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N-Acetyl-L-γ-glutamyl Derivatives of *p*-Nitroaniline, Sulphamethoxazole and Sulphamethizole for Kidney-specific Drug Delivery in Rats

TERUO MURAKAMI, KUMIKO KOHNO, RYOKO YUMOTO, YUTAKA HIGASHI AND NOBORU YATA*

Department of Biopharmaceutics, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minami-ku, Hiroshima 734, Japan

Abstract

Kidney-specific delivery of *p*-nitroaniline, sulphamethoxazole and sulphamethizole after either intravenous administration of the L-γ-glutamyl or *N*-acetyl-L-γ-glutamyl derivatives or the parent drugs has been examined in a rat model.

All L-γ-glutamyl derivatives were converted to the corresponding parent drugs within 60 min whereas the *N*-acetyl-L-γ-glutamyl derivatives were fairly stable in the systemic circulation after parenteral administration. Concentrations of *p*-nitroaniline and sulphamethoxazole 20 min after administration of the parent drugs were somewhat higher in the kidney than in the liver and lung. The concentration of sulphamethizole in the kidney was dramatically higher than those in the hepatic and pulmonary tissue. Kidney-specific delivery of the drugs of interest was evaluated by determining the tissue concentrations of the released parent drug and the total drug levels (i.e. drug levels after hydrolysis of all conjugate to the parent drug). For L-γ-glutamyl-*p*-nitroaniline released renal levels of *p*-nitroaniline and total *p*-nitroaniline concentrations were both higher than those obtained after *p*-nitroaniline dosing. Use of L-γ-glutamylsulphamethoxazole resulted in higher total sulphamethoxazole concentrations in the kidney, but did not lead to an increase in released (unconjugated) sulphamethoxazole levels. In contrast, no kidney-selective distribution was observed for L-γ-glutamylsulphamethizole. Markedly increased kidney distribution was observed for both *N*-acetyl-L-γ-glutamyl-*p*-nitroaniline and *N*-acetyl-L-γ-glutamylsulphamethoxazole and the liver and lung concentrations were correspondingly reduced in comparison with parent drug dosing. Use of the *N*-acetyl-L-γ-glutamyl-*p*-nitroaniline conjugate increased the concentration of *p*-nitroaniline in the kidney to the same extent as did L-γ-glutamyl-*p*-nitroaniline.

In conclusion, *N*-acetyl-L-γ-glutamyl derivatization of certain compounds seems to be useful for kidney-specific drug delivery and preliminary data suggests that lipophilic drugs are better substrates than hydrophilic compounds. Results related to the selectivity of tissue distribution of the derivatives and species differences are discussed.

The enzymatic activity of γ-glutamyl transpeptidase (EC 2.3.2.2) is known to be excellent in the mammalian kidney, and γ-glutamyl derivatives of amino acids are extensively taken up, accumulated and metabolized in the kidney (Orlowski & Szewczuk 1961; Orlowski & Wilk 1976, 1978; Endo 1978). Thus, L-γ-glutamyl derivatives of L-dopamine (gludopa) or of amino acids have been synthesized as kidney-specific prodrugs (Orlowski & Wilk 1976, 1978; Wilk et al 1978). Gludopa is distributed selectively to the kidney, releases dopamine by sequential action of L-amino acid decarboxylase (EC 4.1.1.26) and γ-glutamyl transpeptidase, and causes renal selective vasodilatation and increased sodium excretion without causing systemic effects in experimental animals (Barthelmebs et al 1990; Boeteng et al 1990; Cummings et

*Present address: Faculty of Pharmaceutical Sciences, Setsunan University, 45-1 Nagaotoge-cho, Osaka 573-01, Japan

Correspondence: T. Murakami, Department of Biopharmaceutics, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minami-ku, Hiroshima 734, Japan.

al 1990; Wang et al 1993; Drieman et al 1994). The relatively kidney-specific dopaminergic action of gludopa in healthy man and in patients with essential hypertension is also reported by many investigators (Worth et al 1985; MacDonald et al 1989; Freestone et al 1990; Lee 1990; Boateng et al 1991). On the other hand, the γ-glutamyl derivative of sulphamethoxazole is less specific to the kidney because of rapid conversion to the parent drug in the systemic circulation. To overcome this Orlowski et al (1980) synthesized *N*-acyl-L-γ-glutamyl derivatives of sulphamethoxazole and reported kidney-selective accumulation of sulphamethoxazole in mice. *N*-Acetyl-L-γ-glutamylsulphamethoxazole is also known to be selectively transported into the kidney by a carrier-mediated mechanism, as was proved by the inhibitory action of the anion transport inhibitor probenecid and the γ-glutamyl transport inhibitor buthionine (Drieman et al 1990a). Other *N*-acetyl-L-γ-glutamyl derivatives of CGP 22979 and 4′-aminowarfarin have also been reported (Drieman et al 1990b; Drieman & Thijssen 1991), although the derivative of 4′-aminowarfarin was not accumulated in the kidney and was excreted extensively into the bile, unchanged, via a carrier-mediated mechanism (Drieman & Thijssen 1991).

In this study kidney-specific delivery of *p*-nitroaniline, sulphamethoxazole and sulphamethizole after intravenous administration of the L-γ-glutamyl or *N*-acetyl-L-γ-glutamyl derivatives or the parent drugs was examined in a rat model. These model drugs were selected because of the different lipophilicities of the parent drugs and their derivatives. Species differences in the kidney distribution of mice and rats were also examined.

Materials and Methods

Materials

p-Nitroaniline (Katayama Chemicals), sulphamethoxazole (Shinomin; Shionogi Pharmaceutical), sulphamethizole (Urocydal; Eisai Pharmaceutical) and L-γ-glutamyl-*p*-nitroanilide monohydrate (Wako Pure Chemicals) were obtained commercially. Other compounds were of reagent grade and were used without further purification.

Synthesis of N-*acetyl-L-γ-glutamyl derivatives*

L-γ-Glutamyl derivatives of sulphamethoxazole and sulphamethizole were synthesized according to the method reported by Orlowski et al (1980). Derivatives were separated on a Dowex-1-acetate column (AG 1 × 2, 200–400 mesh, 3 cm × 33 cm). For the sulphamethoxazole derivative, free L-glutamic acid and sulphamethoxazole were eluted from the column with 0·3 M acetic acid (2 L) and L-γ-glutamylsulphamethoxazole was then eluted with a sufficient amount of 0·5 M acetic acid. For the sulphamethizole derivative, free L-glutamic acid and sulphamethizole were eluted with 0·5 M acetic acid (2 L) and L-γ-glutamylsulphamethizole with a sufficient amount of 1 M acetic acid. Fractions containing the desired products were pooled and the solvent was evaporated by rotary evaporation under reduced pressure. Compounds were recrystallized by dissolving in a small amount of 0·1 M HCl and then adjusting the pH of the solution to 4–5 with 0·1 M NaOH. Chemical structures of the L-γ-glutamyl derivatives were assigned by ^{13}C nuclear magnetic resonance spectroscopy (Jeol JNM-PS-100 spectrometer at 23·15 MHz).

For synthesis of *N*-acetyl-L-γ-glutamyl derivatives, L-γ-glutamyl-*p*-nitroaniline, L-γ-glutamylsulphamethoxazole, or L-γ-glutamylsulphamethizole (4 mmol) was dissolved in Na_2CO_3 solution (0·5 M; 5 mL) and acetic anhydride (5 mmol) was added dropwise to each solution over a 15-min period with stirring at 0°C. The pH of the solution was adjusted to 1 with 3 M HCl and the solution was extracted with ethyl acetate. The organic layer was separated, dried with anhydrous sodium sulphate, the solvent was evaporated to dryness under reduced pressure, and the product was recrystallized from a mixture of ethyl acetate and hexane.

Measurement of partition coefficients

Apparent partition coefficients of the parent drugs and their derivatives were measured between chloroform and pH 7·4, 0·05 M Tris-HCl buffer solution at 25°C. The initial concentration of each compound in Tris-HCl buffer was 500 μM. For *N*-acetyl-L-γ-glutamyl-*p*-nitroaniline and *N*-acetyl-L-γ-glutamylsulphamethoxazole, 1 mL of compound solution was equilibrated with 100 mL chloroform. For other compounds 1 mL of compound solution was equilibrated with 8 mL chloroform.

Animal study

Male Wistar rats, 180–230 g, were anaesthetized by intraperitoneal injection of sodium pentobarbital (25 mg kg^{-1}) and kept supine on a surface controlled at 37°C.

Drug solution for animal study was prepared as follows. *p*-Nitroaniline (250 μmol) was dissolved in *N*,*N*-dimethylformamide (0·1 mL). For injection this solution was mixed with 1·3 mL propylene glycol and 0·6 mL sterile purified water. L-γ-Glutamyl-*p*-nitroaniline (250 μmol) was dissolved in 2 M HCl (0·2 mL) and this solution was mixed with 1·7 mL propylene glycol and 0·1 mL of 0·5 M

Na_2CO_3 to adjust the pH of the solution to neutral. *N*-Acetyl-L-γ-glutamyl-*p*-nitroaniline (250 μmol) was dissolved in 0·5 M Na_2CO_3 (0·4 mL) and this solution was mixed with 1·5 mL propylene glycol and 0·1 mL 2 M HCl. Solutions of sulphamethoxazole, sulphamethizole or their derivatives for injection were prepared by dissolving the drug in sterile purified water (2 mL) at a concentration of 250 μmol with the help of equimolar NaOH. Drug solutions (2 mL) were intravenously administered into the rat tail-vein at a dose of 250 μmol kg^{-1} in exactly 2 min. Blood (0·5 mL) was taken from a jugular vein, and was haemolysed by adding deionized water (1·8 mL). After deproteinization with trichloroacetic acid solution (15%; 2 mL) and centrifugation at 3000 rev min^{-1}, the supernatant was used for analysis of drug concentrations.

In separate experiments, rats were killed by decapitation 20 or 60 min after injection of drug, and the liver, lung and kidney were removed to determine drug concentration. Isolated tissues were homogenized with a 5-fold volume of chilled deionized water on ice and then centrifuged at 4°C. The supernatant was deproteinized with trichloroacetic acid solution (15%) and again centrifuged. The supernatant was used for analysis.

Analytical method

Concentrations of unchanged *p*-nitroaniline, sulphamethoxazole and sulphamethizole in blood and tissues were determined by diazotization. Total drug concentrations, including the parent drug, its derivative and other conjugate metabolite(s), if any, in blood and tissues were also determined by the same method after hydrolysis. For *p*-nitroaniline and related compounds hydrolysis was performed by adding NaOH (0·5 M; 1 mL) to the samples and heating the mixture on a boiling water bath for 30 min to give the parent drug. For sulphamethoxazole and related compounds samples were mixed with NaOH (2 M; 1 mL) and the mixture was boiled for 90 min. For sulphamethizole and related compounds samples were mixed with HCl (2 M; 1 mL) and the mixture was boiled for 30 min. The stability of each parent drug and the complete hydrolysis of their derivatives to the parent drugs under these hydrolysis conditions were proved in separate experiments.

Results

Partition coefficients

Table 1 lists partition coefficients of the parent drugs and the synthesized derivatives between chloroform and pH 7·4 Tris-HCl buffer solution; the melting points of the compounds are also listed. The partition coefficients of the derivatives of *p*-nitroaniline and sulphamethoxazole were markedly less than those of the parent drugs; this was not so for sulphamethizole derivatives.

Blood profiles after intravenous administration

Concentrations of a parent drug and total drug in blood after intravenous administration of the parent drug or an equimolar dose of its derivative were measured at appropriate time intervals (Figures 1–3). The levels of total drug represent levels after hydrolysis of all conjugates to the parent compound. All L-γ-glutamyl derivatives were converted to the corresponding parent drugs within 60 min whereas *N*-acetyl-L-γ-glutamyl derivatives were fairly stable in the systemic circulation after parenteral administration. Distribution volumes of *N*-acetyl-L-γ-glutamyl-*p*-nitroaniline and *N*-acetyl-L-γ-glutamylsulphamethoxazole, as evaluated from their blood levels, were much higher than those of *p*-nitroaniline and sulphamethoxazole, respectively, whereas blood levels of *N*-acetyl-L-γ-glutamylsulphamethizole were the same as for sulphamethizole. The elimination rate constant of *N*-acetyl-L-γ-glutamyl-*p*-nitroaniline was found to be increased.

Table 1. The melting points and partition coefficients of the compounds.

Compound	Mp (°C)	Partition coefficient ($\times 10^3$)
p-Nitroaniline	150·0	13500
L-γ-Glutamyl-*p*-nitroaniline	184·5–185·0	1·71
N-Acetyl-L-γ-glutamyl-*p*-nitroaniline	245·0–248·0	0·672
Sulphamethoxazole	169·0–170·0	67·2
L-γ-Glutamylsulphamethoxazole	185·0–187·0	2·65
N-Acetyl-L-γ-glutamylsulphamethoxazole	210·0–212·0	0·0346
Sulphamethizole	210·3	6·84
L-γ-Glutamylsulphamethizole	182·5	3·57
N-Acetyl-L-γ-glutamylsulphamethizole	221·0	6·58

The partition coefficient was determined between chloroform and 0·05 M Tris-HCl buffer (pH 7·4) at 25°C.

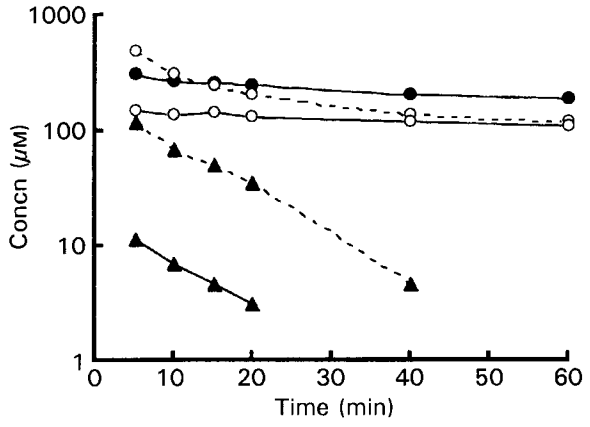

Figure 1. Blood concentrations of *p*-nitroaniline and total drug after intravenous administration of 250 μmol kg^{-1} *p*-nitroaniline (●), L-γ-glutamyl-*p*-nitroaniline (○), or *N*-acetyl-L-γ-glutamyl-*p*-nitroaniline (▲) to rats. Solid line, *p*-nitroaniline released; dotted line, total (*p*-nitroaniline and its derivatives). The total value represents the concentration of *p*-nitroaniline after hydrolysis of all compounds to the parent compound. Each value is the mean of results from three or four trials.

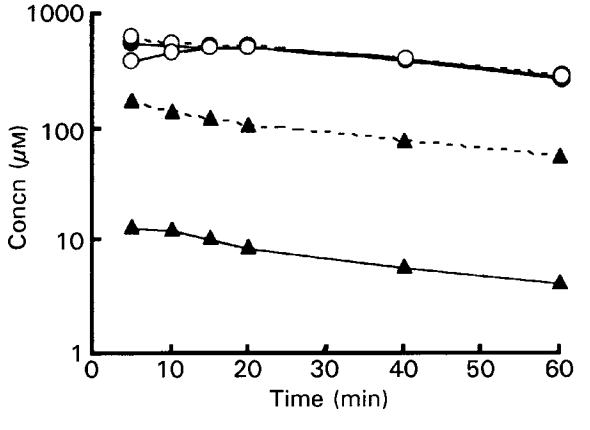

Figure 2. Blood concentrations of sulphamethoxazole and total drug after intravenous administration of 250 μmol kg^{-1} sulphamethoxazole (●), L-γ-glutamylsulphamethoxazole (○), or *N*-acetyl-L-γ-glutamylsulphamethoxazole (▲) to rats. Solid line, sulphamethoxazole released; dotted line, total (sulphamethoxazole and its derivatives). The total value represents the concentration of sulphamethoxazole after hydrolysis of all compounds to the parent compound. Each value is the mean of results from three or four trials.

Tissue distribution after intravenous administration

Concentrations of *p*-nitroaniline and sulphamethoxazole 20 min after administration of the parent drug were somewhat higher in the kidney than in the liver and lung (Table 2). On the other hand, the concentration of sulphamethizole in the kidney was dramatically higher than in hepatic or pulmonary tissue, indicating that sulphamethizole is eliminated rapidly mainly by a renal route. Total tissue drug levels after administration of *p*-nitroaniline, sulphamethoxazole or sulphamethizole would include levels of the parent drug and other conjugate metabolites, if any. However, there was little difference between the tissue concentrations of total and parent (unchanged) drug for all three compounds, findings which suggest that the three compounds are not significantly metabolized (conjugated) in these tissues.

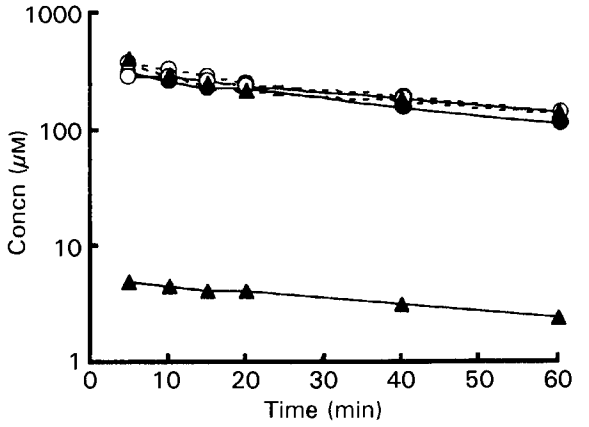

Figure 3. Blood concentrations of sulphamethizole and total drug after intravenous administration of 250 μmol kg^{-1} sulphamethizole (●), L-γ-glutamylsulphamethizole (○), or *N*-acetyl-L-γ-glutamylsulphamethizole (▲) to rats. Solid line, sulphamethizole released; dotted line, total (sulphamethizole and its derivatives). The total value represents the concentration of sulphamethizole after hydrolysis of all compounds to the parent compound. Each value is the mean of results from three or four trials.

Kidney-specific delivery of the drug of interest was evaluated by determining tissue concentrations of the released parent drug and total drug levels in the liver, lung and kidney 20 and 60 min after intravenous administration. For L-γ-glutamyl-*p*-nitroaniline released renal levels of *p*-nitroaniline and total *p*-nitroaniline levels were both significantly (1·4-fold) higher than those obtained after dosing with *p*-nitroaniline, although the tendency was less for hepatic and pulmonary distribution. Such changes in tissue distribution were remarkable after *N*-acetyl-L-γ-glutamyl-*p*-nitroaniline dosing. The concentrations of released *p*-nitroaniline and total *p*-nitroaniline levels in the kidney 20 min after administration were significantly higher than those obtained after administration of *p*-nitroaniline or L-γ-glutamyl-*p*-nitroaniline.

Administration of L-γ-glutamyl-sulphamethoxazole resulted in higher total sulphamethoxazole levels in the kidney but there was no increase in released (unconjugated) sulphamethoxazole levels. Administration of *N*-acetyl-L-γ-glutamylsulphamethoxazole resulted in markedly increased kidney distribution and a corresponding decrease in liver and lung concentrations compared with administration of the parent drug; this was also true for *N*-acetyl-L-γ-glutamyl-*p*-nitroaniline. Thus, relatively

Table 2. Tissue distribution in rats after intravenous administration of 250 μmol kg^{-1} *p*-nitroaniline, sulphamethoxazole, sulphamethizole and their L-γ-glutamyl and *N*-acetyl-L-γ-glutamyl derivatives.

Tissue	Time (min)	Parent compound		L-γ-Glutamyl derivative		*N*-Acetyl-L-γ-glutamyl derivative	
		Parent (nmol g^{-1})	Total (nmol g^{-1})	Parent (nmol g^{-1})	Total (nmol g^{-1})	Parent (nmol^{-1} g^{-1})	Total (nmol g^{-1})
p-Nitroaniline							
Liver	20	263·2 ± 70·2	265·1 ± 80·3	172·7 ± 54·5	201·3 ± 58·2	18·2 ± 20·4*	73·3 ± 21·5*
	60	278·3 ± 100·9	280·1 ± 104·2	184·3 ± 64·3	178·5 ± 55·5	ND*	57·4 ± 12·6*
Lung	20	209·1 ± 27·3	213·4 ± 30·8	154·5 ± 44·0	187·3 ± 46·4	ND*	17·7 ± 4·8*
	60	167·0 ± 29·6	200·0 ± 30·2	121·7 ± 13·9	147·8 ± 12·7	ND*	ND*
Kidney	20	327·3 ± 23·6	330·4 ± 25·4	460·0 ± 47·3†	472·3 ± 49·5†	654·5 ± 116·4†	841·8 ± 35·7†
	60	594·8 ± 80·0	568·3 ± 78·7	521·7 ± 170·4	655·7 ± 128·8	47·0 ± 34·8*	87·0 ± 42·3*
Sulphamethoxazole							
Liver	20	196·8 ± 22·8	228·5 ± 24·1	214·4 ± 20·2	260·1 ± 23·3	10·5 ± 8·3*	193·3 ± 21·1
	60	203·4 ± 30·5	228·8 ± 18·3	191·5 ± 11·9	195·3 ± 12·1	15·2 ± 8·5*	84·7 ± 27·3*
Lung	20	247·8 ± 17·6	248·3 ± 16·7	270·7 ± 21·4	272·3 ± 24·1	ND*	15·4 ± 7·2*
	60	262·7 ± 16·9	254·2 ± 28·4	262·7 ± 13·9	265·1 ± 14·4	ND*	49·2 ± 18·8*
Kidney	20	333·9 ± 29·9	334·5 ± 25·8	379·6 ± 20·0	418·3 ± 22·4†	302·3 ± 83·5	741·7 ± 101·5†
	60	318·6 ± 18·6	347·5 ± 22·0	322·0 ± 30·5	350·8 ± 44·7	127·1 ± 84·7*	361·0 ± 79·4*
Sulphamethizole							
Liver	20	57·1 ± 15·6	57·0 ± 21·3	103·2 ± 14·9†	155·8 ± 18·3†	18·3 ± 2·5*	220·8 ± 31·4†
	60	28·0 ± 15·1	27·5 ± 17·3	47·3 ± 7·5	64·5 ± 12·2	14·2 ± 3·3	102·4 ± 21·5†
Lung	20	194·1 ± 23·3	198·4 ± 30·4	77·9 ± 12·8*	81·5 ± 15·3*	ND*	85·7 ± 19·4*
	60	47·3 ± 15·5	49·9 ± 13·7	38·7 ± 9·7	36·9 ± 11·8	ND*	50·8 ± 18·8
Kidney	20	1246·8 ± 545·5	1363·6 ± 681·7	1558·4 ± 221·2	1550·5 ± 230·6	311·7 ± 77·9*	766·2 ± 108·1
	60	849·5 ± 98·9	907·5 ± 103·9	671·0 ± 78·5	688·0 ± 92·1	117·2 ± 21·4*	229·0 ± 15·3*

Each value is the mean ± s.e.m. (n = 3). Total drug levels are levels after hydrolysis of all conjugates to the parent compound. ND = not detected. † Significantly higher than for parent drug; * significantly lower than for parent drug.

specific kidney distribution of sulphamethoxazole was achieved by *N*-acetyl-L-γ-glutamyl-derivatization, although the concentration of released sulphamethoxazole in the kidney was comparable with that after sulphamethoxazole dosing.

Use of the L-γ-glutamyl- and *N*-acetyl-L-γ-glutamyl derivatives of sulphamethizole failed to result in kidney-specific distribution, although the pulmonary distribution was significantly reduced. In contrast, increased sulphamethizole levels and total sulphamethizole levels in the liver were found 20 min after administration of L-γ-glutamylsulphamethizole. Total sulphamethizole levels in the liver were further increased after administration of *N*-acetyl-L-γ-glutamylsulphamethizole, in contrast with the behaviour of *N*-acetyl-L-γ-glutamyl-*p*-nitroaniline and *N*-acetyl-L-γ-glutamylsulphamethoxazole.

Discussion

The activities of γ-glutamyl transpeptidase and acylase are reported to be especially high in the kidney but low in the liver and lung of mammals including mice, rats and man (Orlowski & Szewczuk 1961; Endo 1978; Orlowski et al 1980). These different enzyme activities give a clue to the development of kidney-selective prodrugs with γ-glutamyl or acyl functional groups, or both, in the molecular structure. Orlowski et al (1980) have reported the marked accumulation of sulphamethoxazole in the kidney of mice after administration of *N*-acetyl-L-γ-glutamylsulphamethoxazole.

In this study, the feasibility of kidney-specific drug delivery by use of L-γ-glutamyl- or *N*-acetyl-L-γ-glutamyl-derivatization was examined in a rat model with three different model compounds of different lipophilicity, including sulphamethoxazole. L-γ-Glutamyl-derivatization resulted in some kidney-specific drug delivery for *p*-nitroaniline but not for sulphamethoxazole or sulphamethizole (Table 2). After administration of the *N*-acetyl-L-γ-glutamyl derivatives of *p*-nitroaniline and sulphamethoxazole, concentrations of released parent drug in the liver and lung 20 min after administration were extremely low in comparison with those after parent drug dosing, indicating that sequential hydrolysis by acylase and γ-glutamyl transpeptidase is less active in the liver and lung than in the kidney. Thus, kidney-specific distribution was observed for *N*-acetyl-L-γ-glutamyl-*p*-nitroaniline and this led to an increase in the concentration of *p*-nitroaniline in the kidney. Drieman et al (1990a)

suggested active uptake of *N*-acetyl-L-γ-glutamylsulphamethoxazole by the kidney. The uptake mechanism might also be similar for the kidney distribution of *N*-acetyl-L-γ-glutamyl-*p*-nitroaniline. In the current study distribution of *N*-acetyl-L-γ-glutamylsulphamethoxazole in a rat model was relatively kidney-specific and distribution to the liver and lung was avoided. In contrast, kidney-specific drug delivery was not observed for *N*-acetyl-L-γ-glutamylsulphamethizole, although the pulmonary distribution of sulphamethizole was significantly reduced in comparison with administration of sulphamethizole. These findings might suggest that *N*-acetyl-L-γ-glutamyl-derivatization is effective for rather lipophilic drugs such as *p*-nitroaniline and sulphamethoxazole but not for poorly lipophilic drugs such as sulphamethizole.

The rapid disappearance of total *p*-nitroaniline levels from the systemic circulation after administration of *N*-acetyl-L-γ-glutamyl-*p*-nitroaniline (Figure 1) suggests the possibility of urinary elimination of the derivative in addition to the kidney-specific distribution. This is supported by the rapid disappearance of total *p*-nitroaniline levels from the kidney (Table 2). In a separate experiment, we determined the urinary excretion of *p*-nitroaniline for 60 min and found that almost 100% of the dose was recovered in the urine (as total *p*-nitroaniline) after administration of *N*-acetyl-L-γ-glutamyl-*p*-nitroaniline, whereas only 8·4% of the dose was recovered after administration of *p*-nitroaniline.

After administration of *N*-acetyl-L-γ-glutamylsulphamethizole or L-γ-glutamylsulphamethizole the total sulphamethizole level in the liver was significantly higher than after administration of the parent compound (Table 2). The reason is unclear at present, but increased liver distribution and extensive biliary excretion of unchanged *N*-acetyl-L-γ-glutamyl-4′-aminowarfarin has already been reported (Drieman & Thijssen 1991). As with *N*-acetyl-L-γ-glutamyl-4′-aminowarfarin, *N*-acetyl-L-γ-glutamylsulphamethizole might also be preferentially taken up by the liver. The preferential accumulation of some *N*-acetyl-L-γ-glutamyl derivatives in the liver must be further investigated.

As described, the kidney-specific delivery of sulphamethoxazole in mice by use of *N*-acetyl-L-γ-glutamylsulphamethoxazole has already been reported by Orlowski et al (1980), although they reported only the levels of released parent drug. In their report the concentration of sulphamethoxazole in the kidney was 1·5-times higher after administration of *N*-acetyl-L-γ-glutamylsulphamethoxazole than after administration of sulphamethoxazole; this is different from our current results with rats. As confirmation we re-examined the tissue distribution of sulphamethoxazole after administration of sulphamethoxazole, L-γ-glutamylsulphamethoxazole and *N*-acetyl-L-γ-glutamylsulphamethoxazole in mice. The concentration of released sulphamethoxazole in the kidney after administration of *N*-acetyl-L-γ-glutamylsulphamethoxazole dosing was 1·4-times higher than after administration of sulphamethoxazole, in good agreement with the report of Orlowski et al (1980). Also, *N*-acetyl-L-γ-glutamylsulphamethoxazole was completely converted to sulphamethoxazole in the kidney and no *N*-acetyl-L-γ-glutamylsulphamethoxazole and other metabolites were detected. Thus, there must be species differences in the distribution of *N*-acetyl-L-γ-glutamylsulphamethoxazole or the conversion of the derivative to parent drug, or both, in the kidney. It is necessary to take account of species differences when designing prodrugs, and to expect tissue-localized enzyme activity.

In conclusion, *N*-acetyl-L-γ-glutamyl-derivatization could be useful for kidney-specific drug delivery and preliminary data suggest that lipophilic drugs are better substrates than hydrophilic compounds. From the standpoints of tissue selectivity, the stability of *N*-acetyl-L-γ-glutamyl derivatives in the tissues and species differences it will be necessary to investigate drugs with a great variety of physicochemical characteristics.

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