Lundberg, J. M., Franco-Cereceda, A., Hua, X., Hokfelt, T., Fischer, J. A. (1985) Co-existence of Substance P and calcitonin gene-related peptide-like immunoreactivities in sensory nerves in relation to cardiovascular and bronchoconstrictor effects of capsaicin. Eur. J. Pharmacol. 108: 315–319

Palmer, J. B. D., Cuss, F. M. C., Mulderry, P. K., Ghatei, M. A., Springall, D. R., Cadieux, A., Bloom, S. R., Polak, J. M., Barnes, P. J. (1987) Calcitonin gene-related peptide is localised to human airway nerves and potently constricts human airway smooth muscle. Br. J. Pharmacol. 91: 95–101

Tippins, J. R., Morris, H. R., Panico, M., Etienne, T., Bevis, P., Girgis, S., MacIntyre, I., Azria M., Attinger, M. (1984) The myotropic and plasma-calcium modulating effects of calcitonin gene-related peptide (CGRP). Neuropeptides 4: 425–534

J. Pharm. Pharmacol. 1988, 40: 211–212
Communicated August 7, 1987

Kinetic mechanism for the intestinal absorption of ofloxacin

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Abstract—The absorptive behaviour of ofloxacin, a quinolone antibacterial agent, was studied following recirculation in small intestine of both male and female rats, at initial doses ranging from 0·125 to 5 mg mL^{-1}. A saturable Michaelis-Menten process is suggested to explain the intestinal absorption. No significant differences were found in the absorption parameters per metabolic weight unit.

Drug absorption through the small intestine is the result of passage across a cellular layer—the brush-border epithelium of intestinal villi. Overton's first rule assumes that the permeability of membranes to small molecules is directly related to their lipid solubility. According to Schanker et al (1958), the degree of ionization of a drug also conditions its absorption, since the oil/water partition coefficient is higher in the un-ionized than in the ionized state of a drug. If a drug is not liposoluble enough to pass through cell membranes by simple diffusion, carrier proteins may improve the absorption. This process fits Michaelis-Menten saturating kinetics and may not require metabolic energy.

Studies on drug absorption kinetics are often aimed at determining apparent absorption rate constants (k_{app}) for a series of initial concentrations of a drug as indicators for its disappearance from the intestinal lumen with time. The constants should include both true absorption and degradation rate constants for the overall process and can be used as starting point for the estimation of true kinetic parameters such as carrier affinity or maximum absorption rate.

The 4-quinolone antibiotic group has a wide antibacterial spectrum, both in-vitro and in experimental infective processes, which resembles the activity of β-lactam antibiotics and aminoglycosides (Neu & Labthavikul 1982). Although the mechanism of the quinolones' antibacterial actions is not yet fully understood, it may be possible that the effect lies in the inhibition of bacterial gyrase enzyme, there by inducing death of the microorganism (Smith 1983).

Previously Prieto et al (1987), observed no significant changes in the intestinal absorption of a second-generation quinolone, ofloxacin DL-8280, in male and female rats, as opposed to findings with other drugs (Foradada et al 1974). We have now attempted to ascertain from among the possible mechanisms of intestinal absorption whether free diffusion and saturating transport are involved in the uptake of ofloxacin.

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Materials and methods

Adult Wistar rats of either sex were used. Body weight was measured twice weekly and gave a sigmoid growth curve (Hammond 1932) similar to other species. Previous data on growth (Prieto et al 1987) showed no significant differences in body weight between male and female rats at weaning, but significant differences in the plateau region of the growth curve as well as the growth rate from the weaning to the adult state. The average values with coefficient of variation were:

Initial weight: males 41·79CV32·28, females 39·25CV35·73. (NS) and
final weight: males 331·52CV22·02, females 263·38CV18·46 ($P < 0·001$).

Perfusion technique. Animals were anaesthesized using urethane (1·5 kg^{-1}) 1 h before starting the experiments. A recirculating perfusion technique was used (Tsuji et al 1978;Ponz et al 1980). An intestinal length of segment of 20 cm taken from the pyloric sphincter was cannulated after ligation of the bile duct, washed with 50 mL of drug-free buffer solution, and perfused with drug solution at known initial concentration, both solutions being pre-heated to 37°C and maintained at this temperature. The perfusion rate was 2 mL min^{-1}. Successive samples were taken at 5 min intervals for 90 min during recirculation of the drug solution. The perfusing medium consisted of 0·05 M phosphate buffer pH 7·4 with ionic strength adjusted to 0·15 by sodium sulphate. In all experiments 50 mL of the different drug concentrations in this buffer solution were perfused.

High-performance liquid chromatography (HPLC) was used to analyse the ofloxacin content of the samples, from which the apparent absorption rate of the drug was assessed. Three males and three females were used in the corresponding perfusion experiments for each one of seven ofloxacin concentrations.

In the HPLC analysis, a low-polarity mobile phase (hydro-alcoholic mixture 90:2:8 (v/v of methanol,—0·05 M phosphate buffer pH 6·0—water) was used. The stationary phase consisted of a polar 5-CN-group column.

Results and discussion

Recirculation of different initial doses of ofloxacin (0·125 to 5·000 mg mL^{-1}) gave results corresponding to the ofloxacin concentration remaining unabsorbed with time. Linear regression of the natural logarithm of concentration versus time gave slope values of the apparent absorption rate constants (K_{app}, min^{-1}).

Table 1. Values obtained for K_{app} (min^{-1}) and K'_{app} ($min^{-1}\,g^{-1}$) from the initial ofloxacin concentrations (C, mg mL^{-1}) perfused. Student's *t*-values are shown for each pair of data, and significant differences (at 0·05 level) are represented by an asterisk.

C mg mL^{-1}	$K_{app} \times 10^3 \pm$ s.e. $\times 10^5$			$K'_{app} \times 10^5 \pm$ s.e. $\times 10^8$		
	Males	Females	*t*	Males	Females	*t*
0·125	0·582 ± 0·417	0·528 ± 0·379	9·585*	0·835 ± 3·283	0·835 ± 3·480	0·000
0·250	1·051 ± 1·698	0·952 ± 0·960	5·075*	1·604 ± 9·292	1·607 ± 12·252	0·195
0·500	2·366 ± 5·310	1·759 ± 1·286	11.11*	3·002 ± 0·577	3·025 ± 4·256	5·355*
1·000	4·165 ± 5·365	3·343 ± 15·605	4·981*	5·471 ± 14·075	5·424 ± 4·177	3·201
1·500	4·339 ± 4·057	4·000 ± 5·077	5·216*	6·936 ± 5·239	6·994 ± 19·402	2·886
2·000	7·723 ± 35·687	6·512 ± 3·957	3·373	8·301 ± 6·009	8·298 ± 6·667	0·334
5·000	10·874 ± 21·962	8·542 ± 8·957	9·832*	11·274 ± 221·685	10·919 ± 70·089	1.526

K_{app} values were studied in either males or females for each ofloxacin concentration perfused. For a given ofloxacin concentration, K_{app} values in the males were significantly different from those in the females. The results seemed to agree with previous findings (Foradada et al 1974), but further analysis referring K_{app} values to the weight of the animal used revealed a series of apparent absorption rate constants K'_{app} ($min^{-1}\,g^{-1}$), which were not significantly different for males and females. This points to the significance of weight changes as a result of the absorptive processes rather than to genetic components.

K_{app} and K'_{app} values for the corresponding initial concentrations of ofloxacin used are shown in Table 1. An analysis of such values reveals the direct relation existing among them. This was studied by supposing two different models to fit the data pairs K'_{app}-concentration (C). The corresponding equations as well as the linear regression parameters are as follows:

(a) Linear model (simple diffusion)

$$K'_{app} = K\,C + b \qquad (1)$$

slope: $2{\cdot}104 \times 10^{-5} \pm 3{\cdot}867 \times 10^{-6}$
intercept: $2{\cdot}258 \times 10^{-5} \pm 8{\cdot}346 \times 10^{-6}$
correlation coefficient: 0·925

(b) Linearized hyperbolic model (saturable transport)

$$\frac{1}{K'_{app}} = \frac{K_m}{V_{max}}\frac{1}{C} + \frac{1}{V_{max}} \qquad (2)$$

slope: $1{\cdot}432 \times 10^{4} \pm 8{\cdot}690 \times 10^{1}$
intercept: $4{\cdot}908 \times 10^{3} \pm 3{\cdot}042 \times 10^{2}$
correlation coefficient: 0·999

The above parameters were obtained by fitting data pairs from both male and female rats. The best correlation found for equation (2) allows us to suggest that a saturable Michaelis-Menten process is involved in the intestinal absorption of ofloxacin rather than a simple diffusion mechanism. The kinetic parameters found for this saturable process are shown in Table 2, and may be considered as general parameters for the absorption of ofloxacin by both males and females.

Table 2. K_m and V_{max} values as obtained from Michaelis-Menten plots of K_{app} or K'_{app} vs ofloxacin initial concentrations, together with their respective standard errors. The significance level at $P = 0{\cdot}005$ ($S_{0{\cdot}005}$) is shown as *(significant) and N.S. (not significant) for the comparison males-females of the kinetic parameters, according to the Chi-square test. Below are the final results for males and females altogether, considering K'_{app} values.

	K_{app}		K'_{app}	
	K_m	V_{max}	K_m	V_{max}
Males	3·804 ± 1·030	$1{\cdot}937 \times 10^{-2} \pm 3{\cdot}029 \times 10^{-3}$	2·864 ± 0·049	$2{\cdot}020 \times 10^{-4} \pm 2{\cdot}852 \times 10^{-6}$
Females	3·110 ± 0·742	$1{\cdot}417 \times 10^{-2} \pm 1{\cdot}842 \times 10^{-3}$	2·565 ± 0·180	$1{\cdot}901 \times 10^{-4} \pm 9{\cdot}076 \times 10^{-6}$
$S_{0{\cdot}005}$		*		N.S.
Males			2·879 ± 0·083	$2{\cdot}032 \times 10^{-4} \pm 4{\cdot}782 \times 10^{-6}$
Females				

References

Foradada, A., Riera, P., Martin, A., Plá-Delfina, J. M. (1974) Absorción intestinal *in situ* de medicamentos. Cien. & Ind. Farm. 6(10): 300–320

Hammond, J. (1932) Growth and Development. 1st ed. Oliver & Boyd Publ., Edinburgh, pp 32–66

Neu, H. C., Labthavikul, P. (1982) *In vitro* activity of norfloxacin, a quinolinecarboxylic acid compared with that of β-lactams, aminoglycosides, and trimethoprim. Antimicrob. Agents Chemother. 22: 23–27

Ponz, F., Ilundain, A., Lluch, M. (1980) Method for successive absorptions with intestinal perfusion *in vivo*. Rev. Esp. Fisiol. 35: 97–104

Prieto, J. G., Barrio, J. P., Gómez, G., Melcón, B. (1987) Prediction of the effect of ofloxacine on intestinal absorption: effect of the body weight and substrate concentration. Comp. Biochem. Physiol. C. in press

Schanker, L. S., Tocco, D. J., Brodie, B. B., Hogben, C. A. (1958) Absorption of drugs from the rat small intestine. J. Pharmacol. Exp. Ther. 123: 81–88

Smith, J. T. (1983) Chemistry and mode of action of 4-quinolone agents. Arbeitstagung der Sektion "Antimikrobielle Chemotherapie" der Paul-Ehrlich-Gesellschaft für Chemotherapie e. V. 1983. Munich.

Tsuji, A., Miyamoto, E., Kagami, I. (1978) GI absorption of β-lactam antibiotics I: kinetic assessment of competing absorption and degradation in GI tract. J. Pharm. Sci. 67(12): 1701–1704