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Prodrug approach of orotic acid using an absorption model

Walter Fürst [1], Reinhard Neubert [1], Thomas Jurkschat [1] and Lothar Lücke [2]

[1] *Sektion Pharmazie der Martin-Luther-Universität, Halle and* [2] *VEB Fahlberg-List, Magdeburg (G.D.R.)*

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Summary

A series of ester prodrugs of orotic acid were synthesized. Using in vitro methods, in particular, an absorption model system, the *n*-butyl ester of orotic acid was found to be the ester prodrug with optimal physicochemical properties. Pharmacokinetic studies on rabbits confirmed these results. The bioavailability of orotic acid after oral administration of the *n*-butyl ester prodrug was found to be 3.4-times higher as compared to the methylglucamine salt of orotic acid. A similar increase in bioavailability was predicted based on the in vitro half life for transport in the absorption model.

Introduction

The central position of orotic acid in the biosynthesis of pyrimidine derivatives has led to certain studies on its therapeutic use. A synthetic supply is necessary when more orotic acid is consumed than can be synthesized by the organism.

To date, orotic acid has been used to reduce serum lipids, in hepatotherapy and in the therapy and prophylaxis of pathological changes of the myocardium and in the field of geriatrics.

Orotic acid was reported to improve the learning process (Matthies, 1971). Furthermore, orotic acid was found to increase the transport of cations, e.g., magnesium, across the cell membrane. A review of orotic acid was published by Falk (1985).

On the other hand, following oral administration orotic acid is poorly absorbed (Walther et al., 1979) because of its low lipophilicity.

A rational way to improve the absorption of hydrophilic drugs would be to manipulate the physiochemical properties of the drugs by selecting drug derivatives with the potential for increasing the rate and extent of absorption (Bundgaard and Hansen, 1981; Sazaki et al., 1985; Johannsen et al., 1986; Koch and Sloan, 1987a,b; Mori et al., 1988).

Using a series of aliphatic esters of orotic acid, the present study was undertaken with the aim of elucidating the effect of variation of the ester alkyl chain length on both lipid and water solubility.

An absorption model described previously (Fürst and Neubert, 1981; Fürst et al., 1982) has been used to determine the alkyl ester of orotic acid with the optimal physicochemical properties. Using this in vitro apparatus it is possible to study the dissolution and lipid permeation in a single

Correspondence: W. Fürst, Sektion Pharmazie der MLU, Weinbergweg 15, DDR-4010 Halle/Saale, G.D.R.

model system. The bioavailability of optimal orotic acid alkyl ester has been examined in rabbits.

Materials and Methods

Orotic acid was obtained from VEB Fahlberg List. In all experiments orotic acid or its methylglucamine salt (**1**) were used. All other reagents were purchased from VEB Laborchemie Apolda.

UV spectra were recorded on a Specord UV/VIS (Zeiss, Jena). UV absorbance was measured on a VSU-2/P spectrophotometer (Zeiss, Jena).

Synthesis

Preparation of orotic acid alkyl esters with alkyl chain length < 4. These alkyl esters of orotic acid were prepared by adding 18.7 g of orotic acid to 100 ml alkanol which was saturated with HCl. The mixture was heated on a water bath. The unchanged residue of orotic acid was separated by filtration. When the filtrate was cooled alkyl esters precipitated. Substances were washed with methanol and dried. Crystalline solids: 83–94% yield.

Preparation of alkyl esters of orotic acid with alkyl chain length > 3. To 100 ml of the alkanol 18.7 g orotic acid and 5 ml concentrated sulfuric acid were added. The solution was boiled under reflux for 10 h using an oil bath. Then the solution was cooled and the orotic acid alkyl ester crystallised. The substances were washed with methanol and dried. Crystalline solids, 85–95% yield. Melting points are listed in Table 1.

Analytical assays

In vitro studies. In all in vitro studies the UV absorption of the compounds was monitored at 284 nm.

Pharmacokinetic studies. The concentration of orotic acid in plasma and urine was measured according to the method described by Stajner et al. (1968). Plasma samples were deproteinized with uranylacetate. Then the orotic acid was converted into 5,5-dibrombarbituric acid by bromination. Following debromination the resulting barbituric acid reacted with *p*-dimethylaminobenzaldehyde to form a coloured product which was extracted with *n*-butyl acetate. The absorbance of this coloured product was measured at 458 nm using a spectrophotometer (VSU-2/P, VEB Carl Zeiss, Jena).

Hydrolysis of the ester prodrug

The hydrolysis of the *n*-butyl ester of orotic acid (**6**) was studied in plasma at 37 °C. Samples were taken at several intervals. The ester was separated from plasma by ether extraction. The ester in the ether phase was hydrolysed and the orotic acid was determined according to the method described above.

Determination of water solubility

The solubility of orotic acid and its prodrugs was determined in water at 18 + 0.1 °C. Suspensions of the drug or prodrugs were stirred for 24 h. After filtration, filtrates were diluted with ethanol. The concentration of the derivatives was determined by measuring the UV absorption at 284 nm.

Determination of apparent partition coefficients

The *n*-octanol/water system was used in order to determine the partition coefficients of orotic acid and its alkyl esters. A volume of 5 ml of a solution of each compound (50 μg/ml) in distilled water and 5 ml *n*-octanol were added to a glass test tube. The tubes were equilibrated for 24 h on a shaker (Thys 2). Both the aqueous and *n*-octanol phases were assayed spectrophotometrically for substance content.

The apparent partition coefficients were calculated in the following way:

$$PC = \frac{[\text{prodrug}]_{n\text{-octanol}}}{[\text{prodrug}]_{\text{water}}} \quad (1)$$

where PC is the partition coefficient and [prodrug] the concentration of prodrugs in water and *n*-octanol, respectively.

Absorption model studies

Dissolution and lipid membrane transport of orotic acid and synthesized prodrugs were studied in a two-compartment absorption model where

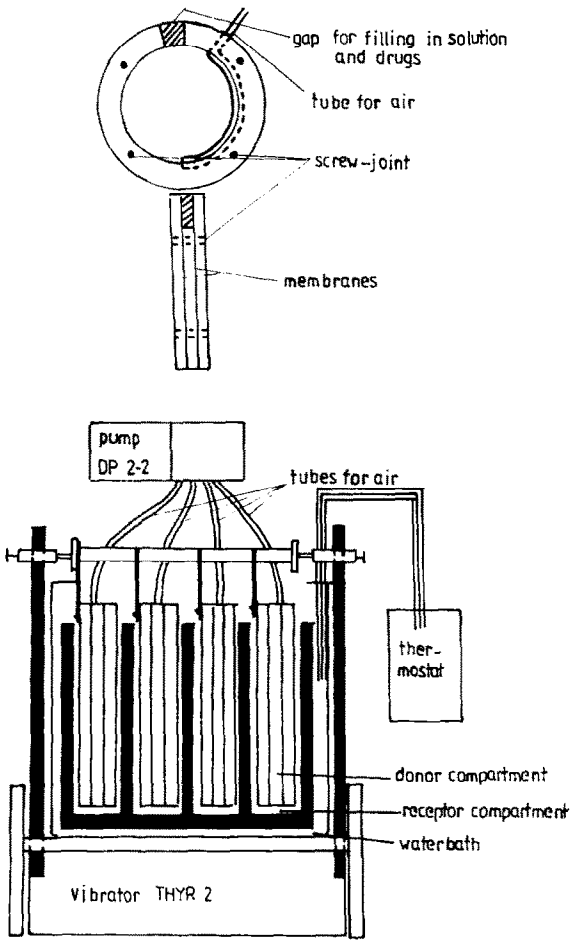

Fig. 1. (a) Donor compartment, (b) absorption model.

the donor compartment (DC = 50 ml) and receptor compartment (RC = 400 ml) were separated by a dodecanol collodion membrane (see Fig. 1). The technique for preparation of the membranes has been described previously (Fürst et al., 1982). The effective permeation area was 160 cm^2.

Orotic acid (50 mg) and an equivalent amount of prodrug, respectively, were added to the DC, then the donor HCl (pH 1.2) and receptor (borate buffer, pH 7.4) phase were placed in the compartments. Samples (2 ml) were removed from the RC periodically over 5 h. The UV absorption of the samples was monitored at 284 nm. Air was pumped into the DC in order to prevent deposition of the undissolved substance on the bottom of the DC. The phases of DC and RC were agitated by vibration using a vibrator (Thyr 2). The half-life of transport of the substances into the RC was calculated for evaluation of the experiments. For this purpose a simplified form of Fick's first law was derived:

$$K_t = \frac{1}{t} \ln \frac{C_{CE}}{C_{CE} - C_{RC}} \quad (2)$$

where K_t denotes the transport constant, t time, C_{RC} the concentration in RC and C_{CE} the concentration at equilibrium.

C_{CE} is defined as:

$$C_{CE} = \frac{M_O}{V_{DC} + V_{RC}} \quad (3)$$

where M_O is the dose of used drug (prodrug) and V_{DC}, V_{RC} are the volumes of DC and RC, respectively.

The half life of transport ($t_{1/2}$) is defined as:

$$t_{1/2} = \frac{\ln 2}{K_t} \quad (4)$$

Pharmacokinetic studies in rabbits

Healthy rabbits were used weighing 2.5–3.0 kg. The rabbits were fasted for 24 h before each study. On the day of study, the rabbits were placed in a special restraining box. Drug solution was injected into the ear vein. For oral studies, the amount of the derivatives equivalent to 60 mg/kg orotic acid was suspended in 3.0 ml water. The suspension was injected by gavage through a plastic feeding tube which had been inserted orally to reach the stomach. Blood (5 ml) was drawn before application for use in the calibration curves. The normal time course for sampling was 20, 40, 60, 120, 180 and 240 min. The samples were centrifuged at 3000 rpm for 5 min. The plasma was extracted for assay.

Calculation of pharmacokinetic parameters

The pharmacokinetic parameters were calculated according to the model-independent method of Weiss (1982).

Results and Discussion

The physicochemical properties of both orotic acid and prodrugs were evaluated in order to determine the derivative with optimal properties for in vivo use.

Water solubility

The water solubility of orotic acid used as the methylglucamine salt (**1**) and the prodrugs (**2–12**) is listed in Table 1. With the exception of prodrug **2** water solubility of the derivatives decreased from 14330 μg/ml for **1** to 14.5 μg/ml for **12** continuously with increasing length of the alkyl ester chain. However, water solubility of the derivatives with a branched ester chain was higher than those of the unbranched. The relation between water solubility and number of carbon atoms in the ester chain of the branched as well as of the unbranched compounds appeared to follow an exponential dependence (see Fig. 2). The compounds with a water solubility below a value of 100 μg/ml seemed to be less suitable for in vivo use.

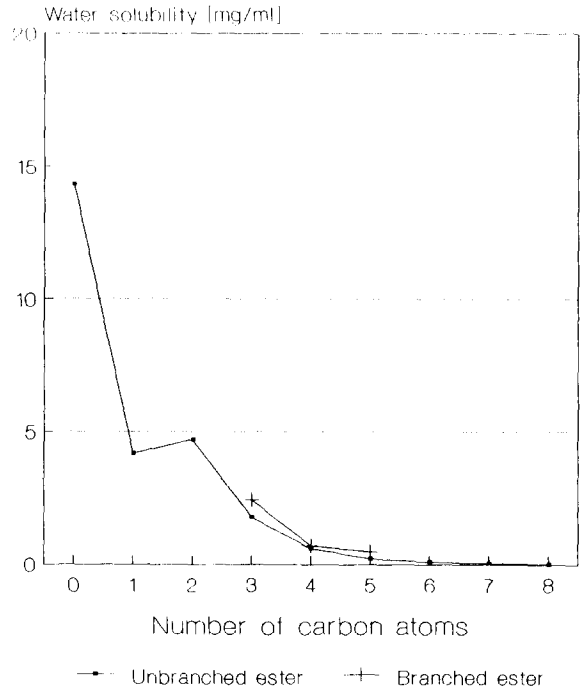

Fig. 2. Relationship between the water solubility and the number of carbon atoms in the ester chain.

Apparent partition coefficients

The *n*-octanol/water partition coefficients of the studied derivatives are listed in Table 1. In contrast to water solubility, the partition coefficient of the prodrugs increased with increasing ester chain length. The partition coefficients of the

TABLE 1

Properties of the synthesized ester prodrugs of orotic acid

Derivative	Melting point (°C)	Solubility in water (μg/ml)	Partition coefficient (*n*-octanol/water)	Half-life of transport in absorption model (h)
(1) R = H [a]		14330	0.02 [b]	21.0
(2) R = CH_3	240.5–242	4200	4.3	7.56
(3) R = C_2H_5	293–294	4700	4.8	3.28
(4) R = C_3H_7	234–236	1785	12.7	2.58
(5) R = $CH(CH_3)_2$	217–218	2425	6.5	1.68
(6) R = C_4H_9	185–186	600	21.4	1.45
(7) R = $CH_2CH(CH_3)_2$	221–222.5	720	10.2	2.37
(8) R = C_5H_{11}	180–181	228	41.3	2.00
(9) R = $(CH_2)_2CH(CH_3)_2$	182–184	480	30.1	2.03
(10) R = C_6H_{13}	171–172	98	57.5	4.44
(11) R = C_7H_{15}	168–169.5	45	73.3	19.4
(12) R = C_8H_{17}	161–163	14.5	78.0	–

[a] Used as methylglucamine salt.
[b] Orotic acid.

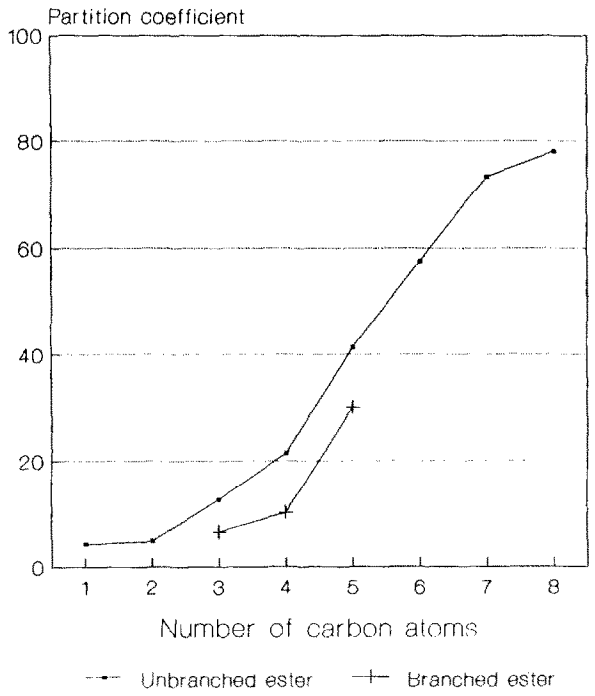

Fig. 3. The relationship between the partition coefficient and the number of carbon atoms in the ester chain.

unbranched derivatives were higher than those of the corresponding branched ones. For the branched alkyl ester prodrugs there was a sigmoidal relationship between the partition coefficient and number of carbon atoms of the ester chain (see Fig. 3). For the branched alkyl ester the same relationship seemed to exist.

Absorption model studies

In previous studies the absorption model was used to optimize oral drug formulations (Fürst et al., 1982). In the present study, this model system should be used for a prodrug approach. As there is a decrease in water solubility and an increase in partition coefficient of the prodrugs, the model system must allow the study of interaction between dissolution and partition processes. Therefore, 50 mg of solid were used in the donor compartment (DC) and the concentrations of the drug and prodrug, respectively, in the receptor compartment (RC) were determined after dissolution, partition into and transport through the membrane lipid. A large effective membrane area (160 cm^2) as well as RC (400 cm^3) were used in order to prevent transport through the membrane becoming rate limiting. Half-lives of transport ($t_{1/2}$) of the studied derivatives are shown in Table 1. A relationship was found between $t_{1/2}$ and the number of carbon atoms in the ester chain, exhibiting a minimum at four carbon atoms. However, there was no difference between $t_{1/2}$ of **5** and **6** (see Table 1).

Hydrolysis of the ester in plasma

The half-life of prodrug **6** in the plasma of rabbits at 37 °C was found to amount to 97 + 2 min. Furthermore, the relation between orotic acid and **6** was examined following i.v. administration of **6** (see Fig. 4).

After 30 min prodrug **6** could not be detected in plasma. Within this period the plasma levels of orotic acid were approx. 3-times higher than those of **6**.

Pharmacokinetic studies

The bioavailability of orotic acid was examined in rabbits following oral administration of the ester which was found in absorption models to be most suitable for in vivo use in comparison to the oral administration of **1**.

As shown in Fig. 5, plasma levels of orotic acid in rabbits were markedly higher after oral administration of prodrug **6** than after those of no. **1**.

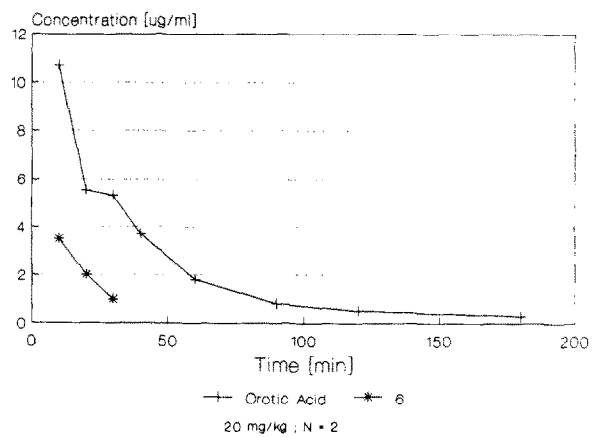

Fig. 4. Plasma levels of orotic acid and **6** after i.v. administration of **6**.

TABLE 2

Pharmacokinetic parameters of orotic acid following administration of prodrugs **1** *and* **6** *in rabbits*

Substance	Dose (mg/kg)	Administration route	MRT (±SD) (h)	AUC (μg h ml^{-1}) (±SD)	C_{max} (μg/ml) (±SD)	t_{max} (h)	B.A. [a] (%)	n
1	20.0	i.v.	0.81 ±0.03	11.2 ±0.95				12
1	135	oral	2.08 ±0.11	4.5 ±0.15	1.35 ±0.56	2.0	16.2	7
6	81.5	oral	1.60 ±0.04	15.4 ±1.0	8.30 ±1.4	0.33	53.5	7

[a] Absolute bioavailability.

The C_{max} of orotic acid was 6-times and the AUC 3.4-times higher after oral administration of prodrug **6** than those after administration of **1**. There was also a significant decrease in t_{max} when **6** was administered.

Table 3 shows the relative bioavailability of orotic acid determined in rabbits and also a corresponding value calculated from the in vitro data. The bioavailability of orotic acid following oral administration of **6** was used as standard and set to 100%. In order to predict bioavailability of a drug after administration of several formulations and prodrugs, respectively, from in vitro data such as $t_{1/2}$, a parameter must be calculated analogous to the bioavailability. Therefore, it is necessary to take into consideration the relation between $t_{1/2}$ and model absorption time. Using the following equations the amount M_{RC} transported in the absorption model can be calculated:

$$M_{DC} = M_O e^{-T0.693/t_{1/2}} \quad (4)$$

$$M_{RC} = M_O - M_{DC} \quad (5)$$

where M_O is the dose of the drug, M_{DC} the amount of the drug in DC (dissolved and not dissolved), M_{RC} the amount of the drug transported into RC and T the model absorption time.

Assuming a model absorption time of 8 h and according to Eqns 4 and 5, an increase in bioavailability of orotic acid by a factor of about 4.4 was predicted when prodrug **6** was administered orally in comparison to the administration of **1**. As shown in Table 3 and discussed above, in the results of the rabbit study an increase in bioavailability of orotic acid by a factor of 3.4 was found after oral

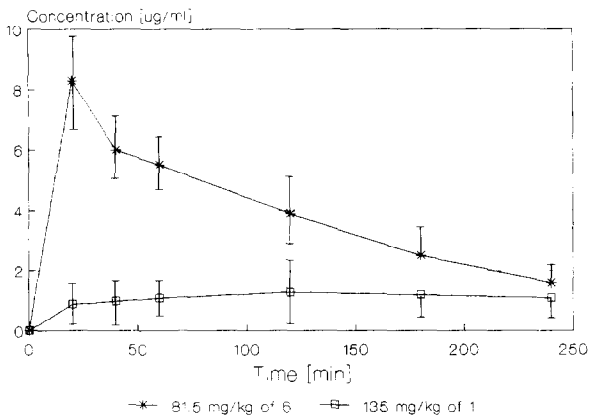

Fig. 5. Plasma levels of orotic acid after oral application of **1** and **6**.

TABLE 3

Comparison of bioavailability orotic acid determined in rabbits and calculated using in vitro data

Orally administered substance	Relative bioavailability (%)	
	In vitro absorption model	In vivo rabbits
6 [a]	100	100
1	22.7	29.2

[a] Bioavailability of orotic acid after oral administration of prodrug **6** was set to 100%.

administration of prodrug **6** in comparison to those of prodrug **1**. Thus, it seems possible to predict relative bioavailability following administration not only of oral drug formulations (Fürst et al., 1982) but also of prodrugs using this absorption model system.

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