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J. Pharm. Pharmacol. 1982, 34: 817–819
Communicated March 30, 1982

0022–3573/82/120817–03 $02.50/0

Evidence for a saturable component in isoniazid transfer across rat small intestine in vitro

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It is well known that isoniazid is a drug that is rapidly absorbed after oral administration (Genazzani et al 1966; Gelber et al 1969). As a weak base it is probably absorbed mainly in the small intestine (Schanker 1961). Studying isoniazid transport across rat small intestine in vitro, Barley et al (1972) concluded that the drug was transported by passive diffusion. However, its good water solubility and the almost total dissociation of its hydrazine moiety (pK_a = 10·8) at physiological pH cannot be regarded as favourable for penetration through lipid barriers like intestinal epithelium by passive diffusion. Since Barley et al (1972) based their conclusion on experiments with a limited number of relatively high isoniazid concentrations, we have re-examined the problem of intestinal transfer of isoniazid using a wider range of concentrations (0·5–50 mM) and a modified perfusion technique of rat small intestine in vitro.

An everted isolated segment of the rat proximal ileum, approximately 10 cm long was suspended in 70 ml phosphate buffer ($KH_2PO_4/Na_2HPO_4.12H_2$, pH = 6·0) representing the mucosal compartment into which isoniazid was introduced at the beginning of the experiment. Continuous bubbling by air ensured mixing of the entire volume of this compartment. The serosal side of the segment was continuously washed by Ringer solution from a reservoir (volume 500 ml) by means of a peristaltic pump (Desaga Resomat 14700, Dibbern, FRG) at a rate of 3·5 ml min^{-1}. The solution in the reservoir was continuously mixed by means of a magnetic mixer assuring a constant concentration in all of the system. The temperature of both solutions was maintained at 37 °C thermostatically.

The concentrations of isoniazid in the serosal compartment were continuously recorded for 10 min after its introduction into the mucosal compartment, by means of a u.v. monitor (Chiratic, Chirana, Czechoslovakia) at 266 nm the wavelength of absorption maximum. The rate of isoniazid transfer across the intestinal barrier was expressed as the increase in amount of drug in the serosal compartment per unit time (μg min^{-1}). The experimental design is depicted in Fig. 1.

The following models were considered as possible alternatives to describe the dependence of transport rates of isoniazid on its mucosal concentrations:

(a) A simple first order process (Model I) representing the usual assumption made on the drug's absorption from the gastrointestinal tract (Ellard et al 1972):

$$v = p.c_m$$

where v is the initial transport rate measured in μg min^{-1}, p a 'permeability' constant having the dimension 'volume/unit of time' and c_m the initial drug concentration in the mucosal compartment.

(b) A capacity-limited process of the Michaelis-Menten type (Model II):

$$v = \frac{c_m}{K + c_m} \cdot V_{max}$$

where v and c_m are defined as above, V_{max} is the maximal transport rate and K represents that drug concentration in the mucosal compartment corresponding to the transport rate which is half of the maximal ($V_{max}/2$).

(c) A combination of both—the capacity-limited and first order processes—described by the relationship (Model III):

$$v = \frac{c_m}{K + c_m} \cdot V_{max} + p.c_m$$

Fitting of the experimental data to model equations was performed using unweighted non-linear regression analysis based on the Gauss-Newton computing algorithm. Asymptotic standard deviations (s.d.) of the

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model parameters were calculated according to the formula:

$$s.d._i = Sa_{ii}$$

where $s.d._i$ is the asymptotic standard deviation of the i-th parameter, S the sum of squared deviations of the observed and calculated values divided by the corresponding degrees of freedom (number of observations —number of model parameters) and a_{ii}— the i-th diagonal element of the inverse to the 'variance-covariance' matrix resulting from the Gauss-Newton computing algorithm.

The goodness of fit of the experimental data to the assumed model equations was evaluated by means of the coefficient of determination R^2 indicating the fraction of the total variance of observed values (Y_{obs}) which can be explained by the theoretical relationship (Y_{calc}) represented by the assumed model

$$R^2 = 1 - \frac{\Sigma(Y_{obs} - Y_{calc})^2}{\Sigma(Y_{obs} - y)^2}$$

where:

$$\bar{y} = \frac{\Sigma Y_{obs}}{n}$$

To test whether or not the sums of squared deviations of Model III have been sufficiently reduced to justify fitting with additional parameters, the F ratio test (Boxenbaum et al 1974) has been used in the form:

$$F = \frac{SS_{II} - SS_{III}}{SS_{III}} \cdot \frac{df_{III}}{df_{II} - df_{III}}$$

where SS_{II} and SS_{III} as well as df_{II} and df_{III} are sums of squared deviations and the number of degrees of freedom for Model II and III, respectively. The calculated F has been compared with the tabular value for the corresponding numbers of degrees of freedom at the 5% and 1% levels of probability.

The isoniazid transport rates estimated from 10 min recordings after introduction of varying concentrations of the drug into the mucosal compartment are given in Table 1. It is evident that under given experimental conditions the rate of isoniazid transport varies with the initial concentration in the mucosal compartment.

Table 1. Rates of isoniazid transport across the everted isolated segment of the rat small intestine in vitro.

Concn of drug in mucosal compartment		Drug transport rate*
mM	μg ml^{-1}	μg min^{-1}
0·52	71·43	14·75*
1·04	142·86	67·20
2·08	285·72	101·65
4·16	571·44	132·80
6·25	857·14	196·75
12·50	1714·28	239·35
25·00	3428·56	344·30
50·00	6857·12	533·34

* The transport rates are means of 2–3 separate experiments.

Table 2. Estimated parameters for models of isoniazid transport across the rat small intestine in vitro.

Parameter	Dimension	Model II Estimate (s.d.)	Model III Estimate (s.d.)
V_{max}	μg min^{-1}	656·80 (76·96)	212·32 (33·84)
K	μg ml^{-1}	2451·64 (650·70)	440·77 (151·90)
p	μl min^{-1}	—	45·64 (5·20)

Plotting of the estimated transport rates against concentration revealed that the relationship between these variables is clearly biphasic: when increasing initial isoniazid concentration the increase in drug transport rate is obviously greater at lower (0·5–2·1 mM) than at higher concentrations (6·25–50 mM) (Fig. 2).

The curvilinear character of this relationship led to the rejection of the assumption that isoniazid transport across the rat small intestinal wall could be represented exclusively by a simple first order process (Model I) in the whole range of drug concentrations used by us.

On the other hand, when we analysed the data according to the simple Michaelis-Menten equation (Model II) a relatively good fit of the theoretical relationship to experimental data was obtained (coefficient of determination 0·9669, 0·9948 and sum of squares of deviations 6172·9, 967·6 for Models II and III respectively, see also Fig. 2 dotted line). Nevertheless, the goodness of fit was further improved when a linear term (Fig. 2 solid line) characterizing a first order process was included in the model (Model III): the value of the coefficient of determination indicated that only 0·52% of data variation remained unexplained by the theoretical relationship. The reduction of sum of squares of deviations from the theoretical relationship appeared highly statistically significant when judged by the F-test ($F = 26·897$, $P < 0·01$). In spite of the fact that the value of the 'permeability' constant in Model III is low, it is evident from Table 2 that its presence led to a drastic reduction in parameter values of the capacity-limited component of Model III compared with Model II.

The results of our experiments presented unambiguous evidence for a capacity-limited component in

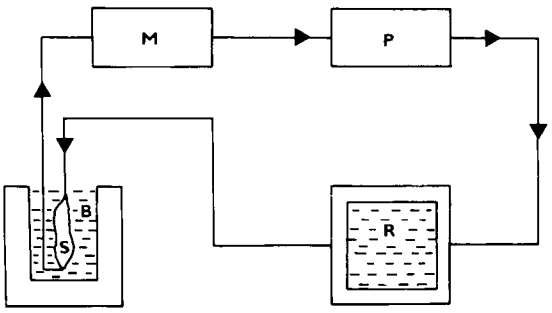

FIG. 1. The experimental design used to study the relationship between isoniazid concentration and its rate of transport across the rat small intestine in vitro. S—isolated segment of rat ileum, B—buffer solution, R—Ringer solution, M—monitor, P—pump.

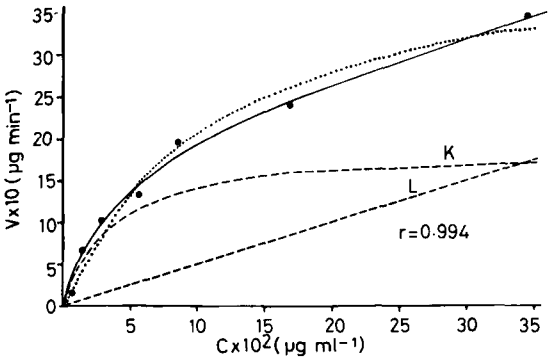

FIG. 2. The fitting of isoniazid transfer rates across small intestine: (. . . .) Michaelis-Mented equation (Model II) and (——) decomposition of the overall transport rate (Model III) to individual components: a capacity-limited (K) and first order process (L).

isoniazid transport across the rat small intestine. It seems that a 'carrier-mediated' mechanism similar to that postulated for various sugars and aminoacids could operate even in isoniazid transport. This is supported by the fact that the value of K when expressed in nM (3·2) is in the range of apparent transport K_m's presented by Caspary (1977).

However, the statistically highly significant superiority of Model III over Model II in describing the experimental data, suggests that passive diffusion, probably represented by the linear component, cannot be excluded from participation in isoniazid penetration through the intestinal barrier. The low transport capacity of this component is in good agreement with physicochemical properties of the drug. It may be also the reason why its contribution to the 'overall' transport of isoniazid becomes appreciable only at higher drug concentrations when the capacity-limited process is already saturated. In this manner the results of our analysis are in no way in contradiction to the findings of Barley et al (1972) who worked with higher concentrations of the drug and thus were unable to detect the saturable component of its transport.

A combination of a capacity-limited and linear components in intestinal transport has been already described for a series of water-soluble compounds of natural origin (Debnam & Levin 1975; Atkins & Gardner 1977). An indication of a saturable rate-limiting step in intestinal transport has also been noted in kinetic studies by Tsuji et al (1977, 1978) for ampicillin and some other rapidly absorbed aminopenicillins at lower concentrations. Although the nature of the capacity-limited component in isoniazid transport across the rat small intestine requires further investigation, the physiological significance of the combination of the two different transport mechanisms is obvious: at low concentrations the 'overall' transport rate is much accelerated by the contribution of the capacity-limited component compared with the situation where only the simple 'first order' process characterized by a low 'permeability' constant would operate.

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