respectively.

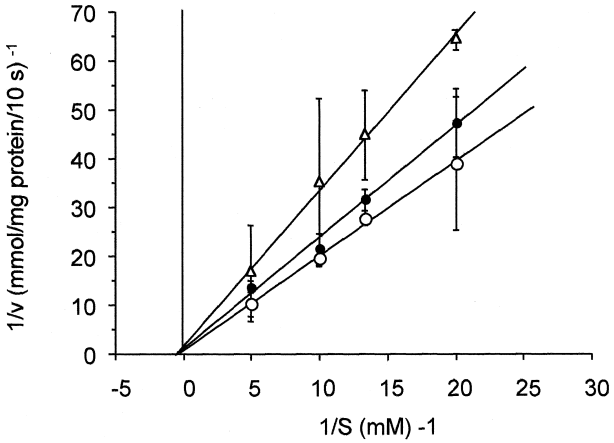

Fig. 2. Kinetic analysis of inhibition of the brush border urate–anion exchanger by E3040. The uptake assay was carried out in incubation medium containing different concentrations of E3040 and unlabeled urate. The data represent the means ± S.D. for three to four membrane preparations.

3.3. Mechanism of inhibition of E3040 OH^- gradient-dependent urate uptake into brush border membrane vesicles

Fig. 2 shows a Lineweaver–Burk plot of urate uptake into brush border membrane vesicles. E3040 increased the apparent K_m for urate uptake without influencing the apparent V_{max}. The apparent K_i (kinetic constant) was

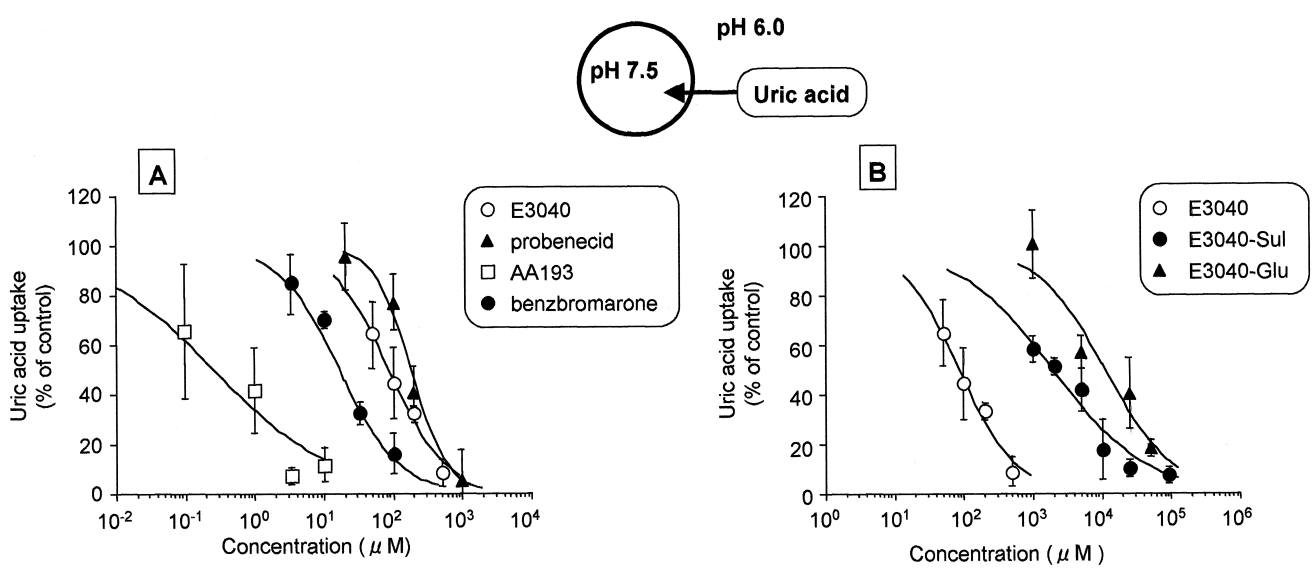

Fig. 1. Effect of uricosuric agents and E3040 (panel A) and E3040 and its conjugates (panel B) on OH^- gradient-dependent urate uptake into brush border membrane vesicles. The 10-s uptake assay was carried out in incubation medium containing different concentrations of uricosuric agents and E3040 (E3040, probenecid, AA193 and benzbromarone in panel (A); E3040, E3040 sulfate and E3040 glucuronide in panel (B)). The data represent the means ± S.D. of the percentage of the control uptake for three to four membrane preparations.

estimated by simultaneous nonlinear regression fitting to be 2.0 ± 1.9 μM.

4. Discussion

The order of potency of these agents was consistent with their uricosuric effect in DBA/2A mice (Yamada et al., 1999b). Clinically important uricosuric agents, such as probenecid, benzbromarone and AA193, have been reported to inhibit the urate–anion exchanger of brush border membranes in the proximal tubules of rats (Dan and Koga, 1990; Roch-Ramel et al., 1994; Dan et al., 1991). Therefore, our results suggest that E3040 has a uricosuric effect in the proximal tubules, since E3040 inhibited OH^- gradient-dependent urate uptake into brush border membrane vesicles, as did the other uricosuric agents.

The IC_{50} for E3040 (89.6 ± 0.7 μM) was about $20\times$ smaller than that for E3040 sulfate (1948.9 ± 1.2 μM) and $110\times$ smaller than that for E3040 glucuronide ($10\,265.7 \pm 1.4$ μM). These findings agree with our previous in vivo studies in rats and DBA/2N mice (Yamada et al., 1999a,b).

Based on the results for the Lineweaver–Burk plot of urate uptake into brush border membrane vesicles, E3040 may be a competitive inhibitor of OH^- gradient-dependent urate uptake into brush border membrane vesicles.

In conclusion, the present studies show that E3040 inhibits the urate–anion exchanger in the brush border membranes of the rat renal proximal tubules. Moreover, E3040 is a much more potent inhibitor of urate uptake into brush border membrane vesicles than its two conjugates. These findings support the previous results that the uricosuric effect seen after intravenous administration of E3040 may be mainly due to E3040 (Yamada et al., 1999a,b).

5. Uncited reference

Kessler et al., 1978

References

Blomstedt, J.W., Aronson, P.S., 1980. pH Gradient-stimulated transport of urate and *p*-aminohippurate in dog renal microvillus membrane vesicles. J. Clin. Invest. 65, 931–934.

Bessey, O.A., Lowry, O.H., Brock, M.J., 1964. A method for the rapid determination of alkaline phosphatase with fine cubic millimeters of serum. J. Biol. Chem. 164, 321–329.

Dan, T., Koga, H., 1990. Uricosurics inhibit urate transport in rat renal brush border membrane vesicles. Eur. J. Pharmacol. 187, 303–312.

Dan, T., Onuma, E., Tanaka, H., Koga, H., 1991. A selective uricosuric action of AA-193 in rats: comparison with its effect on PAH secretion in vivo and in vitro. Arch. Pharmacol. 343, 532–537.

Diamond, H.S., 1978. Uricosuric drugs. Uric Acid. In: Kelley, W.N., Weiner, I.M. (Eds.), Handb. Exp. Pharmacol. vol. 51 Springer, New York, pp. 459–484.

Frankfurt, S.J., Weinman, E.J., 1977. The effect of probenecid on urate transport in the rat kidney. Proc. Soc. Exp. Biol. 155, 554–557.

Guggino, S.E., Martin, G.J., Aronson, P.S., 1983. Specificity and modes of the anion exchanger in dog renal microvillus membranes. Am. J. Physiol. 244, F612–F621.

Itoh, T., Koyano, S., Nakamura, H., Tsuda, Y., Yamada, H., 1998. Lack of renal secretion of carbenicillin in rats: poor affinity to the organic anion transporter at renal brush border membrane. Biopharm. Drug Dispos. 19, 251–258.

Jorgensen, P.L., 1974. Isolation of $(Na^+ + K^+)$-ATPase. Methods Enzymol. 32, 277–290.

Kahn, A.M., Aronson, P.S., 1983. Urate transport via anion exchange in dog renal microvillus membranes. Am. J. Physiol. 244, F56–F63.

Kahn, A.M., Weinman, E.J., 1985. Urate transport in the proximal tubule: in vivo and vesicle studies. Am. J. Physiol. 249, F789–F798.

Kahn, A.M., Branhamm, S., Weinman, E.J., 1983. Mechanism of urate and *p*-aminohippurate transport in rat renal microvillus membrane vesicles. Am. J. Physiol. 245, F151–F158.

Kakkar, T., Boxenbaum, H., Mayersohn, M., 1999. Estimation of K_i in a competitive enzyme-inhibition model: comparisons among three methods of data analysis. Drug Metab. Dispos. 27, 756–762.

Kessler, M., Acuto, O., Storelli, C., Murer, M., Semenza, G., 1978. A modified procedure for the rapid preparation of efficiently transporting vesicles from small intestinal brush border membranes. Biochem. Biophys. Acta 506, 136–154.

Kramp, R.A., Lenoir, R., 1975. Distal permeability to urate and effects of benzofuran derivatives in the rat kidney. Am. J. Physiol. 228, 875–883.

Quigley, J.P., Gotterer, G.S., 1969. Distribution of $(Na^+–K^+)$-stimulated ATPase activity in rat intestinal mucosa. Biochem. Biophys. Acta 173, 456–468.

Roch-Ramel, F., Werner, D., Guisan, B., 1994. Urate transport in brush-border membrane of human kidney. Am. J. Physiol. 266, F797–F805.

Yamada, H., Kotaki, H., Furitsu, H., Sawada, Y., Iga, T., 1999a. Mechanism of the uricosuric action of the anti-inflammatory drug, E3040 used to treat inflammatory bowel disease: I. Study using a rat model of hyperuricemia. Biopharm. Drug Dispos. 20, 77–83.

Yamada, H., Kotaki, H., Furitsu, H., Sawada, Y., Iga, T., 1999b. Mechanism of the uricosuric action of E3040, a drug used to treat inflammatory bowel disease: Part II. Study using DBA/2N mice. Biopharm. Drug Dispos. 20, 271–276.

Yamaoka, K., Tanaka, H., 1985. A nonlinear multiple regression program, MULTI2 (BAYES), based on Bayesian algorithm for microconputers. J. Pharm. Dyn. 8, 246–256.

Weiman, E.J., Knight, R., McKenzie, R., Ekinoyan, G., 1976. Dissociation of urate from sodium transport in the rat proximal tubule. Kidney Int. 10, 295–300.