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Drug delivery to the brain. DOPA prodrugs based on a ring-closure reaction to quaternary thiazolium compounds

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Abstract

In order to explore the possibility of an alternative redox chemical delivery system to a dihydropyridine-pyridinium interconversion system, we prepared DOPA prodrugs coupled with thiazolium precursors, like thiamine disulfides, by forming an ester bond with the amino acid carboxylic moiety while protecting the catechol function with pivalyl groups. The disposition of the prodrugs was evaluated by measuring the concentrations of DOPA regenerated after intravenous administration of the prodrugs and the results were compared with those for DOPA itself. The plasma levels of DOPA demonstrated no significant differences between DOPA and the prodrugs. In contrast, however, brain levels of DOPA were remarkably elevated following administration of the prodrugs. Among the prodrugs examined, ZiPr-DOPA(P)$_2$ was found to most efficiently facilitate delivery of DOPA to brain and this compound showed 30- and 3.7-fold greater increases in the AUC and MRT of DOPA in brain, respectively, than did DOPA itself. These findings suggest that a redox ring-closure system to a quaternary thiazolium can be used as an alternative chemical delivery system to the brain.

Keywords: Drug delivery system; Prodrug; Brain; Thiazolium; Thiamine disulfide; DOPA; Redox reaction

1. Introduction

Bodor and co-workers have established redox chemical delivery systems (CDS) to the brain based on dihydropyridine-pyridinium salt interconversion systems (Bodor et al., 1981). They have applied the system to several drugs including peptides, e.g., enkephalin (Bodor et al., 1992), and there are also some other research groups working along similar lines (Chu et al., 1990; Boddy et al., 1991; Torrence et al., 1993). In addition to being very interested in this concept, we are also concerned by the technical limitations of this approach, the facile oxidation of the dihydropyridine-modified prodrugs by oxygen in air as well as in solution, making even i.v. formulation difficult (Shanmuganathan et al., 1994).

To improve the stability of prodrugs, we have observed the conversion of *cis*-2-formylaminoethenylthio derivatives to the corresponding quaternary thiazolium, and considered that it could be applied to an alternative drug delivery system to brain. Thiamine disulfide (TDS) is one of the most familiar representatives of the *cis*-2-for-

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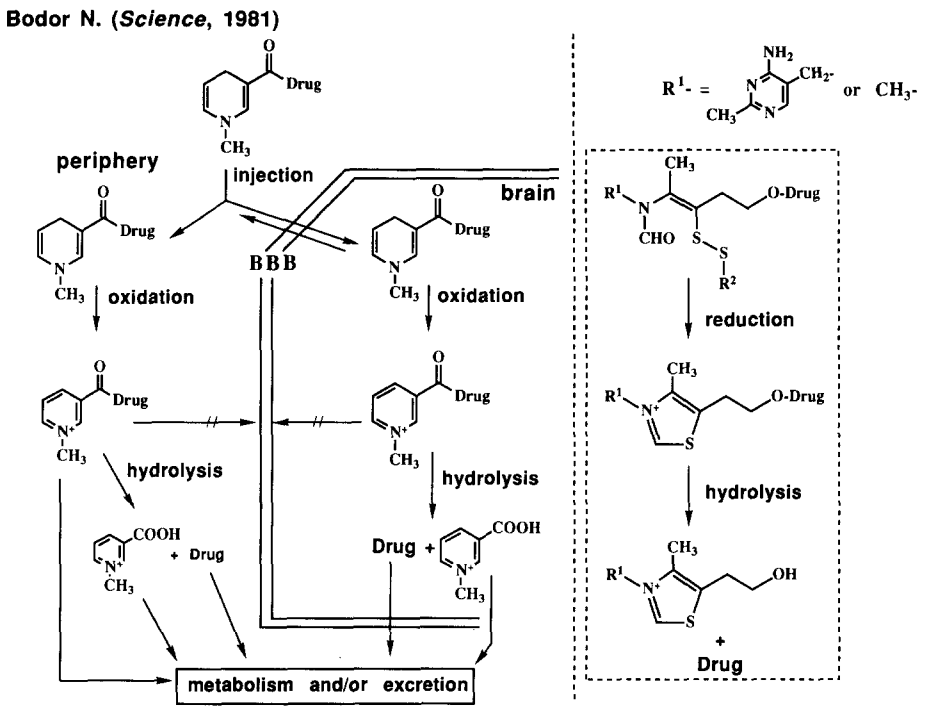


Fig. 1. Sequential metabolism of a redox chemical delivery system based on a ring-closure reaction to thiazolium (within the dashed area) in comparison with Bodor's dihydropyridine/pyridinium system.

mylaminoethenylthio derivatives (Zima and Williams, 1940; Fujiwara and Watanabe, 1952; Matsukawa and Yurugi, 1952) and has been clinically used as a fat-soluble precursor of thiamine (vitamin B1) which has a quaternary thiazolium moiety in its structure. The conversion reaction, which proceeds mainly via glutathione and hemoglobin (Hamada et al., 1967; Utsumi et al.,

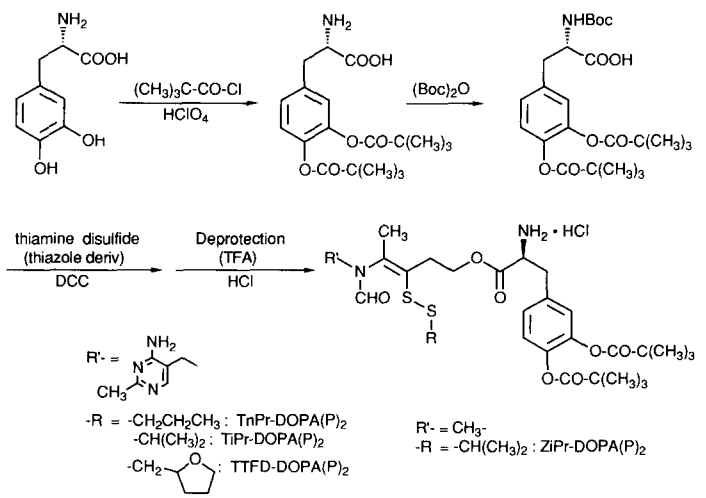


Fig. 2. Reaction scheme of the synthesis of DOPA prodrugs with thiazolium precursors in their molecules.

1968), is similar to that of Bodor's CDS, however, the reactions of TDS with thiamine and Bodor's CDS are reduction and oxidation, respectively.

There are some conjugates of TDS, including pyridoxine (Iwanami et al., 1968) or baclofen (Masaki et al., 1989). However, these conjugates were not primarily designed for the ring-closure reaction of TDS. The objective of the present study was to explore the possibility of thiazolium precursors as an alternative carrier for redox chemical delivery systems to the brain.

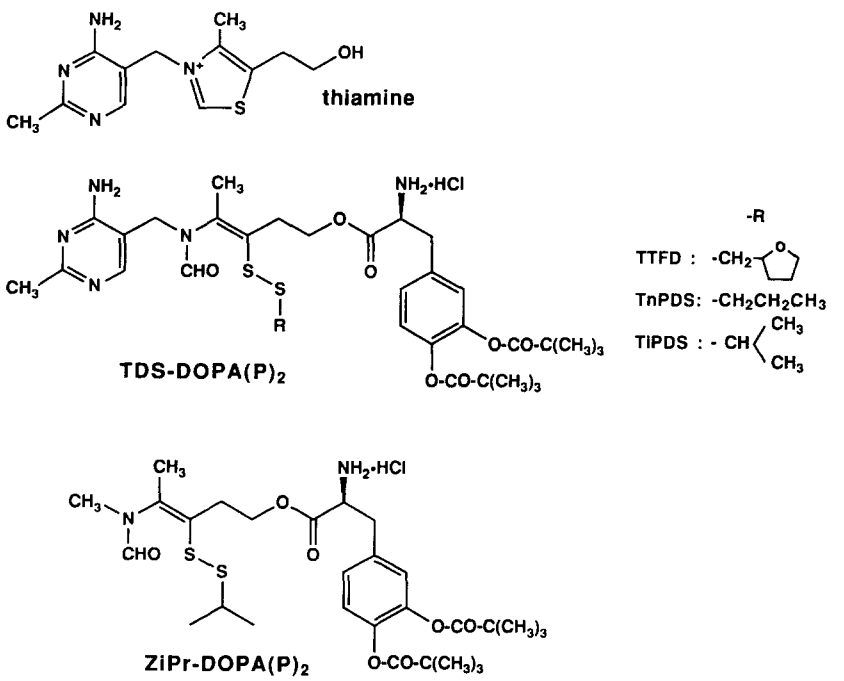
Fig. 1 illustrates the schematic conversion of the redox ring-closure system to brain by referring to that of Bodor and co-workers. Generally, the reduction of disulfide is considered to proceed more rapidly than the hydrolysis (drug release) while both reactions would occur simultaneously in the body.

We selected L-3,4-dihydroxyphenylalanine (DOPA) as a model drug. DOPA has been used for the treatment of Parkinson's disease in an oral dosage form. DOPA is considered to be a precursor of dopamine, a neurotransmitter in the brain, which can scarcely be transported across the blood-brain barrier (BBB). Recently, it has been demonstrated that DOPA itself also acts as a neurotransmitter (Misu and Goshima, 1993). We synthesized the ester prodrugs from DOPA and thiazolium precursors including TDS, as illustrated in Fig. 2 and determined concentrations of DOPA and the metabolites in the brain, plasma and other organs following intravenous administration of 5 mg/kg of DOPA or an equimolar dose of the prodrugs in rats. In comparison with DOPA, the prodrugs not only increased the delivery of DOPA to brain, but also prolonged the mean residence time (MRT) of DOPA in brain. Other metabolites detected are also discussed.

2. Materials and methods

2.1. Materials

DOPA and 3,4-dihydroxybenzylamine were purchased from Sigma Chemical Co. (St. Louis, MO, USA), thiamine hydrochloride and alumina activated for catecholamine measurement from Wako Pure Chemical Industries Ltd (Osaka,



Scheme 1. Structures of thiamine and the prodrugs.

Japan), and 4-methyl-5-thiazoleethanol from Aldrich Chemical Co. Inc. (Milwaukee, WI, USA). Other chemicals used were of reagent grade.

2.2. Synthesis

Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained on a Varian VXR-500S (500 MHz, FT mode). Samples were dissolved in an appropriate solvent and chemical shifts (δ) reported in ppm relative to tetramethylsilane as an internal standard. Column chromatography was performed with Wakogel C-200 (silica gel, Wako Pure Chemical Industries, Ltd, particle size, 75–150 μm).

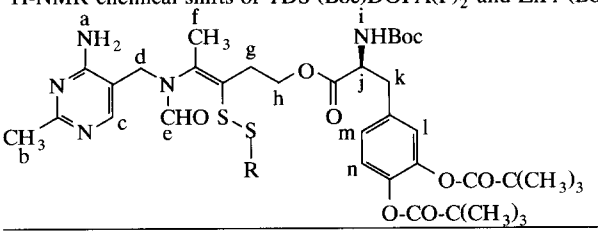
2.2.1. TDSs (N-(2-alkyldithio-4-hydroxy-1-methyl-1-butenyl)-N-[(4-amino-2-methyl-5-pyrimidyl)methyl]formamide)

TDSs were synthesized according to the method reported by Matsukawa et al. (1953) To an aqueous solution of thiamine hydrochloride and 3 × equimolar sodium hydroxide was added an equimolar amount of the corresponding sodium alkylthiosulfate which was obtained from sodium thiosulfate and the corresponding alkyl halide. The resultant viscous substance was extracted with chloroform. The solvent was removed under reduced pressure and the white residue obtained was crystallized from an appropriate solvent.

2.2.2. (Boc)DOPA(P)$_2$ (N-tert-butyloxycarbonyl-3,4-dipivalyloxy-L-phenylalanine)

To 250 ml of a mixed solution (water/dioxane = 1:2) of 3,4-dipivalyloxy-L-phenylalanine perchlorate (DOPA(P)$_2$ · HClO$_4$, 36 g, 77.3 mmol), which was synthesized according to the method of Bodor et al. (1977), was added an excess of sodium bicarbonate and the mixture then ice-cooled. To the solution was added di(*tert*-butyl) dicarbonate (20 g, 91 mmol) followed by stirring for 2 h at

Table 1
^{1}H-NMR chemical shifts of TDS-(Boc)DOPA(P)$_2$ and ZiPr-(Boc)DOPA(P)$_2$ with its chemical structure



Pos.,no. [a]	TTFD ($CDCl_3$)	TnPr (DMSO)	TiPr (DMSO)	ZiPr ($CDCl_3$)
a,2H	5.97,broad	6.78,broad	6.79,broad	–
b,3H	2.45,s	2.27,s	2.27,s	–
c,e,2H	7.90,7.81,s	7.90,7.87,s	7.93,7.88,s	7.90,s
d,2H	4.8,4.1,broad	4.40,broad	4.40,broad	1.93 (3H,s,CH_3)
f,3H	1.95,s	2.04,s	2.04,s	2.94,s
g,2H	2.88,broad	2.83,m	2.84,broad m	2.92–2.86,m
h,2H	4.24,4.12,	4.09,m	4.07,m	4.21,m
i,1H	5.18,broad	7.39,d	7.37,d	–
j,1H	4.52,m	4.20,m	4.20,m	4.54,m
k,2H	3.06,m	3.01,2.88,m	3.02,2.89,m	3.06,d
l,m,n,3H	7.07–6.93,m	7.18–7.09,m	7.18–7.09,m	7.06–6.91,m
Boc,9H	1.42,s	1.33,s	1.34,s	1.43,s
Pivalyl,18H	1.33,s	1.27,1.26,s	1.28,1.26,s	1.330,1.328,s
R	3.88 (1H,m),	2.32 (2H,t)	2.59 (1H,m)	2.92–2.86 (1H,m)
	3.84,3.72 (1H,m)	1.40 (2H,m)	1.02 (6H,d)	1.26 (6H,d)
	2.56 (2H,d),2.0	0.85 (3H,t)		
	(1H,m),1.89 (2H,m),1.54 (1H,m)			

[a] Position of the structure and number of proton.

room temperature. The solution was neutralized with an aqueous solution of citric acid and condensed under reduced pressure. The syrupy residue obtained was dissolved in ethyl acetate, washed with water a couple of times, dried over anhydrous sodium sulfate and the solvent was evaporated in vacuo. Purification via silica gel column chromatography gave a colorless foam of (Boc)DOPA(P)$_2$ (21.5 g, 46.2 mmol, 59.7%). ^{1}H-NMR (in CD_3OD): δ ppm 7.2–7.0 (3H,m,aromatic), 4.35 (1H,q,α-CH), 3.18 and 2.94 (each 1H, each dd,-CH_2-Ph), 1.40 (9H,s,Boc), 1.33 (18H,s, pivalyl-CH_3).

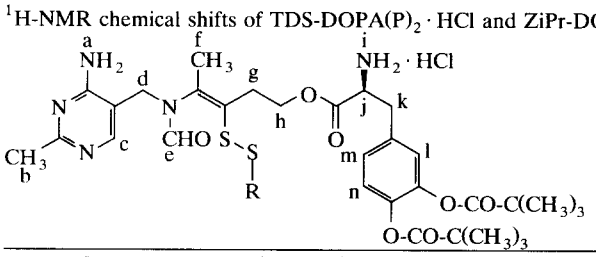
2.2.3. General procedure for the synthesis of TDS-(Boc)DOPA(P)$_2$ (3-alkyldithio-4-[(4-amino-2-methyl-5-pyrimidinyl)methyl]formylamino-3-pentenyl 2-(tert-butyloxycarbonyl)amino-3-(3,4-dipivalyloxyphenyl)propionate)

(Boc)DOPA(P)$_2$ (5 mmol), TDS (5.5 mmol) and 4-dimethylaminopyridine (1 mmol) were dissolved in a mixture of acetonitrile (20 ml) and tetrahydrofuran (20 ml), and the solution was then ice-cooled. To the solution was added dicyclohexyl carbodiimide (5.2 mmol), then stirred for about 3 h in an ice bath. A white precipitate was filtered off, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography, giving a white foam of TDS-(Boc)DOPA(P)$_2$ (3.5–4.5 mmol). ^{1}H-NMR data are summarized in Table 1 with its chemical structure.

2.2.4. General procedure for the synthesis of TDS-DOPA(P)$_2$ · HCl (3-alkyldithio-4-[(4-amino-2-methyl-5-pyrimidinyl)methyl]formylamino-3-pentenyl 2-amino-3-(3,4-dipivalyloxyphenyl)propionate hydrochloride)

TDS-(Boc)DOPA(P)$_2$ (5 mmol) was ice-cooled and then trifluoroacetic acid (20 ml) was added dropwise, the resultant solution being stood in an ice bath for about 1 h. To an aqueous suspension (100 ml) of sodium bicarbonate was added the trifluoroacetic acid solution dropwise, the resultant white viscous precipitate was extracted with ethyl acetate, the organic layer was washed with water, and then dried over anhydrous sodium sulfate. The solvent was evaporated under re-

Table 2
^{1}H-NMR chemical shifts of TDS-DOPA(P)$_2$ · HCl and ZiPr-DOPA(P)$_2$ · HCl with its chemical structure



Pos.,no. [a]	TTFD (CD_3OD)	TnPr (DMSO)	TiPr (DMSO)	ZiPr (DMSO)
a,i,4H	–	8.6,7.2,broad	8.7,7.2,broad	8.53,3H,broad
b,3H	2.41,s	2.32,s	2.33,s	–
c,e,2H	7.97,7.85,s	7.93,2H,s	7.96,2H,s	7.99,7.83,s
d,2H	4.6,broad	4.42,broad	4.42,broad	3.00,2.84,s
f,3H	2.04,s	1.99,s	2.00,s	1.92,1.85,s
g,2H	2.93,broad m	2.79,t	2.79,t	2.81,m
h,2H	4.29,4.15,broad	4.15,4.05,m	4.12,4.03,m	4.23–4.11,m
j,1H	4.33,m	4.36,t	4.36,t	4.33,m
k,2H	3.21,t	3.20,3.08,m	3.21,3.09,m	3.19,3.09,m
l,m,n,3H	7.23–7.13,m	7.2–7.16,m	7.2–7.17,m	7.19,7.16,s
pivalyl,18H	1.342,1.339,s	1.28,1.27,s	1.28,1.27,s	1.28,1.27,s
R	3.88 (1H,m),	2.38 (2H,t)	2.66 (1H,m)	2.93 (1H,m)
	3.81,3.71 (1H,m)	1.42 (2H,m)	1.04 (6H,d)	1.20 (6H,d)
	2.63 (2H,d),2.00	0.86 (3H,t)		
	(1H,m),1.89 (2H,m), 1.57 (1H,m)			

[a] Position of the structure and number of proton.

duced pressure, the colorless syrup obtained being purified by silica gel column chromatography. The colorless syrup obtained (4 mmol) was dissolved in ethanol (100 ml), 1 N HCl (4 ml) added, and concentrated under reduced pressure. The residue was dissolved in a small amount of chloroform and the solution was added dropwise into ether (200 ml) with stirring. The resultant white precipitate was collected by filtration and dried under reduced pressure (TDS-DOPA(P)$_2$ · HCl, 3–3.5 mmol). ^{1}H-NMR data are summarized in Table 2 with its chemical structure.

2.2.5. Me-MTE$^+$ I$^-$ (N,4-dimethyl-5-[2-(hydroxy)ethyl]thiazolium iodide)

4-Methyl-5-thiazoleethanol (100 g, 0.7 mol) and methyl iodide (180 g, 1.3 mol) were mixed and refluxed for 2 h. After evaporation of excess methyl iodide, to the residual brown syrup was added ether (400 ml), followed by trituration a couple of times, yielding Me-MTE$^+$ I$^-$ (202 g, 0.7 mol, 100%). ^{1}H-NMR (in CD_3OD): δ ppm 4.13 (3H,s,CH_3-N), 3.81 (2H,t, -CH_2CH_2-O-), 3.11 (2H,t,-CH_2CH_2-O-), 2.51 (3H,s,CH_3-C =).

2.2.6. ZiPr (N-methyl-N-[4-hydroxy-1-methyl-2-(2-propyl)dithio-1-butenyl]formamide)

To a 50 ml aqueous solution of sodium hydroxide (8.0 g, 200 mmol) and Me-MTE$^+$ I$^-$ (28.6 g, 100 mmol) was added sodium isopropyl thiosulfate (43 g). The resultant oily substance was extracted with chloroform and the organic layer was dried over sodium sulfate and concentrated under reduced pressure. Purification of the syrup by column chromatography with silica gel gave a colorless syrup of ZiPr (20.7 g, 83 mmol, 83%). ^{1}H-NMR (in $CDCl_3$): δ ppm 7.99 and 7.95 (1H,each s,-N-CHO), 3.78 (2H,t,-CH_2CH_2-OH), 2.97 (3H,s,CH_3-C = C), 2.93–2.87 (3H,m,-CH_2CH_2-OH and -$CH(CH_3)_2$), 2.00 (3H,s,CH_3-N-CHO), 1.27 (6H,d,-CH(CH_3)$_2$).

2.2.7. ZiPr-(Boc)DOPA(P)$_2$ (4-methylformylamino-3-(2-propyl)dithio-3-pentenyl 2-(tert-butyloxycarbonyl)amino-3-(3,4-dipivalyloxyphenyl) propionate)

(Boc)DOPA(P)$_2$ (7.0 g, 15 mmol), ZiPr (3.72 g, 14.9 mmol), and 4-dimethylaminopyridine (245 mg, 1.5 mmol) were dissolved in acetonitrile (30 ml) and the solution was ice-cooled. To this solution was added dicyclohexyl carbodiimide (3.2 g, 15.5 mmol), followed by stirring for 3 h in an ice-cooled water bath. A white precipitate was filtered off and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography, to give a white foam of ZiPr-(Boc)DOPA(P)$_2$ (8.26 g, 11.9 mmol, 79.5%).

2.2.8. ZiPr-DOPA(P)$_2$ · HCl (4-methylformylamino-3-(2-propyl)dithio-3-pentenyl 2-amino-3-(3,4-dipivalyloxyphenyl)propionate hydrochloride)

ZiPr-(Boc)DOPA(P)$_2$ (4.6 g, 7.2 mmol) was ice-cooled and trifluoroacetic acid (15 ml) added dropwise, the resultant solution being stood for 0.5 h in an ice bath. To a 200 ml aqueous suspension of sodium bicarbonate was added the trifluoroacetic acid solution dropwise and a white viscous precipitate was extracted with ethyl acetate. The organic layer was washed with water, and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure and the colorless syrup obtained was purified by silica gel column chromatography. The colorless syrup obtained (3.95 g, 6.6 mmol) was dissolved in ethanol (100 ml), 1 N HCl (6.6 ml) added, and then concentrated under reduced pressure. The residue was dissolved in a small amount of ether and the solution was added to 200 ml of *n*-hexane, the resultant white precipitate being collected by filtration and dried under reduced pressure (3.6 g, 5.7 mmol, 78.9%).

2.3. Method of administration of prodrugs in rats

Male Sprague-Dawley (SD) rats (200–250 g body weight; age 7 weeks) were used and were allowed free access to water and food (standard laboratory chow). Rats were anesthetized with diethyl ether. An aqueous solution containing 5 mg/ml of DOPA or an equimolar dose of prodrug was injected in the femoral vein at a volume of 1 ml/kg (5 mg/kg of DOPA = 25.4 μmol/kg). At various times after administration, the rats were anesthetized again and exsanguinated through the abdominal aorta with a heparinized syringe, and the brain or other organs were col-

lected and rapidly ice-cooled after a quick rinse in saline. The blood samples were also immediately ice-cooled and centrifuged to obtain plasma. The plasma and organs obtained were frozen at −30°C prior to quantitative analysis by the method described below.

2.4. Alumina adsorption method for HPLC analysis

A high-performance liquid chromatographic (HPLC) method with electrochemical detection was performed according to the method reported by Miwa et al. (1986), following adsorption of catechols on activated alumina and elution. Not only DOPA but also its metabolites including dopamine, dopac, epinephrine, and norepinephrine could be determined by this method. No transformation of the prodrugs to DOPA was observed during the treatment.

HPLC was performed with a system consisting of a model 880-PU pump, a model 880-50 degasser and a model 850-AS auto sampler (all from Japan Spectroscopic Co., Ltd, Tokyo, Japan) and an electrochemical detector (Shimadzu Corp., Kyoto, Japan, L-ECD-6A). The potential of the electrochemical detector was set at 0.7 V against the Ag/AgCl reference electrode. The working electrode was of glassy carbon and the auxiliary electrode was of stainless steel. A reversed-phase column (Tosoh Corp. Tokyo, Japan, TSK gel ODS-80TM, 5 μm particle size, 150 × 4.6 mm i.d.) was used at 30°C. The mobile phase was 0.1 M potassium phosphate (pH 3.1) containing methanol (8%), sodium heptanesulfonate (300 mg/l, as an ion-pair reagent), and EDTA (0.1 mM), and the flow rate was 0.8 ml/min.

3. Results

3.1. Synthesis of TDS-DOPA(P)$_2$

The catechol group of DOPA was acylated with pivalyl chloride in the presence of perchloric acid (Bodor et al., 1977) to yield DOPA(P)$_2$ as the perchlorate. *N*-protection of DOPA(P)$_2$ was performed with di(*tert*-butyl) dicarbonate in aqueous dioxane as the solvent in the presence of sodium bicarbonate. The resultant (Boc)-DOPA(P)$_2$ and TDSs were condensed with dicyclohexyl carbodiimide in the presence of 4-dimethylaminopyridine to give the conjugates, TDS-(Boc)DOPA(P)$_2$. The *N*-deprotection of the conjugates was performed with trifluoroacetic acid under ice-cooled conditions, and after neutralization with aqueous sodium bicarbonate and purification with silica gel column chromatography, the amines were converted to the hydrochlorides.

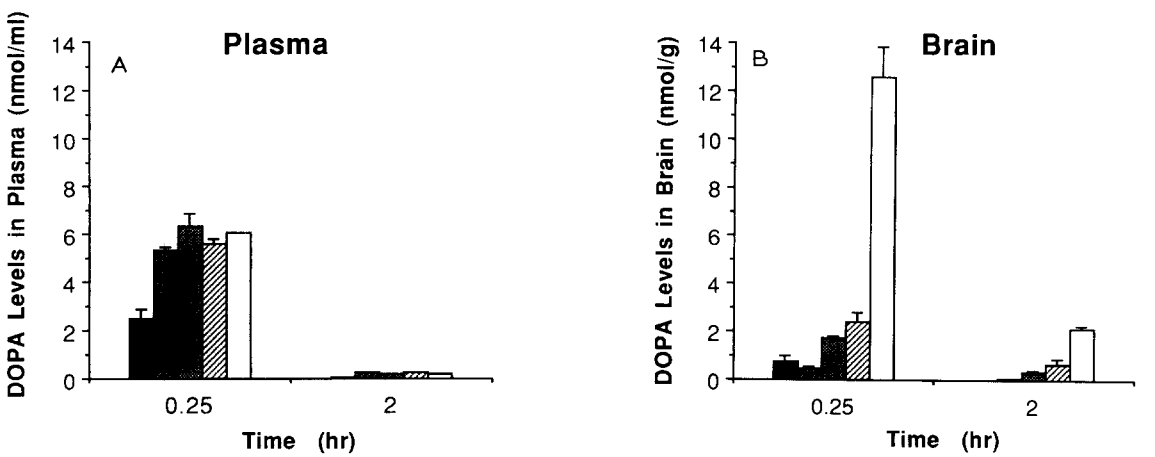


Fig. 3. DOPA concentrations in plasma (A) and brain (B) following intravenous administration of DOPA or the prodrugs at the two sampling points, 15 min and 2 h. Data are expressed as the means with error bars for standard deviations of three rats. (■) DOPA; (▨) TTFD-DOPA(P)$_2$; (▩) TnPr-DOPA(P)$_2$; (▨) TiPr-DOPA(P)$_2$; (□) ZiPr-DOPA(P)$_2$.

3.2. Synthesis of ZiPr-DOPA(P)2

N-methylation of 4-methyl-5-thiazoleethanol was performed with methyl iodide. The quaternary thiazolium was converted to the corresponding disulfide according to the method of Matsukawa et al. (1953) using sodium isopropyl thiosulfate.

In the ^{1}H-NMR spectrum of the disulfide which possesses a formamide function, the peak attributed to the hydrogen of the formamide was observed in a split pattern. The ratio of the split pattern varied according to the solvent used (data not shown) and the total integral of the peaks was equivalent to one proton, suggesting that it is caused by the rotational isomers of the *N*-formyl group. It is also observed in the conjugate with DOPA, but not in the case of TDS.

The disulfide and (Boc)DOPA(P)$_2$ were condensed, and the conjugate obtained was deprotected and converted to its hydrochloride as described above.

3.3. Evaluation of DOPA and the prodrugs at two sampling points

To evaluate DOPA delivery systems to the brain, DOPA or prodrugs were administered intravenously to rats, and the concentrations of DOPA regenerated in the brain and plasma were determined.

Fig. 3A and B show DOPA concentrations in the plasma and brain, respectively, at 15 min and 2 h after intravenous administration of DOPA (5 mg/kg) or an equimolar dose of prodrugs. Fig. 3A demonstrates that at the 15 min sampling point, all the prodrugs tested gave similar plasma concentrations of DOPA (4.8–6.1 nmol/ml); however, their levels were about double those following administration of DOPA itself. In contrast, at the 2 h sampling point, the concentrations (DOPA or the prodrugs) declined to below 0.35 nmol/ml. In the context of the plasma concentrations of DOPA, there were no significant differences among either the prodrugs or between the prodrugs and DOPA.

As shown in Fig. 3B, which illustrates DOPA concentrations in the brain, the prodrugs indicated a characteristic disposition compared with DOPA. Higher concentrations of DOPA in brain were observed following intravenous administration of the prodrugs, especially in the case of ZiPr-DOPA(P)$_2$, and levels of 0.9–2.1 nmol/g were maintained at 2 h, corresponding to 2.6–11-fold greater values than those in plasma at the same sampling point. Intravenous administration

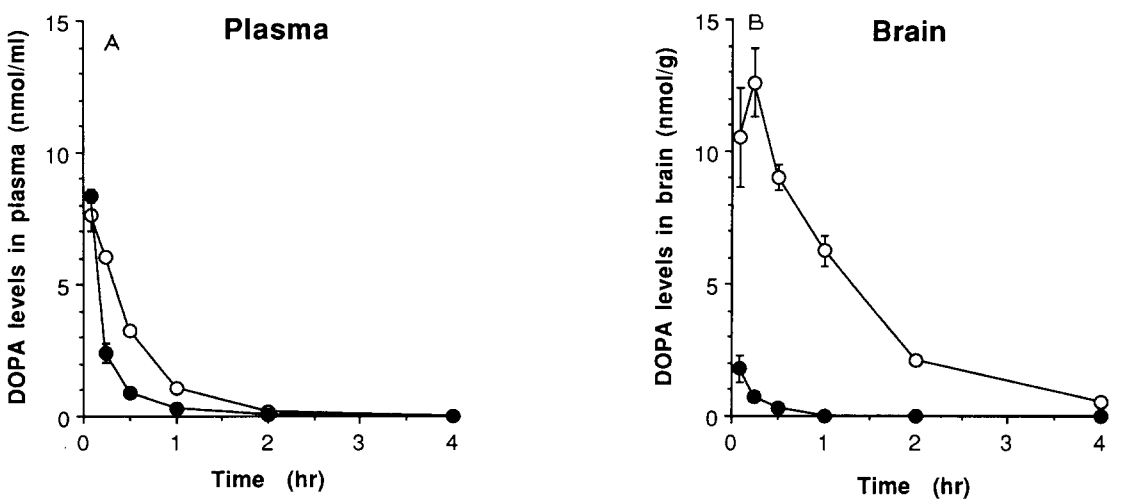


Fig. 4. DOPA concentration-time profiles in plasma (A) and brain (B) following intravenous administration of DOPA (●) or ZiPr-DOPA(P)$_2$ (○). Data are expressed as the means with error bars for standard deviations of three rats.

of DOPA resulted in a level of 1.7 nmol/g of DOPA in the brain at the 15 min sampling point; however, it could no longer be detected at the 2 h point.

In addition, irrespective of whether DOPA or the prodrugs were used, DOPA concentrations in whole blood were about half of those in plasma (data not shown). This suggests that DOPA observed in blood can be attributed to that in the plasma, not to red blood cells, since plasma accounts for about half the volume of blood.

3.4. Comparison between DOPA and ZiPr-DOPA(P)$_2$

To obtain more information about ZiPr-DOPA(P)$_2$ delivering the largest amount of DOPA to brain, we monitored the time course changes of DOPA levels following administration of this prodrug as well as DOPA. Fig. 4A and B indicates the concentration-time profiles of DOPA in plasma and brain, respectively, following intravenous administration of DOPA or an equimolar dose of ZiPr-DOPA(P)$_2$.

DOPA concentrations in plasma after intravenous administration of DOPA reached 8 nmol/ml at the 5 min sampling point, subsequently decreasing rapidly. A similar disposition in plasma was found after administration of ZiPr-DOPA(P)$_2$, showing a less sustained manner (Fig. 4A).

In contrast, as illustrated in Fig. 4B, high concentrations of DOPA in the brain were observed following administration of ZiPr-DOPA(P)$_2$, indicating the peak level at the 15 min point, while only a minor increase in DOPA concentrations in the brain was detected immediately after administration of DOPA itself for a brief period. The area under the brain DOPA concentration-time curves from time zero to 4 h (AUC) and the MRT obtained following administration of ZiPr-DOPA(P)$_2$ were 30- and 3.7-fold larger, respectively, than those of DOPA. Actually, at all sampling points measured, ZiPr-DOPA(P)$_2$ gave higher DOPA levels in brain than those in plasma, unlike DOPA itself.

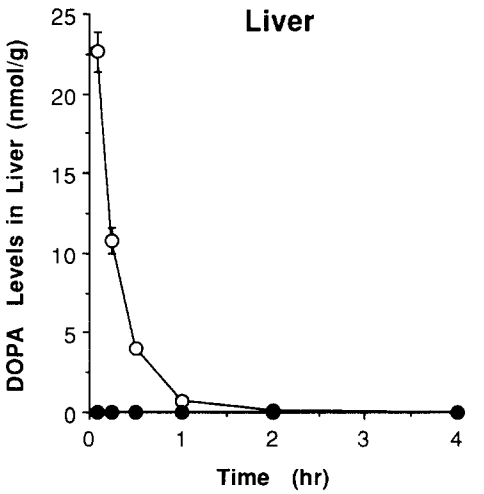


Fig. 5. DOPA concentration-time profiles in liver following intravenous administration of DOPA (●) or ZiPr-DOPA(P)$_2$ (○). Data are expressed as the means with error bars for standard deviations of three rats.

3.5. DOPA levels in liver and muscle

To permit the evaluation of the disposition of DOPA in other peripheral organs, we observed

Table 3
Comparison between DOPA and ZiPr-DOPA(P)$_2$ of AUC (nmol h g^{-1} or nmol h ml^{-1}) and MRT (h) of DOPA regenerated in several tissues following intravenous administration

	DOPA		ZiPr-DOPA(P)$_2$		Ratio (prodrug/DOPA) [a]	
	AUC	MRT	AUG	MRT	AUG	MRT
Brain	0.52 (1.0)	0.27 (1.0)	15.77 (1.0)	1.00 (1.0)	30.33	3.70
Plasma	2.19 (4.2)	0.35 (1.3)	4.57 (0.3)	0.55 (0.6)	2.09	1.57
Liver	0 (–)	0 (–)	7.27 (0.5)	0.33 (0.3)	–	–
Muscle	9.46 (18.2)	0.58 (2.2)	7.78 (0.5)	0.44 (0.4)	0.82	0.76

[a] Ratio of AUC and MRT for ZiPr-DOPA(P)$_2$ to those of DOPA, respectively.
Values in parentheses indicate the ratios of the values of the tissues to those of brain.

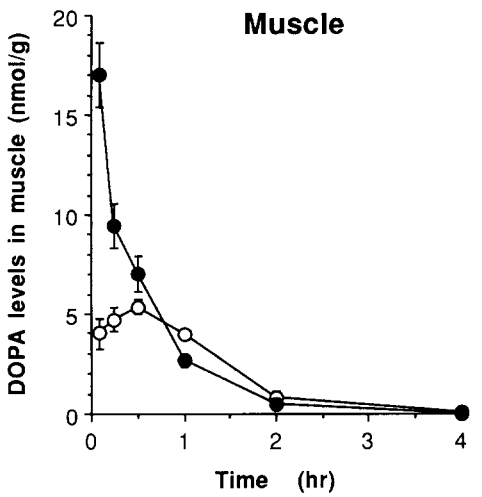


Fig. 6. DOPA concentration-time profiles in muscle following intravenous administration of DOPA (●) or ZiPr-DOPA(P)$_2$ (○). Data are expressed as the means with error bars for standard deviations of three rats.

DOPA concentration in liver and femoral muscle resected from another leg injected with the compounds. The concentration-time profiles in liver and muscle are illustrated in Fig. 5 and 6, respectively, and the AUC and MRT values based on the DOPA concentrations of each tissue are listed in Table 3.

As shown in Fig. 5, at the initial sampling point after intravenous administration of ZiPr-DOPA(P)$_2$, a high distribution of DOPA in the liver was evident, being double those in the brain, and the levels decreased rapidly up to 1 h. On the other hand, intravenous administration of DOPA provided no detectable increase in DOPA concentrations in the liver at any points measured.

Fig. 6 illustrates that, initially, DOPA itself yielded higher levels of DOPA in muscle than did ZiPr-DOPA(P)$_2$ while the levels became comparable after the 0.5 h sampling point. The AUC in the muscle following administration of DOPA was 18-fold larger than that in the brain; however, after administration of ZiPr-DOPA(P)$_2$, the AUC in the muscle was half of that in the brain (Table 3).

3.6. DOPA and dopamine levels in brain

Since DOPA is readily metabolized to a number of catechols including catecholamines in the body, we also determined some of them. The time-course changes in the levels of dopamine, an active metabolite of DOPA as a neurotransmitter, as well as DOPA in brain following intravenous administration of DOPA and ZiPr-

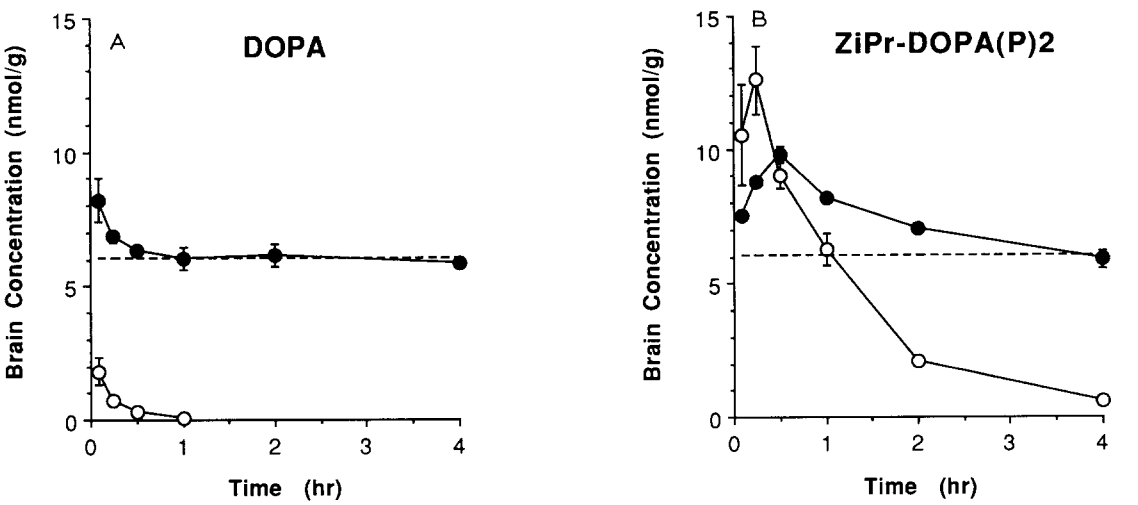


Fig. 7. Time course of changes in dopamine levels (●) as well as DOPA (○) in brain following administration of DOPA (A) or ZiPr-DOPA(P)$_2$ (B). Data are expressed as the means with error bars for standard deviations of three rats. The dashed lines in each graph indicate the dopamine level of untreated rats (n = 4).

$DOPA(P)_2$ are illustrated in Fig. 7A and B, respectively. The mean levels of dopamine in the brain of intact rats were 6.06 nmol/g ($n = 4$), while DOPA failed to be detected in those rats. As shown in Fig. 7A, a slight increase in dopamine levels in the brain corresponding to those of DOPA were detected for about 0.5 h after intravenous administration of DOPA. In contrast, a large and continuous increase in dopamine levels was found for about 2 h after administration of ZiPr-$DOPA(P)_2$, as illustrated in Fig. 7B. The maximal dopamine level was observed at the 30 min sampling point, indicating some delay by about 15 min as compared with that of DOPA following administration of ZiPr-$DOPA(P)_2$. This prodrug enhanced both the levels and duration not only of DOPA but also of dopamine in brain.

4. Discussion

Bodor and co-workers have reported a considerable body of results on brain-specific drug delivery systems based on a dihydropyridine-quaternary pyridinium redox system (for example, reviewed by Brewster and Bodor, 1992). In those reports, they clearly discussed the biotransformation of prodrugs by determining almost all of the relevant drugs including the quaternary 'locked-in' compounds. However, our present report deals solely with regenerated DOPA and its metabolites, since the HPLC analysis with an electrochemical detector could not detect *O*-acylated catechols. This indicates that the total amount of DOPA derivatives, including the prodrugs and quaternary intermediates in brain or other samples collected, remained unknown. Attempts to resolve these problems are currently under investigation. Nevertheless, it has been demonstrated that a new type of redox chemical delivery system based on a ring-closure reaction like the conversion of thiamine disulfide to quaternary thiamine may have implications for interesting properties suggestive of future applications.

DOPA levels in the tissues at the two sampling points, 15 min and 2 h after intravenous administration, can translate to the amount of initial distribution and subsequent level in the tissues, respectively. DOPA was eliminated from plasma by 2 h after administration irrespective of DOPA or the prodrugs. In sharp contrast, a considerable increase in the brain distribution of DOPA was observed after administration of ZiPr-$DOPA(P)_2$, and a large amount of DOPA was retained in the brain even after 2 h. Such an increase in distribution and retention of DOPA in brain was also evident following administration of either TnPr-$DOPA(P)_2$ or TiPr-$DOPA(P)_2$, although the potency was not as high in comparison with ZiPr-$DOPA(P)_2$. These findings imply the following; (1) in the context of DOPA distribution to brain, the pyrimidine ring of thiamine is not indispensable; (2) a ring-closure system to a quaternary thiazolium following reductive cleavage of the disulfide is remarkably favorable for retainment of the prodrug in brain, whether thiamine or thiazolium, in lieu of a dihydropyridine/pyridinium system.

However, we were troubled by the question as to why the distribution to brain varied according to the prodrugs tested. There is strong support for the contention that the passive transport of a compound through the BBB is associated with the lipophilicity and the molecular weight of the compound (Levin, 1980; reviewed by Rapoport, 1992). We did not determine the octanol/water partition coefficients of the prodrugs. However, the pyrimidine ring of thiamine, of which the molecular weight is 106 ($C_5H_6N_3$), behaves as a weak base in the formation of a salt. Although it facilitates the water solubility of thiamine disulfide, the increase in both water solubility and molecular weight does not contribute to the passage of the prodrugs across the BBB. In other words, it is considered that ZiPr-$DOPA(P)_2$, which does not carry the pyrimidine moiety with a reduced molecular weight, is more suitable for entering brain.

In liver, DOPA concentrations dramatically increased immediately after intravenous administration of ZiPr-$DOPA(P)_2$, while they decreased rapidly. This phenomenon in the liver is possibly attributed to the high susceptibility to metabolism in the liver since intravenous administration of DOPA itself failed to increase DOPA levels in the liver at any sampling points. On the other

hand, DOPA concentrations in muscle following administration of ZiPr-DOPA(P)$_2$ paralleled those following administration of DOPA; however, when ZiPr-DOPA(P)$_2$ was given, DOPA in muscle reached the peak level at 30 min post-administration. Therefore, it is considered that regeneration of DOPA from ZiPr-DOPA(P)$_2$ in muscle seems to occur more slowly than in the liver. In terms of the ratio of AUC and MRT of the prodrug toward those of DOPA, respectively, the prodrug enhances delivery of DOPA to brain and decreases distribution of DOPA to muscle (Table 3).

Takeuchi et al. (1963) as well as other researchers have reported that thiamine disulfides are instantaneously distributed and accumulated into red blood cells, suggesting that the prodrugs which bore thiamine disulfides as a promoiety tended to be accumulated in red blood cells. Furthermore, Stella and Himmelstein (1985) reported that the depletion of glutathione in red blood cells by reaction with TTFD could present a problem. However, Utsumi et al. documented that red blood cell glutathione was recovered by glutathione reductase within several minutes although it was temporarily depleted by thiamine disulfides (Kohno et al., 1968; Utsumi et al., 1968). The present results demonstrated that DOPA levels in whole blood were about half of those corresponding to plasma and this finding indicates that the DOPA prodrugs with thiamine disulfide in its molecule and at least DOPA were not accumulated in red blood cells.

A marked increase in dopamine levels in brain, as well as DOPA, was also observed following administration of ZiPr-DOPA(P)$_2$, suggesting that a pharmacological effect attributed to dopamine can be expected; this is now under investigation. Although we measured the DOPA and dopamine levels in whole brain, it is very important to determine the concentrations in nerve or endothelial cells, or in an extracellular fluid in brain with regard to the pharmacological efficacy.

The increase in dopamine levels in brain seems to be insufficient as compared with that in DOPA following intravenous administration of ZiPr-DOPA(P)$_2$. The flux of amino acids across the BBB is bidirectional (Pardridge and Oldendorf, 1977), and the net flux of unmetabolized DOPA is from brain to blood as plasma DOPA concentrations fall. Thus, the half-life of unmetabolized DOPA in the brain would be brief (Nutt and Fellman, 1984). Furthermore, dopamine is not the terminal metabolite of DOPA but an intermediate previous to norepinephrine, epinephrine or dopac, etc. The elimination of DOPA from brain and metabolism of dopamine in brain partly cause the discrepancy in the increase in DOPA and dopamine levels in brain following administration of the prodrug.

Usually, the treatment of Parkinson's disease with DOPA is practiced in an oral dosage form, therefore, the clinical application of the DOPA prodrugs in an injection dosage form described above appears to be impractical. However, through pursuing the disposition of DOPA after administration of DOPA or the prodrugs, we also believe that a redox ring-closure system to a quaternary thiazolium can be used as a novel and alternative chemical delivery system to brain.

5. Conclusion

A novel brain delivery system (prodrug) based on a redox ring-closure reaction to a quaternary thiazolium was synthesized using DOPA as a model drug. Intravenous administration of the prodrug, in comparison with DOPA itself, facilitates not only delivery of DOPA to brain but also retention of both DOPA and dopamine, the active metabolite of DOPA, in brain. It can be used as an alternative to a dihydropyridine-pyridinium redox system.

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