[Chem. Pharm. Bull. 16(1) 13—16 (1968)]

UDC 615.778-033 : 547.82 : 612-084

Studies on Gastrointestinal Absorption of Nalidixic Acid[1)]

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(Received December 17, 1966)

1) *In situ* and *in vitro* absorption experiments of nalidixic acid from the gastrointestinal tracts of rats were studied.

2) Results obtained from both methods were in favor of pH–partition hypothesis. Namely, absorption rate of unionized form of nalidixic acid was faster than that of ionized form of nalidixic acid.

3) Absorption rate of nalidixic acid *in situ* was faster than that of its drug *in vitro* by ten times. This might be due to the volume of solution, the length of the intestine and other physiological conditions of rats.

Drug absorption from the gastrointestinal tract has been discussed by many investigators. A better understanding of drug absorption has become important for establishing the proper methods of drug administration.

Nalidixic acid, which is 1–ethyl–4–oxo–7–methyl–1,4–dihydro–1,8–naphthyridine–3–carboxylic acid (Fig. 1), was found to be effective against a variety of gram–negative bacteria *in vitro*, including *Escherichia coli*, *Salmonella typhimurium* and *Neisseria gonorrheae*,[3-5)] and then this has been demonstrated its remarkable clinical ability in a vast range. The published work on the absorption and excretion of nalidixic acid has been directed towards the estimation of blood level and urinary excretion,[6-8)] and little attention has been paid to the absorption rate studies of nalidixic acid from the gastrointestinal tract at various pH value.

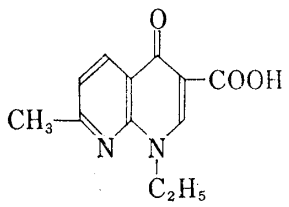

Fig. 1. Structure of Nalidixic Acid (NA)

In this report both *in situ* and *in vitro* absorption experiments of nalidixic acid from the gastrointestinal tracts of rats were carried out. Results obtained from both methods were compared. Nalidixic acid was absorbed very rapidly from the gastrointestinal tracts. Effect of the gastrointestinal pH on the absorption of nalidixic acid is discussed. Relationship between the absorption rate and the apparent partition coefficients of nalidixic acid is also discussed.

Experimental

Materials——Nalidixic acid, mp 227—230°. All other chemicals used were of reagent grade.

1) A part of this paper was presented at the 86th Annual Meeting of the Pharmaceutical Society of Japan, Toyama, 1966.
2) Location: *Narihira-5-chome, Sumida-ku, Tokyo.*
3) G.Y. Lesher, E.J. Froelich, M.D. Gruett, and J.H. Bailey, *J. Med. Pharm. Chem.*, **5**, 1063 (1962).
4) E.W. McChesney, E.J. Froelich, G.Y. Lesher, A.V.R. Crain, and D. Rosi, *Toxicol. and Appl. Pharmacol.*, **6**, 292 (1964).
5) K. Shimizu, T. Harada, M. Hatakeyama, O. Kumii, T. Zindachi, E. Yamada, and K. Shimada, *Chemotherapy*, **12**, 384 (1964).
6) W.E. Moore, G.A. Portmann, H. Stander, and E.W. McChesney, *J. Pharm. Sci.*, **54**, 36 (1965).
7) G.A. Portmann, E.W. McChesney, H. Stander, and W.E. Moore, *J. Pharm. Sci.*, **55**, 59 (1966).
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Equipments——A Hitachi-Perkin-Elmer spectrophotometer, model 139, Toa Denpa pH meter, model HM-5A, circulating perfusion pump (Ishii Shoten, Tokyo) and circulation apparatus described by Wiseman and Smith with minor modification[9] were employed.

Absorption of Nalidixic Acid from the Stomach and Small Intestine *In Situ*

1) Procedure——Male Wistar rats weighing 200 to 230 g were fasted for a whole night prior to the experiments. Water was given freely and coprophagy was prevented by using cages with wide-mesh floors. And the method of Schanker, *et al.*[10-14] was followed. Buffer systems used for drug solution were as follows;

0.1 N HCl–NaCl (isotonic) for pH 1,
citric acid–Na_2HPO_4 (isotonic) for pH 3 and 5,
KH_2PO_4–Na_2HPO_4 (isotonic) for pH 7 and 9.

Initial concentration of nalidixic acid was 20γ/ml for pH 1,3,5,7 & 9 and 200γ/ml for pH 7 and 9. A 30 ml of drug solution was used for the circulation experiments of intestine. Phenol red, which was expected to be unabsorbed, was dissolved in the drug solution to indicate any volume change. And the absorption rate constants were calculated by following equation;

$$K=2.303\times\frac{1}{t}\log\left(\frac{C_o\ \text{drug}}{C_t\ \text{drug}}\times\frac{C_t\ \text{Phenol red}}{C_o\ \text{Phenol red}}\right) \quad (1)$$

where C_o is the initial concentration and C_t is the concentration at time (t). Equation (1) was driven by setting the absorption rate from the gastrointestinal tract to be first order, which was confirmed experimentally. At the end of the experiments, the entire stomach or intestine was excised and washed with 0.9% NaCl solution. After washing, tissue was homogenized. The homogenate was then assayed for naphthyridine.

2) Analytical Method——When the concentrated sample was used at pH 7 and 9, one ml of the sample was taken and diluted to an appropriate volume with distillated water in a volumetric flask. Concentration of nalidixic acid was determined by adding 1 ml of 0.5M acetate buffer, pH 5.0, to 1 ml of the sample or the dilution in a syringe. After 5 ml of chloroform was added to the solution, the mixture was shaken vigorously for a few minutes and then centrifuged. The chloroform phase was separated and optical density for free naphthyridine was determined at 334 mμ.

Absorption of Nalidixic Acid from the Small Intestine of Rats *In Vitro*

1) Procedure——The experimental procedure was almost the same as described by Nogami, *et al.*[9] Each half hour the aliquots of 2 ml were taken out and analyzed for 2 hours.

Inner solution (mucosal side)——Drug solutions used were the same as *in situ* experiments.

Outer solution (serosal side)——A 80 ml of 0.9% NaCl solution was used. Nalidixic acid absorption was assumed to be penetrated passively and it was also assumed that the volume change was negligible. And the absorption rate constants were calculated by following equation:

$$k'=-2.303\frac{1}{t}\log\left(\frac{C_m}{C_o}\right) \quad (2)$$

Since $C_m=C_o-C_s$, so equation (2) becomes,

$$K=-2.303\frac{1}{t}\log\left(1-\frac{C_s}{C_o}\right) \quad (3)$$

C_m :Nalidixic acid concentration in mucosal side
C_s :Nalidixic acid concentration in serosal side
C_o :Initial concentration of nalidixic acid

2) Analytical Method——Concentration of nalidixic acid was determined by adding 1 ml of 0.5M acetate buffer, pH 5.0, to 2 ml of the sample or the dilution in a syringe. After 10 ml of chloroform was added to the solution, the same procedure described before was carried out.

Determination of Apparent Partition Coefficients——Twenty ml of drug solution at various pH was shaken with 20 ml of organic solvent at 28°,[15] and drug content was determined in water layer after equilibrium was reached. And apparent partition coefficients were calculated by the following equation:

9) H. Nogami and T. Matsuzawa, *Chem. Pharm. Bull.* (Tokyo), **9**, 532 (1961).
10) L.S.Schanker, P.A. Shore, B.B.Brodie, and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **120**, 528 (1957).
11) L.S. Schanker, D.J. Tocco, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **123**, 81 (1958).
12) K. Kakemi, T. Arita, and S. Ohashi, *Yakugaku Zasshi*, **82**, 348 (1962).
13) K. Kakemi, T. Arita, H. Sezaki, and T. Nadai, *Yakugaku Zasshi*, **83**, 871 (1963).
14) K. Kakemi, T. Arita, H. Sezaki, and N. Takasugi, *Chem. Pharm. Bull.* (Tokyo), **13**, 551 (1965).
15) M. Samejima, I. Sugimoto, and I. Utsumi, The 86th Annual Meeting of the Pharmaceutical Society of Japan, Toyama, Apr., 1966, Abstract p. 196.

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$$P=a\frac{\text{Initial Concentration of Water Layer–Equilibrium Concentration of Water Layer}}{\text{Equilibrium Concentration of Water Layer}}$$

Britton–Robinson buffer systems were used for this experiment. Drug concentration of water layer was determined by measuring the optical density at 335 mμ after addition of N NaOH. Organic solvents used were isoamylacetate and benzene.

Determination of Apparent pK_a——The UV method of Yoshioka, *et al.*[16] was carried out.

Results and Discussion

Apparent partition coefficients of nalidixic acid at variuos pH is shown in Fig. 2. The apparent pK_a of nalidixic acid was 5.9 at 28°. And the pH–profile of absorption rate from rat stomach *in situ* is shown in Fig. 3. Result was obtained in favor of pH–partition hypothesis.

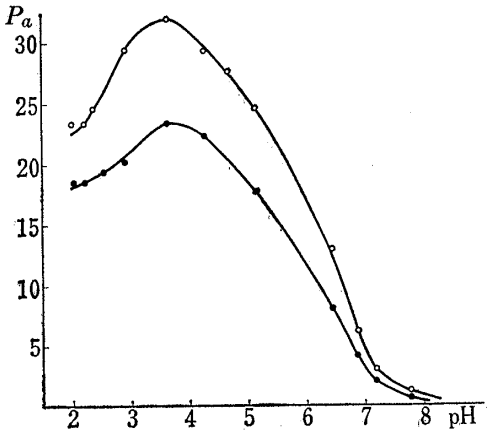

Fig. 2. Relationship between Apparent Partition Coefficient(Pa) and pH

$$Pa=\frac{\text{Concentration in organic solvent}}{\text{Concentration in water}}$$

—●— Isoamylacetate
—○— Benzene

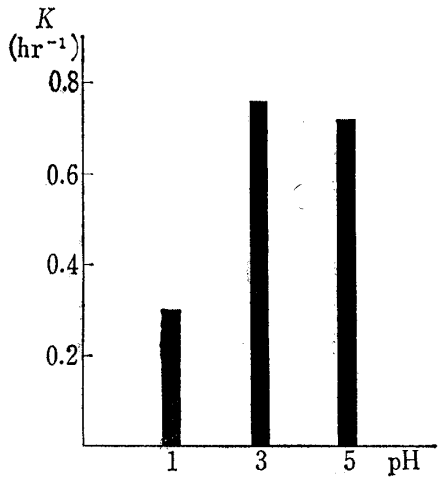

Fig. 3. Absorption of NA from Rat Stomach

Animals No.: 3
Initial Concentration of NA: 2 mg %

Namely, nalidixic acid was absorbed more slowly at the pH shown low partition coefficients, where the drug is ionized to a considerable extent. The slow absorption of nalidixic acid at pH 1 might be due to the formation of the protonated form of nalidixic acid at this pH value.

Absorption of nalidixic acid from stomach could not be negligible for oral administration in spite of low solubility of nalidixic acid at the pH of stomach.

The pH–profile of absorption rate from rat small intestine *in situ* is given in Fig. 4. The absorption rate was found to decrease with increasing pH. Absorption rate of unionized form of nalidixic acid was faster than that of ionized form of nalidixic acid. But ionized form of nalidixic acid was also absorbed with a considerable speed. There is a rough relationship between the degree of absorption rate of nalidixic acid and its apparent partition coefficients.

The pH–profile of absorption rate from rat small intestine *in vitro* is shown in Fig. 5. The pH–effect of absorption rate *in vitro* resembled to that of *in situ* experiments. The absorption rate of nalidixic acid *in situ* was faster than that of nalidixic acid *in vitro* by ten times.

This might be due to the volume of drug solution, the length of the intestine and other physiological conditions of rats. Accumulation of nalidixic acid at the intestinal tissue was negligibly small during *in situ* experiments. The total amount of the drug found at serosal

16) M. Yoshioka, K. Hamamoto, and T. Kubota, *Bull. Chem. Soc. Japan*, **35**, 1723 (1962).

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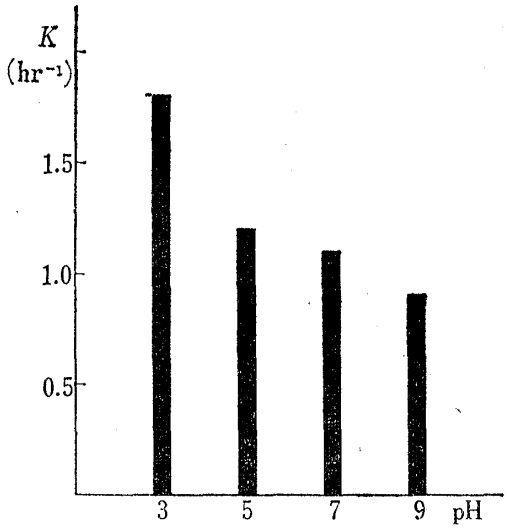

Fig. 4. Absorption of NA from Rat Small Intestine (*in situ*)

Animals No.: 3
Initial Concentration of NA : 2 mg %

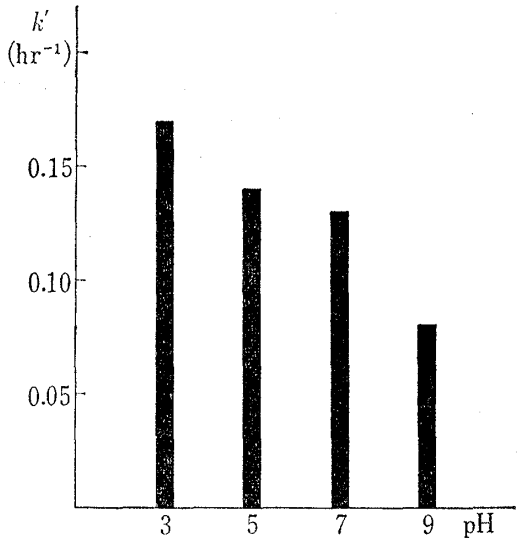

Fig. 5. Absorption of NA from Rat Small Intestine (*in vitro*)

Animals No.: 3
Initial Concentration of NA: 2 mg %

side and mucosal side was nearly equal to the amount used. So accumulation of nalidixic acid at the intestinal tissue was negligible at *in vitro* experiments.

Effect of initial concentration of nalidixic acid on the absorption rate is given in Table I. No effect of initial concentration on absorption rate was observed at these experimental conditions.

Table I. Absorption of NA from Rat Small Intestine

pH	Initial Concentration mg %	*K* (*in situ*) (hr^{-1})	*k'* (*in vitro*) (hr^{-1})	*K* (*in vitro*) (hr^{-1})
3	2	1.8	0.17	0.18
5	2	1.2	0.14	0.14
7	2	1.1	0.13	0.11
7	20	1.1	0.12	0.13
9	2	0.9	0.08	0.09
9	20	1.0	0.07	0.07

K (*in situ*): from Eq. 1 *k'* (*in vitro*): from Eq. 2 *K* (*in vitro*): from Eq. 3

Stability of nalidixic acid in gastrointestinal tract were examined by using the sample circulated 2 hours at pH 7. One spot was found in this sample by paperchromatography.[4] And no increase of optical density at 334 mμ was found at the analysis after acid hydrolysis of the sample.[4] Accordingly, formation of conjugated drug was negligible. And so nalidixic acid seemed to be stable at these experimental conditions.

Further studies are indicated in the absorption, distribution, metabolism and excretion of nalidixic acid after the administration of various preparations.

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