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Furosemide prodrugs: synthesis, enzymatic hydrolysis and solubility of various furosemide esters

Niels Mørk [1], Hans Bundgaard [1], Michael Shalmi [2] and Steen Christensen [2]

[1] *Department of Pharmaceutical Chemistry, Royal Danish School of Pharmacy, Copenhagen (Denmark)*
[2] *Department of Pharmacology, University of Copenhagen, Copenhagen (Denmark)*

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Summary

Various esters of furosemide were prepared and assessed as potential prodrugs with the aim of enhancing the peroral absorption characteristics of the parent drug. The esters studied included a neutral alkyl ester, alkyl esters containing an amino group, glycolamide esters and an *O*-acyloxymethyl ester. The esters showed widely different susceptibilities to undergo enzymatic hydrolysis in human plasma and rat liver homogenate, the propionyloxymethyl ester being the most reactive compound. The amino-containing esters were most soluble in water whereas all the esters were more lipophilic than furosemide as expressed by octanol-pH 7.4 buffer partition coefficients.

Introduction

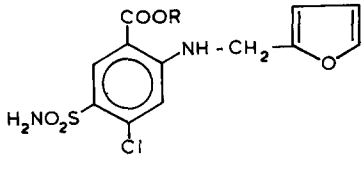
Furosemide (frusemide) (I) is a widely used loop diuretic which is most often administered orally. The pharmacokinetic and pharmacodynamic properties of furosemide have been well documented in both healthy volunteers and patients with specific diseases. The extensive literature on these objects has been throughly reviewed by Benet (1979), Cutler and Blair (1979) and recently by Hammarlund-Udenaes and Benet (1989). As described in these reviews several studies have shown that furosemide is only incompletely (40–60%) absorbed following peroral administration and in addition, the bioavailability both in terms of rate and extent of absorption shows a high degree of inter- and intraindividual variability. Various possible reasons for the relatively low bioavailability have been considered such as acid-catalyzed degradation of the drug in the stomach, first-pass metabolism in the gut wall or in the liver, dissolution-limited absorption and site-limited or site-specific absorption but no firm explanation can presently be given (Hammarlund-Udenaes and Benet, 1989). Data obtained in rats (Chungi et al., 1979; Lee and Chiou, 1983) appear to indicate that the occurrence of a site-related absorption from the stomach or the upper part of the gastrointestinal tract is the most likely explanation for the incomplete and variable absorption pattern of furosemide.

Because of the short duration of the diuretic effect of conventional furosemide tablets or caps-

Correspondence: H. Bundgaard, Department of Pharmaceutical Chemistry, Royal Danish School of Pharmacy, 2 Universitetsparken, DK-2100 Copenhagen, Denmark.

ules (Benet, 1979), various slow-release preparations have been developed. A disadvantage of these preparations is, however, that the bioavailability of furosemide is further reduced compared to conventional preparations (Beermann, 1982; Ebihara et al., 1983; Uchino et al., 1983; Verhoeven et al., 1988). In these studies the relative availability of slow-release products was found to be about 50% of that obtained with conventional tablets.

A potentially useful means to improve the per-oral bioavailibility characteristics of furosemide may be development of prodrug derivatives. If derivatives showing a complete absorption could be developed it might then also be possible to prepare controlled-release products possessing a higher bioavailability than those products presently being in clinical use. As recently concluded by Verhoeven et al. (1988) from studies in man the absorption problems of furosemide hinder an easy approach for formulating oral controlled-release dosage forms. Furosemide contains a carboxylic acid function and an obvious approach to obtain prodrug derivatives is to make esters of the acid group. In this paper, the synthesis of various esters of furosemide (II–VIII) is described along with data on the enzymatic hydrolysis of the esters and their aqueous solubilities and lipophilicities. Results from bioavailability studies of the esters will be reported subsequently.



I R = H

II R = -C2H5

III R = -CH2CON(CH3)2

IV R = -CH2CON(C2H5)2

V R = -CH2CON⟨⟩N-CH3, HCl

VI R = -CH2CH2N⟨⟩O, HCl

VII R = -CH2CH2CH2N(CH3)2, HCl

VIII R = -CH2OCOCH2CH3

Materials and Methods

Apparatus

Melting points were determined in capillary tubes and are uncorrected. ^{1}H-NMR spectra were run on a Varian 360L instrument using tetramethylsilane as an internal standard. Measurements of pH were performed at the temperature of study using a Radiometer Type PHM 26 instrument. High-performance liquid chromatography (HPLC) was carried out using a system consisting of a Shimadzu LC-6A pump, a variable-wavelength UV detector Waters type Lambda Max 481 operated at 270 nm and a Rheodyne 7125 injection valve with a 20 µl loop. Microanalyses were performed at the Microanalytical Laboratory, Leo Pharmaceutical Products, Ballerup, Denmark.

Chemicals

Furosemide was purchased from Sigma (St. Louis, U.S.A.) and was used as received. Buffer substances and solvents used were of reagent grade.

Synthesis of furosemide esters II–VIII

The ethyl ester (II), *N,N*-dimethylglycolamide ester (III) and *N,N*-diethylglycolamide ester (IV) were prepared as reported in a previous paper (Bundgaard et al., 1988).

N-Methylpiperazinoglycolamide ester, hydrochloride salt (V) A mixture of furosemide (1.65 g, 5 mmol), triethylamine (1.4 ml, 10 mmol), NaI (75 mg, 0.5 mmol) and 1-methyl-4-chloroacetylpiperazine hydrochloride (1.07 g, 5 mmol) in 10 ml of *N,N*-dimethylformamide was stirred at room temperature for 20 h. Water (50 ml) was added and the solution adjusted to pH 7.5 with 2 M HCl. The mixture was extracted with ethyl acetate (2 × 50 ml) and the combined extracts were washed

with water, dried over anhydrous Na_2SO_4 and evaporated in vacuo. The solid residue obtained was dissolved in methanol-ether. A 2.5 M HCl solution in methanol (2 ml) was added followed by ether. The precipitate formed was filtered off, washed with ether and recrystallized from 90% ethanol to give 1.2 g of the title compound; m.p. 196–198°C.

Anal.: Calc. for $C_{19}H_{23}ClN_4O_6S \cdot HCl$: C, 44.98; H, 4.77; N, 11.04. Found: C, 44.81; H, 4.85; N, 11.12.

Morpholinoethyl ester, hydrochloride salt (VI) A mixture of furosemide (1.65 g, 5 mmol), triethylamine (1.8 ml, 13 mmol), NaI (75 mg, 0.5 mmol) and 2-chloroethylmorpholine hydrochloride (1.11 g, 6 mmol) in 10 ml of *N,N*-dimethylformamide was stirred at room temperature for 24 h. Water (50 ml) was added and the pH of the solution was adjusted to pH 7 with 2 M HCl. A crystalline precipitate formed upon standing at 4°C for 3 h was filtered off and recrystallized from ethanol-water to give 0.9 g of the morpholinoethyl ester of furosemide; m.p. 134–135°C. It was converted to the hydrochloride salt by adding a 2.5 M methanolic HCl solution to a solution of the free base form in ethanol-ether. The salt was recrystallized from 90% ethanol; m.p. 168–170°C.

Anal.: Calc. for $C_{18}H_{22}ClN_3O_6S \cdot HCl$: C, 45.00; H, 4.83; N, 8.75. Found: C, 45.10; H, 4.89; N, 8.74.

3-N,N-Dimethylaminopropyl ester, hydrochloride salt (VII) A mixture of furosemide (1.65 g, 5 mmol), triethylamine (1.4 ml, 10 mmol), NaI (75 mg, 0.5 mmol) and 3-*N,N*-dimethylaminopropyl chloride hydrochloride (0.79 g, 5 mmol) was stirred at room temperature for 22 h. Water (75 ml) was added and the mixture extracted with ethyl acetate (2 × 75 ml). The combined extracts were washed with a 2% Na_2CO_3 solution (pH 10.0) and water, dried over anhydrous Na_2SO_4 and evaporated in vacuo to yield an oil. This was dissolved in methanol-ether. A 2.5 M methanolic HCl solution (2.5 ml) was added followed by ether. The precipitate formed upon standing at 4°C for 2 h was collected, washed with ether and recrystallized from ethanol-acetonitrile-ether to give 0.85 g of the title compound. m.p. 212–213°C.

Anal.: Calc. for $C_{17}H_{22}ClN_3O_5S \cdot HCl$: C, 45.14; H, 5.13; N, 9.29. Found: C, 44.98; H, 5.21; N, 9.23.

Propionyloxymethyl ester (VIII) A mixture of furosemide (16.5 g, 50 mmol), triethylamine (8.4 ml, 60 mmol), NaI (0.75 g, 5 mmol) and α-chloromethyl propionate (6.75 ml, 55 mmol) (prepared as reported by Waranis and Sloan (1987)) in *N,N*-dimethylformamide (70 ml) was stirred for 48 h at room temperature. Water (200 ml) was added and the mixture extracted with ethyl acetate (2 × 200 ml). The combined extracts were washed with a 2% aqueous solution of $NaHCO_3$ and water, dried over anhydrous Na_2SO_4 and evaporated in vacuo. The residue obtained was crystallized from chloroform and finally recrystallized from chloroform-petroleum ether to give 15.1 g of the title compound. m.p. 141–142°C.

Anal.: Calc. for $C_{16}H_{17}ClN_2O_7S$: C, 46.10; H, 4.11; N, 6.72. Found: C, 46.20; H, 4.17; N, 6.65.

The ^{1}H-NMR spectra of the esters (in DMSO-d_6) were in agreement with their structures. The identity of the esters was furthermore confirmed by their quantitative conversion to furosemide by alkaline hydrolysis as revealed by HPLC measurements. The purity of the esters was greater than 99.5% as assessed by TLC and HPLC. In TLC silica gel plates were eluted with ethyl acetate-toluene-acetic acid (3 : 1 : 0.04 v/v).

Analysis of furosemide and its esters

Furosemide and the esters II–VIII were quantitated by an HPLC method capable of separating the esters and furosemide. A Waters Radial-Pak column (8 × 100 mm) packed with Nova-Pak C18 (4-μm particles) in conjunction with a Waters Guard-Pak precolumn packed with μBondapak C18 (10-μm particles) was eluted at ambient temperature with a mobile phase consisting of acetonitrile-methanol-water-85% phosphoric acid-triethylamine (45 : 5 : 50 : 0.1 : 0.1 v/v) at a flow rate of 1.0 ml min^{-1}. Under these conditions furosemide and its esters showed the following retention times: I, 4.2 min; II, 7.8 min; III, 10.8 min; IV, 8.0 min; V, 5.0 min; VI, 9.2 min; VII, 7.6 min.

Hydrolysis kinetics in biological media

The hydrolysis of the furosemide esters was studied in human or rat plasma diluted to 80%

with 0.05 M phosphate buffer of pH 7.4 as well as in 10% rat liver homogenate at 37°C. The liver homogenates were prepared as previously described (Buur and Bundgaard, 1984). The reactions were initiated by adding 50 μl of a stock solution of the esters in acetonitrile or ethanol-water to 5 ml of preheated plasma solution or liver homogenate, the final concentrations of the compounds being about 5×10^{-5} M. The mixtures were kept in a water bath at 37°C and at appropriate intervals samples of 250 μl were withdrawn and added to 500 μl of a 2% solution of $ZnSO_4$ methanol-water (1 : 1 v/v) in order to deproteinize the samples. After mixing and centrifugation at 13000 rpm for 3 min, 20 μl of the clear supernatant was analyzed by HPLC for remaining ester derivative as well as for the parent furosemide as described above. Pseudo-first-order rate constants for the hydrolysis were determined from the slopes of linear plots of the logarithm of residual ester against time.

Both the stock solutions and the reaction solutions were protected from light in order to avoid any photodegradation of the esters (Bundgaard et al., 1988).

Determination of aqueous solubility and partition coefficients

The solubility of the esters in water or buffer solutions was determined at 21°C by adding excess amounts of the compounds to water or buffer solution in screw-capped test-tubes covered with aluminium foil. The mixtures were placed in an ultrasonic water bath for 10 min and then rotated on a mechanical spindle for 20–30 h. It was ensured that saturation equilibrium was established. Upon filtration an aliquot of the filtrate was diluted with water and the mixture analyzed by HPLC.

The partition coefficients of the esters were determined in an octanol-0.05 M phosphate buffer (pH 7.4) system as previously described (Bundgaard at al., 1986).

Results and Discussion

The esters of furosemide (II–VIII) prepared include a simple alkyl ester (II), three glycolamide esters (III–V), two alkyl esters containing a basic amino group (VI and VII) and an *O*-acyloxymethyl ester (VIII). Such esters were expected to possess widely differing properties in terms of aqueous solubility, lipophilicity and ease of enzymatic hydrolysis as indeed was observed. Since furosemide esters are extremely susceptible to undergoing photo-catalyzed degradation in aqueous solution (Bundgaard et al., 1988), all experiments were performed under exclusion of light.

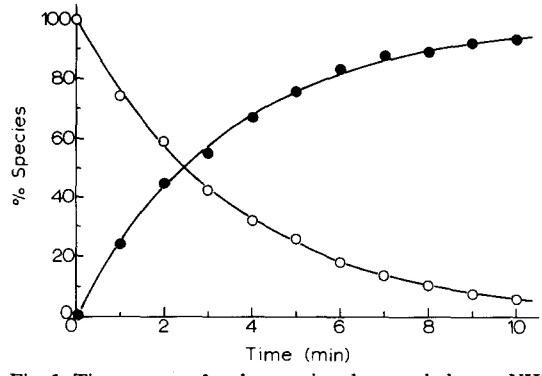


Fig. 1. Time courses for the propionyloxymethyl ester VIII (○) and furosemide (●) during incubation of the ester in 80% human plasma at 37°C.

The various esters were found to be hydrolyzed quantitatively to furosemide in the plasma solutions or in 10% rat liver homogenate as revealed by HPLC analysis of the reaction solutions. An example is shown in Fig. 1. In all cases the progress of hydrolysis followed strict first-order kinetics over several half-lives as illustrated in Fig. 2

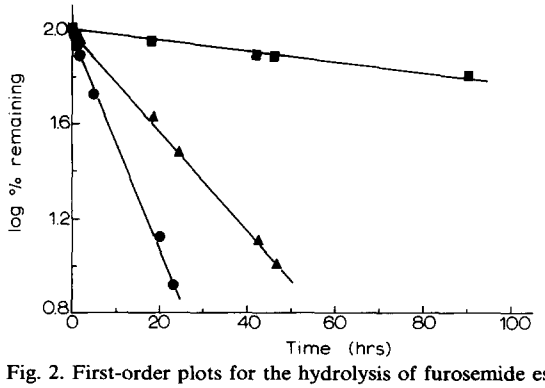


Fig. 2. First-order plots for the hydrolysis of furosemide esters III (▲), IV (■) and VI (●) in 80% human plasma at 37°C.

TABLE 1

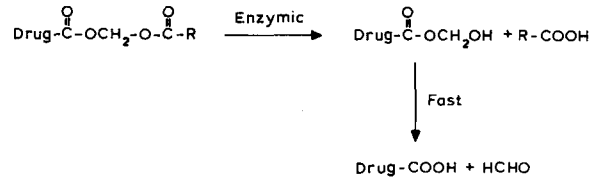
Hydrolysis data for various furosemide esters

Compound	$t_{1/2}$ (h) [a]			
	0.02 M phosphate buffer, pH 7.4	80% human plasma	80% rat plasma	10% rat liver homogenate
II	~1000	>100	4.7	14.2
III	230	14.4	1.6	12.4
IV	270	4.4	0.8	7.1
V	260	>100	3.3	31.4
VI	31	>100	25	50.1
VII	73	~100	19	~100
VIII	2.8	2.5 min	0.6 min	0.4 min

[a] Half-life of hydrolysis at 37°C.

for some esters. The half-lives for the various esters are listed in Table 1. Hydrolysis data for the esters in a 0.02 M phosphate buffer solution of pH 7.40 at 37°C are also included in Table 1.

As can be seen from the rate data the esters show widely different rates of enzymatic hydrolysis. The esters II and V–VII are only slowly hydrolyzed whereas the *N,N*-dialkylglycolamide esters III and IV are fairly rapidly cleaved. The greater reactivity of the latter esters compared with the ethyl ester (II) is in accordance with similar differences in enzymatic reactivity of such esters of benzoic acid, naproxen, salicylic acid and various other carboxylic acids (Bundgaard and Nielsen, 1987, 1988; Nielsen and Bundgaard, 1988, 1989). However, the absolute rate of the plasma-catalyzed hydrolysis of these glycolamide esters of furosemide is markedly lower than that observed with other carboxylic acids (Bundgaard and Nielsen, 1988). A plausible reason for this may be the steric hindrance exhibited by the *ortho*-positioned furfuryl amino group in furosemide esters. In accordance with this expectation is the finding of a very rapid rate of enzymatic hydrolysis of the acyloxymethyl ester VIII. In such a double ester enzymatic hydrolysis can take place at the ester moiety remote from the furosemide acyl group yielding a hydroxymethyl ester which spontaneously dissociates to the acid and formaldehyde (Scheme 1). Such *O*-acyloxymethyl esters have been widely studied and used as prodrug forms



Scheme 1.

for various carboxylic acid agents (for a review, see Bundgaard (1985, 1989)).

For all the esters the half-lives of hydrolysis were markedly shorter in rat plasma than in human plasma (Table 1). More surprising is the finding that rat liver homogenate is less efficient than rat plasma in catalyzing the ester hydrolysis except for ester VIII. In fact, both liver homogenate and human plasma exhibited an inhibitory effect on the rate of hydrolysis of esters VI and VII (Table 1), probably due to binding of the compounds to proteins.

As reported before (Bundgaard et al., 1988) for the esters II–IV, the acid stability of these compounds is greater than that of furosemide. At 60°C the half-lives of hydrolysis of these esters in aqueous solution of pH 2.0 are about 13 h (Bundgaard et al., 1988).

The water solubilities and partition coefficients of the compounds are shown in Table 2. As can be

TABLE 2

Solubility and partition data of furosemide and various furosemide esters

Compound	S [a] (mg ml^{-1})	log P [b]
I	0.040	−1.06
	0.011 [c]	2.03 [d]
II	0.0004	2.67
III	0.0049	1.18
IV	0.0044	2.45
V	1.00 [e]	1.23
VI	1.11 [e]	2.00
VII	10.5 [e]	0.83
VIII	0.0064	2.90

[a] Solubility in water at 21°C.

[b] Partition coefficient (P) between octanol and 0.05 M phosphate buffer (pH 7.4) at 21°C.

[c] Solubility in 0.01 HCl.

[d] Partition coefficient between octanol and 0.01 M HCl.

[e] Solubility of the compound as HCl.

seen from the data all the esters studied are more lipophilic than furosemide at pH 7.4 as determined on the basis of the octanol-buffer partition coefficients. A log P value of 2.03 was determined for furosemide as undissociated acid. The carboxylic acid function in the compound has a pK_a value of 3.9 (Hajdu and Häussler, 1964) and it is thus highly ionized at pH 7.4. If only the undissociated acid species is assumed to partition into the octanol phase, it can be calculated from the P value for this species (log $P = 2.03$) that furosemide should have a log P value of -1.47 at pH 7.4. The slightly higher value actually found (-1.06) indicates that the ionized form also to some extent can partition into the octanol phase.

The water solubility of the neutral esters II–IV and VIII is very low and less than that of furosemide in its undissociated form (Table 2). The esters V–VII contain a protonatable amino group and as expected, these esters in the form of hydrochloride salts show markedly higher water solubilities (from 1 to 10 mg ml^{-1}).

The primary sulphonamide group in furosemide is a weak acid with a pK_a value of 7.5 (Verhoeven et al., 1988) and therefore, an increase in solubility of the neutral esters in weakly basic solution was expected. However, ionization of the sulphonamide group does not appear to affect the solubility significantly as shown from the following solubilities (μg ml^{-1}) observed for ester III at 21°C: 4.9 (pH 6.0), 4.9 (pH 6.9), 5.3 (pH 8.4), 5.4 (pH 9.0) and 6.1 (pH 9.4).

Conclusions

Considering the primary aim of this work which is to develop prodrug derivatives useful for enhancing the peroral bioavailability of furosemide, derivatives possessing both sufficient water solubility and lipophilicity as well as a rapid rate of enzymatic conversion to furosemide during or following absorption should be selected. All the esters studied show a favourable lipophilicity for absorption from the gastrointestinal tract (Yalkowsky and Morozowich, 1980) and some esters (V–VII) also have an adequate water solubility. The latter esters are, however, only slowly hydrolyzed in vitro to the parent drug, and incomplete reconversion of these compounds in vivo may limit the availability of the active parent acid. Conversely, ester VIII is very rapidly hydrolyzed but it is only very slightly soluble in water. Studies are in progress to assess the bioavailability of a number of these furosemide esters in rats and dogs following peroral administration.

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