International Journal of Pharmaceutics, 103 (1994) 1–7

IJP 03317

Research Papers

Evaluation and improvement of bioavailability of a new angiotensin II receptor antagonist, 2-butyl-1-[2′-(1H-tetrazol-5-yl)biphenyl-4-yl] methyl-1H-benzimidazole-7-carboxylic acid by making prodrug [1]

Yoshinobu Yoshimura [a], Norio Tada [a], Keiji Kubo [b], Miwa Miyamoto [c], Yoshiyuki Inada [b], Kohei Nishikawa [b] and Takehiko Naka [d]

[a] *Drug Safety Research Laboratories, Pharmaceutical Development Division,* [b] *Pharmaceutical Research Laboratories I and* [c] *Pharmaceutical Research Promotion, 2-17-85 Juso-Honmachi, Yodogawa-ku, Osaka 532 (Japan) and* [d] *Pharmaceutical Research Division and Discovery Research Laboratories II, Discovery Research Division Pharmaceutical Group, Takeda Chemical Ind. Ltd, 10 Wadai, Tsukuba, Ibaragi 300-42 (Japan)*

(Received 22 March 1993)
(Modified version received 17 May 1993)
(Accepted 22 May 1993)

Key words: Angiotensin receptor antagonist; Prodrug; Bioavailability

Summary

The bioavailability (BA) of a new angiotensin II (AII) receptor antagonist, 2-butyl-1-[[2′-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]-1H-benzimidazole-7-carboxylic acid (**I**) in rats was only 5.7%. No improvement in BA by water solubility and foods was observed. To improve the BA of **I** by making a prodrug, the BA as **I** after oral dosing of the esters of **I** to rats was evaluated. The BAs of the parent compound (**I**) after oral dosing of simple alkyl esters (**II–IV**) and substituted alkyl esters (**V–VII**) of **I** to rats were generally lower than those after dosing of **I**. These esters were scarcely hydrolyzed to **I** in rat plasma and small intestine homogenate. However, the BA of methyl ester (**II**) after oral dosing of **II** was improved to 30%. These results imply that the permeability across the intestinal membrane is increased by the esterification of **I**. On the other hand, the BA of **I** after oral dosing of the pivaloyloxymethyl ester (**VIII**) hydrolyzed to **I** rapidly in vitro was improved considerably to 52.8%. However, the BA as **I** after oral dosing of the *N*-pivaloyloxymethyltetrazole derivative (**IX**) was not improved. From this study, we demonstrate that it is possible to improve BA by making an ester-type prodrug which hydrolyzes to **I** rapidly during the absorption process.

Correspondence to: Y. Yoshimura. Drug Safety Research Laboratories, Takeda Chemical Industries Ltd, 2-17-85 Jusohonmachi, Yodogawa-ku, Osaka 532, Japan.

[1] This work has been presented at the 113th Annual Meeting of the Pharmaceutical Society of Japan, Osaka, Japan, March, 1993.

Introduction

Angiotensin II (AII) receptor antagonists have been demonstrated as new antihypertensive agents (Timmermans et al., 1991). Since the discovery by Furukawa et al. (1981) and Nishikawa

et al. (1991) of non-peptide AII receptor antagonists, 1-benzimidazole-5-acetic acids, many imidazole derivatives have been synthesized (Duncia et al., 1992).

Recently, Kubo et al. (1993a) prepared a series of 1-[[2′-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]-1H-benzimidazole-7-carboxylic acids by drug design on the basis of Furukawa's results. Shibouta et al. (1993) reported that these compounds showed potent antihypertensive activity against spontaneously hypertensive rats and the inhibitory effect of the induced pressor response induced by AII in conscious rats. Among the compounds, 2-butyl-1-[[2′-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]-1H-benzimidazole-7-carboxylic acid (**I**) showed good antihypertensive activity.

As I has two acidic groups, we speculate that the bioavailability (BA) of **I** is too low to use an orally active antihypertensive agent. Thus, we evaluated the BA of **I** after oral dosing of **I** and its derivatives (**II–IX**) as shown in Scheme 1.

Materials and Methods

2-Butyl-1-[[2′-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]-1H-benzimidazole-7-carboxylic acid (**I**) and its derivatives (**II–IX**) were prepared by Takeda Chemical Ind. Ltd (Kubo et al., 1993b). The reagents were of analytical grade and were purchased from Wako Pure Chemical Ind. Ltd (Osaka, Japan).

Instruments and conditions

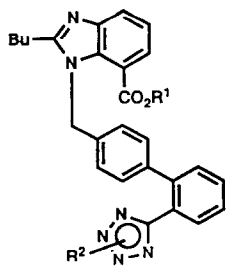
The HPLC system consisted of an LC-9A pump, an SPD-6AV, and an SIL-6A autosampler, all of which were controlled by an SCL-6A controller (all from Shimadzu, Kyoto, Japan). A CR-4AT integrator (Shimadzu) was used for quantitation. A YMC ODS A302 column (150 × 4.6 mm i.d., Yamamura Chemical, Kyoto, Japan) was used. The mobile phase was 0.05 M KH_2PO_4/MeCN (55 : 45, v/v) for **I** and 0.05 M KH_2PO_4/MeCN (2:3, v/v) for **II**, respectively. The flow rate was 0.8 ml/min. Detection was carried out at 258 nm and the injection volume of the sample was 100 μl.

Preparation of standard solution

I was prepared as a methanol solution at a concentration of 1000 μg/ml, which was diluted with 1/15 M phosphate buffer (pH 7.4) to prepare the standard solution in the range of 0.01–100 μg/ml. **II** was also prepared as a standard solution in the range of 0.01–100 μg/ml with the mobile phase mentioned above.

Hydrolysis to I from the ester in vitro

Preparation of enzyme solution A male Sprague-Dawley rat (Clea Japan Inc., Tokyo, Japan) was starved but had free access to water for 16–18 h before experiments. The blood was taken from vena cava inferior under ethereal anaethesia and the rat was killed. The liver and small intestine were removed immediately. The liver was washed with ice-cold saline and homogenized with 4 volumes of saline. After centrifugation of the homogenate at 3000 rpm for 15 min, the supernatant was diluted with saline to pre-



Compound No	R^1	R^2
I	H	H
II	Me	H
III	Et	H
IV	Bu	H
V	CH_2–(N)	H
VI	CH_2CH_2OH	H
VII	CH_2CH_2–(N,O)	H
VIII	CH_2OCO^tBu	H
IX	H	CH_2OCO^tBu

Scheme 1.

pare 2% liver homogenate. The small intestine was washed to expel the luminal contents with ice-cold saline, and homogenized with 4 volumes of saline. After centrifugation at 3000 rpm for 15 min, the supernatant was diluted to prepare 1% small intestine homogenate with saline. Blood was centrifuged to obtain plasma at 3000 rpm for 15 min, and the plasma was diluted with 9 volumes of saline to prepare 10% plasma.

Hydrolysis studies A methanol solution of the ester (concentration 10 μg/ml, 100 μl) was added to plasma (900 μl) or homogenate (900 μl) preheated at 37°C for 3 min, and then incubated at 37°C. An aliquot of the sample (100 μl) was withdrawn at 10, 40, 60, 90 s, 2, 5, 10, 30, and 60 min after incubation, and deproteinized with methanol (300 μl). After centrifugation at 10000 rpm and 4°C for 10 min, the supernatant (300 μl) was evaporated to dryness in vacuo under centrifugation. The residue was dissolved in 1/15 M phosphate buffer (pH 7.4, 200 μl), and injected to HPLC with an autosampler for the determination of the **I** produced.

Administration studies

Male Sprague-Dawley rats (Clea Japan Inc., Tokyo, Japan), weighing about 330 g, were starved but had free access to water for 16–18 h before experiments except for the study on the effect of food on bioavailability.

I was administered intravenously into tail vein at a dose of 1 mg/ml per kg as an aqueous solution with equimolar quantity of $NaHCO_3$. Also, the methyl ester (**II**) was administered intravenously to rats at a dose of 1 mg/ml per kg equivalent to **I** as a solution of dimethylsulfoxide/polyethylene glycol 400 (1:1, v/v). Blood was taken from ophthalmic venus plexus at 0.083, 0.167, 0.25, 0.5, 1, 2, 3, 5, 7, and 24 h after injection, and centrifuged to obtain plasma at 3000 rpm for 10 min.

I was administered to rats orally at a dose of 10 mg/ml per kg as an aqueous suspension of 0.5% gum arabic or an aqueous solution with an equimolar quantity of $NaHCO_3$. Also the ester (**II–IX**) was given orally to rats at a dose of 10 mg/ml per kg equivalent to **I** as an aqueous suspension of 0.5% gum arabic. Blood was taken from the opthalmic venus plexus at 0.25, 0.5, 1, 2, 3, 5, 7 and 24 h after dosing, and centrifuged to obtain plasma. The plasma samples were stored at −20°C until analysis.

The plasma (200 μl) was deproteinized with methanol (600 μl), vortex-mixed for 30 s, and centrifuged at 10000 rpm and 4°C for 10 min. The supernatant (600 μl) was transferred into a 1.5 ml polypropylene tube, and evaporated to dryness in vacuo under centrifugation. The residue was dissolved in 1/15 M phosphate buffer (pH 7.4, 200 μl) for **I** and the mobile phase for **II** and centrifuged at 10000 rpm and 4°C for 10 min. The supernatant was transferred into a 200 μl polypropylene tube for the autosampler. The concentration of **I** was determined by HPLC.

Standard solution (20 μl) was added to rat blank plasma (180 μl), and mixed with a vortex mixer to prepare plasma standard solution in the range of 0.01–10 μg/ml of **I** or **II**. Methanol (600 μl) was added to this plasma solution, and mixed with the vortex mixer. After centrifugation at 4°C and 10000 rpm for 10 min, the supernatant (600 μl) was transferred into a 1.5 ml polypropylene tube, and evaporated to dryness in vacuo under centrifugation. The residue was dissolved in 1/15 M phosphate buffer (pH 7.4, 200 μl) for **I** and the HPLC mobile phase for **II** and centrifuged at 4°C and 10000 rpm for 10 min. The supernatant was transferred into a 200 μl polypropylene tube for the autosampler. The concentration of **I** was determined by HPLC. These recovery tests were carried out during each experiment and used for the calibration curves. The detection limits of **I** and **II** in these methods were 10 ng/ml.

Calculation of pharmacokinetic parameters

The bioavailability (BA) was calculated from the ratio of area under the plasma concentration-time curve after oral administration (AUC) to that after intravenous administration. The AUC was calculated via the trapezoidal rule and AUC (0–∞) was determined from the terminal three points by the log-linear method. Curve fitting was carried out by APAS as presented by Yamaoka and Tanigawara (1983), and the half-life was calculated from the pharmacokinetic parameters obtained via APAS.

TABLE 1

Plasma concentration after intravenous and oral administration of **I** *to rats at doses of 1 and 10 mg / kg, respectively*

Route	Conditions	Plasma concentration (μg/ml) (mean ± SE, n = 3)									AUC_{inf} (μg h ml^{-1})	BA (%)
		0.08	0.17	0.25	0.5	1	2	3	5	7		
i.v.	fasted	11.0	7.99	6.45	2.30	2.05	0.77	0.42	0.29	0.19	8.07	–
		0.5	0.48	0.72	0.17	0.11	0.06	0.03	0.03	0.02		
p.o.	fasted susp.			0.28	0.49	0.52	0.61	0.51	0.37	0.26	4.56	5.7
				0.04	0.09	0.11	0.11	0.11	0.04	0.07		
	fed susp.			0.17	0.28	0.35	0.36	0.30	0.13	0.10	1.69	2.1
				0.09	0.09	0.03	0.08	0.04	0.02	0.03		
	fasted soln			0.67	0.79	0.81	0.74	0.51	0.27	0.22	4.38	5.0
				0.05	0.28	0.09	0.13	0.03	0.02	0.05		

susp, aqueous suspension with 0.5% gum arabic; soln, aqueous solution with equimolar quantity of $NaHCO_3$.

Results

I was administered intravenously to rats at a dose of 1 mg/kg. The plasma concentrations are listed in Table 1 with the area under plasma concentration-time curve for 0 to infinity (AUC_{inf}). After i.v. dosing, **I** disappeared rapidly from the plasma in biphasic fashion with half-lives of 0.14 and 1.62 h simulated by the two compartment model, and was not detected at 24 h afer dosing. AUC_{inf} was 8.07 μg h ml^{-1}.

I was given orally as a 0.5% gum arabic suspension to fasted and fed rats at a dose of 10 mg/kg. The plasma concentrations are shown in Table 1 with the AUC_{inf} and bioavailability (BA) calculated from the ratio AUC_{inf} after oral dosing to that after i.v. dosing. **I** in the plasma of the fasted rats reached a peak concentration (C_{max}) of 0.63 μg/ml at 2 h after dosing, and was not detected at 24 h. AUC_{inf} and BA were 4.56 μg h ml^{-1} and 5.7%, respectively. On the other hand, **I** in the plasma of the fed rats showed a C_{max} of 0.36 μg/ml at 2 h after dosing and was below the detection limit at 24 h. AUC_{inf} and BA were 1.69 μg h ml^{-1} and 2.1%, respectively. No improvement in the BA of **I** by foods was observed.

Further, **I** was administered orally as an aqueous solution with equimolar sodium bicarbonate to fasted rats at a dose of 10 mg/kg. Table 1 also shows the plasma concentrations with AUC_{inf}

TABLE 2

Regenerated half-lives to **I** *from its derivatives in 1% rat small intestine homogenate, 10% plasma, and 2% liver homogenate at 37°C*

No.	R^1	R^2	Medium	Half-life (min) (mean ± SD, n = 3)
II	Me	H	1% SI [a]	stable [b]
			10% plasma	stable [b]
			2% liver	stable [b]
IV	Bu	H	1% SI	stable [b]
V	CH_2-(4-Py)	H	1% SI	stable [b]
VIII	CH_2OCO-t-Bu	H	1% SI	1.08 ± 0.06
			10% plasma	0.69 ± 0.01
			2% liver	3.61 ± 0.23
IX	H	CH_2OCO-t-Bu	1% SI	22.74 ± 0.99
			10% plasma	3.94 ± 0.32
			2% liver	9.80 ± 0.19

Final concentration was 10 μg/ml; half-life was calculated from the elimination rate constant of an ester. [a] Small intestine homogenate. [b] **I** was not detected at 60 min after incubation.

TABLE 3

Plasma concentration of I after oral administration of derivatives of I to fasted rats at a dose of 10 mg / kg as equivalent to I

Compound no.	R^1	Plasma I concentration (μg/ml) (mean ± SE, $n = 3$)								AUC_{inf} (μg h ml^{-1})	BA (%)
		0.25	0.5	1	2	3	5	7	24 h		
I	H	0.28	0.49	0.52	0.61	0.51	0.37	0.26	–	4.56	5.7
		0.04	0.09	0.11	0.11	0.11	0.04	0.07			
II	Me	1.31	2.66	2.99	0.81	0.45	0.22	0.14	0.02	8.83	10.9
		0.16	0.46	0.44	0.02	0.06	0.04	0.00	0.02		
III	Et	0.06	0.08	0.15	0.36	0.37	0.14	0.12	–	1.92	2.4
		0.05	0.05	0.08	0.14	0.19	0.07	0.03			
IV	Bu	0.02	0.02	0.02	0.02	0.03	0.07	0.04	–	0.42	0.51
		0.02	0.02	0.02	0.02	0.03	0.01	0.02			
V	CH_2Py [a]	0.04	0.06	0.09	0.07	0.04	0.04	0.02	–	0.45	0.55
		0.00	0.00	0.01	0.01	0.01	0.02	0.02			
VI	C_2H_4OH	–	–	–	–	0.03	0.06	0.04		0.40	0.50
						0.03	0.02	0.01			
VII	C_2H_4Mo [b]	0.51	1.05	0.93	0.31	0.19	0.12	0.11		3.5	4.3
		0.19	0.31	0.07	0.06	0.03	0.02	0.01			
VIII	Piv [c]	8.82	8.11	6.05	7.70	5.35	2.61	1.16	0.07	42.6	52.8
		1.50	0.67	1.22	1.32	1.16	0.56	0.22	0.00		
IX	Tet-Piv [d]	0.04	0.04	0.01	0.00	–	–	–	–	0.03	0.5
		0.02	0.01	0.00	0.00						

[a] CH_2Py, CH_2–  ; [b] C_2H_4Mo, CH_2CH_2–  ; [c] Piv, CH_2OCO-t-Bu; [d] Tet-Piv.

and BA. **I** in the plasma reached a C_{max} of 0.81 μg/ml at 1 h, and was not detected at 24 h after dosing. AUC_{inf} and BA were 4.38 μg h ml^{-1} and 5.0%, respectively. These results indicate that water solubility does not have an effect on the BA of **I**.

To improve the BA of **I** by making a prodrug, we evaluated BA as **I** for the compounds modified at the carboxyl group (**II–VIII**) and the tetrazole group (**IX**) of **I**. Furthermore, we also investigated the hydrolysis to **I** from these compounds in vitro.

Table 2 shows the hydrolysis rates to **I** from the ester in rat 1% small intestine, 2% liver homogenate at 10% plasma at 37°C. Methyl ester (**II**), butyl ester (**IV**) and 4-pyridinylmethyl ester (**V**) were stable in the 1% small intestine homogenate, and **I** was not detected at 60 min after

TABLE 4

Plasma concentrations of I and II after intravenous and oral administration of II to fasted rats at doses of 1 and 10 mg / kg equivalent to I, respectively

Route	Compd	Plasma concentration (μg/ml) (mean ± SE, $n = 3$)										AUC_{inf} (μg h ml^{-1})
		0.08	0.17	0.25	0.5	1	2	3	5	7	24 h	
i.v.	**II**	1.03	0.79	0.66	0.56	0.36	0.32	0.30	0.28	0.28	0.20	18.33
		0.11	0.44	0.09	0.29	0.04	0.04	0.03	0.04	0.04	0.03	
	I	9.88	6.39	5.26	1.76	0.97	0.50	0.36	0.22	0.16	–	6.05
		0.89	1.77	0.81	0.21	0.18	0.13	0.10	0.07	0.05		
p.o.	**II**			2.38	4.25	2.49	1.05	0.84	0.64	0.59	0.59	53.3
				0.42	0.56	0.49	0.03	0.04	0.04	0.03	0.05	
	I			1.31	2.66	2.99	0.81	0.45	0.22	0.14	0.02	8.83
				0.16	0.46	0.44	0.02	0.06	0.04	0.00	0.02	

incubation of **II** in 1% small intestine homogenate. **II** was also stable in 2% rat liver homogenate and 10% plasma. The compounds bearing a pivaloyloxymethyl moiety at the carboxyl group (**VIII**) or the tetrazolyl group (**IX**) were hydrolyzed to **I** with a half-life of 1.08 and 22.7 min, respectively, in 1% small intestine homogenate. Also, **VIII** and **IX** were hydrolyzed rapidly to **I** in 10% plasma and 2% liver homogenate. The hydrolysis rate of **VIII** was faster than that of **IX**.

Table 3 lists the plasma concentrations of **I** after oral administration of **II**–**IX** to fasted rats at doses of 10 mg/kg equivalent to **I**. Also, Table 4 shows both plasma concentrations of **I** and **II** after intraveneous and oral administration of **II** to fasted rats at doses of 1 and 10 mg/kg, respectively, equivalent to **I**.

Among the simple alkyl esters (**II**–**IV**) and substituted alkyl esters (**V**–**VII**), **I** in the plasma after oral dosing of **II** showed a higher C_{max} (2.99 μg/ml) at 1 h, and was also detected at 24 h. The C_{max} of **I** after dosing of **II** was improved about 4-fold compared with that after dosing of **I**. AUC_{inf} of **I** after dosing of **II** was 8.83 μg h ml^{-1} and BA was 10.9%.

As shown in Table 4, the AUC_{inf} values of **I** and **II** after i.v. dosing of **II** at a dose of 1 mg/kg equivalent to **I** were 6.05 and 18.83 μg h ml^{-1}, respectively. **II** in the plasma after oral dosing of **II** at a dose of 10 mg/kg reached a C_{max} of 4.25 μg/ml at 0.5 h, and was detected at 24 h. The AUC_{inf} values of **I** and **II** were 8.83 and 53.3 μg h ml^{-1}, respectively. From the ratio of $AUC_{p.o.}$ to $AUC_{i.v.}$ of **II**, the BA of **II** was about 30%.

C_{max} values of **I** after oral dosing of **III** and **IV** were low and decreased with increasing carbon number of the ester moiety. BA as **I** after dosing of **III** and **IV** was 2.4 and 0.5%, respectively.

The plasma concentration of **I** after oral dosing of substituted alkyl esters (**V**–**VII**) was lower than that after dosing of **II**. BA as **I** after dosing of **V**–**VII** was 0.55, 0.5, and 4.3%, respectively.

On the other hand, the plasma concentration of **I** after dosing of pivaloyloxymethyl ester (**VIII**) was more improved compared with that after dosing **I**. The C_{max} of **I** was 8.82 μg/ml at 0.25 h after dosing, and BA as **I** after dosing of VIII was 52.8%, which was improved 9-fold compared with that after dosing of **I**. However, the BA of **I** after dosing of **IX**, which has a pivaloyloxymethyl moiety in the tetrazole ring of **I**, was 0.5%.

Discussion

Bioavailability after oral dosing of **I** to rats was only 5.7%. No effect of foods and water solubility on the BA of **I** was observed. **I** has two acidic groups, i.e., the carboxyl group and the tetrazole ring, in the molecule which is a pseudo dicarboxylic acid. We speculated that the low BA of **I** was caused by the dissociation of the two acidic groups. Thus, we attempted to improve the BA of **I** by masking the dissociated groups. In the case of the esterification of the carboxyl group, the BA as **I** after oral dosing of alkyl or substituted alkyl esters was not improved except for the methyl ester (**II**). Also, these esters were scarcely hydrolyzed to **I** in the in vitro study. However, the BA of **II** after dosing of **II** amounted to 30%, which was improved compared with that of I after dosing of **I**. These results indicate that the permeability of **I** through the intestinal membrane was increased by the esterification of **I**. Furthermore, the results suggest that hydrolysis of the ester to **I** had a considerable effect on the BA as **I**. The pivaloyloxymethyl ester (**VIII**), which was hydrolyzed to **I** rapidly in vitro, showed the highest BA as **I**. These data indicate that the 1-acyloxyalkyl and/or 1-alkoxycarbonyloxyalkyl moiety, which have been using to improve the oral absorption of β-lactam antibiotics (Yoshimura et al., 1985, 1986, 1987; Nishimura et al., 1987), is preferred to improve the BA of **I**. However, no improvement in the BA of **I** by pivaloyloxymethylation (**IX**) in the tetrazole ring was observed, although **IX** was hydrolyzed rapidly to **I** in vitro. These results suggest that the carboxyl group is preferred in the masking position compared with the tetrazole ring of **I**.

In conclusion, these results imply that to improve the BA of **I** it is necessary to design an ester which hydrolyzes to **I** readily during the absorption process. We believe that this conclusion is applicable to the improvement of BA of

other AII receptor antagonists bearing a similar structure to that of **I**.

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