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Carrier-mediated processes in blood-brain barrier penetration and neural uptake of paraquat

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Abstract

Due to the structural similarity to N-methyl-4-phenyl pyridinium (MPP^+), paraquat might induce dopaminergic toxicity in the brain. However, its blood-brain barrier (BBB) penetration has not been well documented. We studied the manner of BBB penetration and neural cell uptake of paraquat using a brain microdialysis technique with the HPLC/UV detection in rats. After subcutaneous administration, paraquat appeared dose-dependently in the dialysate. In contrast, MPP^+ could not penetrate the BBB in either control or paraquat pre-treated rats. These data indicated that the penetration of paraquat into the brain would be mediated by a specific carrier process, not resulting from the destruction of the BBB function by paraquat itself or a paraquat radical. To examine whether paraquat was carried across the BBB by a certain amino acid transporter, L-valine or L-lysine was pre-administered as a co-substrate. The pre-treatment of L-valine, which is a high affinity substrate for the neutral amino acid transporter, markedly reduced the BBB penetration of paraquat. When paraquat was administered to the striatum through a microdialysis probe, a significant amount of paraquat was detected in the striatal cells after a sequential 180-min washout with Ringer's solution. This uptake was significantly inhibited by a low Na^+ condition, but not by treatment with putrescine, a potent uptake inhibitor of paraquat into lung tissue. These findings indicated that paraquat is possibly taken up into the brain by the neutral amino acid transport system, then transported into striatal, possibly neuronal, cells in a Na^+-dependent manner.

Theme: DISORDERS OF THE NERVOUS SYSTEM Topic: Neurotoxicity

Keywords: Paraquat; Blood-brain barrier; Amino acid transporter; Sodium dependent uptake; Brain microdialysis

1. Introduction

Paraquat (1,1'-dimethyl-4,4'-bipyridinium) is a widely used non-selective herbicide, which is strongly toxic and is often lethal to animals, including humans [27]. Since the toxic damage to the lung, liver and kidney are responsible for death after acute exposure to this herbicide, the experimental studies on paraquat poisoning have concentrated on its effects in peripheral tissues [5,30]. However, toxic damage to the brain has also been observed in patients who died from paraquat poisoning [11,12]. According to epidemiological studies, environmental factors may contribute to the etiology of Parkinson's disease [1,10,14,16,29,33,34,37,38]. In addition, a number of clinical and experimental studies have increased the interest in the possibility that environmental chemicals or endogenous substances may be related to the development of Parkinson's disease. Although controversial, paraquat is considered to be one of the possible chemicals that can induce Parkinson's disease, since a strong correlation has been found between the incidence of the disease and the amount of paraquat used [18,19,21].

The herbicide paraquat bears structural similarity to the known dopaminergic neurotoxin, N-methyl-4-phenylpyridinium cation (MPP^+) (Fig. 1). Thus, concern has focused on the possibility that paraquat may be related to the development of Parkinson's disease [9]. The neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, destroys dopaminergic neurons of the ventral mesencephalon, in particular those located in the substantia nigra pars compacta, after being converted to the active metabolite, MPP^+ [6,13]. This neurotoxic metabolite must be formed intracerebrally by monoamine oxidase B in glia or non-dopaminergic neurons, since it cannot cross the blood-brain barrier (BBB). However, paraquat has induced brain damage in fatal poisoning cases [11], and induces dopaminergic neuronal damage in experimental animals [4,18], indicating that this herbicide might cross the BBB. However, the possibility that this herbicide itself was responsible for neurotoxic events has been challenged, because the

BBB penetration of paraquat has been controversial [23,25]. The BBB penetration of paraquat has not yet been supported by conclusive findings.

For the study of the BBB penetration of drugs, a number of techniques have been used, including brain homogenate analysis, autoradiography, brain capillary-endothelial cell culture and cerebrospinal fluid sampling, which all have their specific advantages and limitations. Intracerebral microdialysis is a relatively new *in vivo* technique for studying drug transport across the BBB. If surgical and experimental conditions are well controlled, the microdialysis technique would offer decisive evidence of the BBB penetration of paraquat. Thus, in the present study, we examined the BBB penetration of this herbicide using a brain microdialysis technique with the HPLC/UV detection in freely moving rats to obtain conclusive evidence of paraquat transportation across the BBB. We also examined the manner of the BBB penetration and the neural uptake of paraquat.

2. Materials and methods

2.1. Chemicals

Paraquat dichloride and L-valine were obtained from Tokyo Chemical Industry (Tokyo, Japan). MPP^+ iodide was purchased from Research Biochemicals International (Natick, MA, USA). The other reagents were of analytical grade and obtained from Wako (Osaka, Japan).

2.2. Animal Treatments

Male Wistar rats (8 weeks old, 210-260 g; SLC, Shizuoka, Japan) were housed under the condition of constant temperature, humidity and dark/light (12/12h) cycle.

Anesthetized (50 mg/kg i.p., sodium pentobarbital) rats were stereotaxically implanted with 22-gauge cannulae in the left striata at AP + 0.48 mm, L + 3.0 mm from the bregma, and - 3.7 mm from the skull, according to the stereotaxic atlas of Paxinos and Watson [28]. Dummy probes were then placed inside the cannulae. The rats were housed in plastic cages (30 x 30 x 35 cm) with free access to food and water, and a greater than 5-day recovery period was allotted. The animal experiments were done in accordance with the guidelines for care and use of laboratory animals by the Committees of Asahikawa Medical College and Shimane Medical University.

2.3. BBB Penetration

The microdialysis probes with a dialysis area of 3 mm length were of the I-shaped type prepared according to the method of Nakahara et al. [24]. After inserting through the guide cannula, the probe was connected to a microinfusion pump and perfused with Ringer's solution (146 mM Na^+, 1.26 mM Ca^{2+}, 4 mM K^+, 1.0 mM Mg^{2+} and 154.5 mM Cl^-) at a flow rate of 2 µl/min.

After 4 h pre-perfusion with Ringer's solution to recover brain injury due to the probe insertion, the dialysate was collected for 60 min as a blank sample. Then, paraquat dichloride in saline (5, 10 and 20 mg/kg; equivalent to 15.8, 31.6, and 63.1 µmoles/kg, respectively) was administered subcutaneously into the back of the neck. Subsequently, the dialysate collection with a 60-min interval was performed for 180 min. To test the suitability of the brain dialysis technique, the BBB penetration of MPP^+ (10 mg/kg; equivalent to 33.1 µmoles/kg) was also examined. The dialysate sample was directly injected into an HPLC apparatus (Tosoh CCPD, Tokyo Japan) to determine paraquat and MPP^+ levels.

At the end of the perfusion, the blood was collected from the heart under deep pentobarbital anesthesia. The serum was deproteinized with 4 volumes of 0.5 N

perchloric acid and then centrifuged at 10,000 g for 10 min. The supernatant was filtered through a cellulose-acetate filter (pore size, 0.45 µm; DISMIC-3, Advantec, Tokyo, Japan), and a portion (50 µl) of the filtrate was injected into the HPLC.

2.4. BBB Damage by Paraquat

It was considered that paraquat might injure endothelial cells in brain capillaries to destroy the BBB function. To test this, MPP^+ was administered 1 h after a paraquat injection, and then MPP^+ in the dialysate was analyzed. If paraquat destroyed the BBB function, MPP^+ should appear in the brain dialysate.

2.5. Amino Acid Transporter

To examine whether paraquat was carried into the brain via a specific amino acid transporter, L-valine (200 mg/ml) or L-lysine (200 mg/ml) was intraperitoneally pre-administered 30 min prior to the paraquat injection. Since amino acids share the same high affinity BBB transport system [26], the entry of paraquat into the brain should be diminished by the simultaneous presence of high concentration of L-valine or L-lysine. L-Valine and L-lysine are high affinity substrates for neutral and basic amino acid transporters, respectively, which mediate the amino acids across the BBB.

2.6. Uptake into Striatal Cells

Paraquat (50 µM) or MPP^+ (10 µM) was administered to the striatum through the microdialysis probe for 60 min, followed by a sequential 180-min washout with Ringer's solution. Then, the animal was decapitated, and ipsi- and contra-lateral striata were removed immediately. The striatal tissue treated with paraquat was sonicated in 350 µl of 0.2 N perchloric acid, and then centrifuged at 9,000 g for 15 min. The supernatant

was filtered through the cellulose-acetate filter followed by triplicate washes with 200 µl of ethyl acetate. For the MPP^+ treated sample, the tissue was sonicated in 1 ml of 0.2 N perchloric acid followed by the addition of 0. 1 ml of 2 N KOH, and then centrifuged at 9,000 g for 15 min. The supernatant was filtered through the cellulose-acetate filter. A portion (100 µl) of the filtrate was injected into the HPLC.

To clarify whether the brain cell uptake of paraquat was mediated by a specific ion-exchange transporter, the paraquat perfusion and the subsequent washout were performed under Na^+-free Ringer's solution (146 mM Li^+, 1.26 mM Ca^{2+}, 4 mM K^+, 1.0 mM Mg^{2+} and 154.5 mM Cl^-). Also, the participation of the polyamine transporter in the paraquat uptake was examined. Putrescine at 50 µM, which is a high affinity substrate of polyamine transporter, was co-perfused with 50 µM paraquat for 60-min followed by a 180-min washout with Ringer's solution.

2.7. HPLC Conditions

18 18

Paraquat was separated on a reverse phase C column (Puresil 5µ C , 250 x 4.6 mm, Waters), and detected by a UV detector at 290 nm (UV-8010, Tosoh). The mobile phase, 0.1 M phosphoric acid containing 7.5 mM 1-octanesulfonic acid, 0.1 M diethylamine, and 25 µM EDTA, was delivered at a flow rate of 1.0 ml/min. MPP^+

were also separated on the reverse phase C column, followed by the fluorescence detection (Ex 325 nm and Em 375, RF-10A, Shimadzu, Kyoto, Japan). The mobile phase, 110 mM citric acid/sodium acetate buffer (pH 4.25) containing 6.9 mM 1-octanesulfonic acid, 1 mM triethylamine hydrochloride, 25 µM EDTA and 21% acetonitrile (v/v), was delivered at a flow rate of 0.8 ml/min.

2.8. Statistical Analysis

Statistical significances of differences were determined by one factorial ANOVA, followed by the post hoc Dunnett *t* test. The *p* value less than 0.05 was considered to be statistically significant.

3. Results

3.1. BBB penetration of paraquat and MPP^+

Typical HPLC chromatograms of the dialysate samples before and after a 20 mg/kg (63.1 µmoles/kg) paraquat injection are shown in Fig. 2. After systematic administration, paraquat clearly appeared in the dialysate of the striatum (Fig. 2B-D). However, MPP^+ was not detected in the striatum dialysate after its systematic injection (Fig. 3B-D), indicating that the BBB penetration of paraquat did not result from technical brain injury.

Extracellular concentrations of paraquat and MPP^+ in the striatum after

subcutaneous injections were calculated from the recovery of the dialysis probe membrane. The recovery efficiency of the microdialysis probes for paraquat and MPP^+ was tested in 500 ng/ml of each drug solution. The recovery efficiency was 12.1 ± 1.4 or 18.7 ± 2.6% (mean ± S.E.M., n = 4 in each drug) for paraquat or MPP^+, respectively. As shown in Fig. 4, paraquat dose-dependently appeared in the striatal dialysate samples, and disappeared rapidly from the extracellular fluid. The elimination constant and half-time from the brain extracellular fluid were 0.91 h^{-1} and 0.76 h, respectively, in rats that received 20 mg/kg of paraquat. Serum concentrations and the ratio of brain extracellular to blood concentrations at 3 h after administration are summarized in Table 1. The extracellular concentration of paraquat at 3 h after its administration was calculated by the liner regression analysis based on the findings presented in Fig. 4. The serum concentration of MPP^+ in rats that received the 10 mg/kg dose was higher than that of paraquat, indicating that MPP^+ was particularly well absorbed into the circulation system after its subcutaneous administration. The ratio of brain extracellular to blood concentrations of paraquat at 3 h after administration decreased with the increase in blood concentrations ($F(2,13) = 4.472$, $p < 0.05$, Table 1).

3.2. Effect of paraquat on the BBB function

To clarify whether the BBB was resulted from the dysfunction of the BBB due to paraquat toxicity, MPP^+ at a 10 mg/kg dose was administered in rats pre-treated with paraquat (20 mg/kg, 1 h before MPP^+ injection). Then, MPP^+ in the dialysate was analyzed. MPP^+ was not observed in the dialysate of paraquat pre-administered rats (Fig. 3E), indicating that the paraquat was carried into the brain by a specific carrier process, not because of the BBB destruction.

3.3. Carrier mediated transport for the BBB penetration of paraquat

To test whether paraquat was carried through the BBB by a certain amino acid transporter, L-valine or L-lysine was pre-treated before paraquat administration. L-Valine and L-lysine are high affinity substrates for neutral and basic amino acid transporters, respectively. The pre-treatment of L-valine significantly reduced striatal extracellular paraquat levels after its systematic administration compared with the control ($p < 0.01$ or 0.05) and L-lysine pre-treatment ($p < 0.01$) groups (Table 2). The blood concentration of paraquat slightly increased with the L-valine treatment. The ratio of brain extracellular to blood concentrations of paraquat at 3 h after administration in L-valine-treated rats was significantly lower than that in L-lysine-treated rats ($p < 0.05$). The pre-treatment of L-lysine did not affect the extracellular levels of paraquat.

3.4. Paraquat uptake into the striatal cells

When paraquat or MPP^+ was administered to the striatum through a microdialysis probe, a significant amount of paraquat or MPP^+ was detected in the ipsi-lateral but not contra-lateral striata after a sequential 180-min washout with Ringer's solution (Table 3). This uptake of paraquat was significantly inhibited by the low Na^+ condition ($p < 0.02$). On the other hand, treatment with 50 μM putrescine did not influence the striatal uptake of paraquat. The accumulation amount of paraquat was almost equal to that of MPP^+, whose uptake is mediated by the dopamine transporter.

4. Discussion

Although a number of clinical and experimental studies have indicated that paraquat could induce neural damage [4,8,18,32], the possibility that this herbicide itself was

responsible for neurotoxic events has been challenged [23,25,39] because of its structural similarity to MPP^+, which cannot penetrate into the central nervous system. In other words, the BBB penetration of paraquat has not yet been supported by the conclusive findings. Several *in vivo* experimental methods have been employed to examine the BBB penetration of paraquat [7,15,17,40]. However, technical disadvantages exist in the these techniques to evaluate BBB penetration, for example, blood contamination of the samples could not be avoided in the homogenization and autoradiographic methods. Thus, conflicting findings on paraquat BBB penetration have been reported [23,25]. The present results using the brain microdialysis technique with the HPLC/UV detection clearly demonstrated that paraquat penetrates the BBB in a dose-dependent manner. Although an elegant *in vivo* method for studying drug transport across the BBB, brain microdialysis is an invasive technique. However, the tissue damage due to the surgery, such as eicosanoids and local changes of cerebral blood flow and glucose metabolism, are normalized within one day [3,22]. Shortly after the probe insertion, the BBB function around the probe is intact despite the slight brain tissue trauma [3]. In the present study, MPP^+, which is structurally similar to paraquat, was used as a negative marker for the BBB penetration. Since MPP^+ was observed in the blood but not in the dialysate after its systematic administration, the present brain microdialysis method was well controlled to study the BBB penetration of paraquat. Thus, the present findings offered conclusive evidence that paraquat can penetrate the BBB.

An additional query was whether the BBB paraquat penetration was mediated by a specific transporter. MPP^+ was not observed in the dialysate of paraquat pre-administered rats, indicating that the paraquat was not carried into the brain because of the BBB destruction by paraquat itself or a paraquat radical. Considering the structural hydrophilicity of paraquat, the BBB permeability cannot be explained by simple diffusion, which is the major entry mechanism across the BBB for chemicals having high lipid solubility. Despite the structurally similarity, paraquat but not MPP^+ was detected

in the dialysate in the striatum. This finding indicated that the penetration of paraquat into the brain could be mediated by a specific carrier process. The finding, that the ratio of brain extracellular to blood concentrations of paraquat decreased with the increase in blood concentrations, also supported the existence of a carrier mediated process. Lung damage induced by paraquat was suggested to be initiated, at least in part, by an energy dependent accumulation through an uptake system shared by polyamines [36]. However, the polyamine transporters are not expressed in the BBB structure, since the BBB penetration of the polyamines is restricted [35]. From the chemical structure of paraquat, it was assumed that the possible carrier involved in the BBB penetration would be one of amino acid transporters, which are highly expressed in the BBB [15]. The BBB penetration of paraquat was significantly inhibited by the pre-treatment with L-valine, but not with L-lysine. The simultaneous presence of high serum concentration of L-valine would share the same BBB transport system involved in the paraquat penetration into the brain. The evidence, that the ratio of brain extracellular to blood concentrations of paraquat in L-valine-treated rats was lower, also strongly supported that both substances shared the same transport system. L-Valine is high affinity substrate for neutral and basic amino acid transporter. Thus, this finding indicated that paraquat was possibly taken up into the brain by the neutral amino acid transport system expressed in the brain capillaries.

The third question was whether paraquat was taken up into the striatal cells. When paraquat or MPP^+ was administered to the striatum through a microdialysis probe, a significant amount of paraquat or MPP^+ was detected in the striatum even after a sequential 180-min washout with Ringer's solution. This uptake of paraquat was Na^+-dependent. However, putrescine, which is a potent uptake inhibitor of paraquat into lung tissue, did not influence the striatal uptake of the herbicide, indicating that the polyamine transporter was not involved in the neural uptake of paraquat. The kinetic velocity of paraquat accumulation may be as high as MPP^+ [20]. It was reported that

paraquat is rapidly taken up by nerve terminals isolated from mouse cerebral cortices which then induced lipid peroxidation in a concentration dependent manner in the presence of NADPH and ferrous ion [41]. The neutral amino acid transporters are further classified as sodium-dependent and sodium-independent systems [2]. Putting together the data from the present and previous [41] findings, it is suggested that paraquat could be taken up into the nerve terminals by the Na^+-dependent neutral amino acid transporter. The accumulation of MPP^+ has been shown to be dependent on the dopamine transporter, and the kinetic characteristics of MPP^+ uptake are similar to those of dopamine [6,13]. Biogenic amine transporters, including dopamine transporter, also require the presence of external Na^+ ions [31]. However, the involvement of the dopamine transporter in the paraquat accumulation could not be evaluated from the present results.

A number of clinical and experimental studies have increased the interest in the possibility that environmental chemicals, including paraquat, may be related to the development of Parkinson's disease [4,8,18,32]. Although controversial, paraquat is a one of the possible herbicides that induce Parkinson's disease, since a strong correlation has been found between the incidence of the disease and the amount of paraquat used [18,19,21]. Furthermore, paraquat administered systematically to experimental animals induces behavioral and biochemical changes that are compatible with parkinsonian symptoms, such as increase in rigidity, akinesia, tremor, decrease in dopamine concentration and increase in pigmentation [4,8,17]. Thus, further experiments on the neuronal functions under continuous exposure to paraquat for a long period are desired.

In conclusion, the present findings indicated that paraquat is possibly taken up into the brain by the neutral amino acid transport system, then transported into brain cells in a Na^+-dependent manner.

Acknowledgements

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Table 1

The ratio of the extracellular to serum concentration 3 h after paraquat or MPP^+ administration[a]

Dose	n	Serum concentration (nmoles/ml)	Striatal extracellular level (nmoles/ml)	Ratio
Paraquat				
5 mg/kg	3	1.42 ± 1.50	0.28 ± 0.06	0.208 ± 0.064
10 mg/kg	5	3.88 ± 0.79	0.36 ± 0.09	0.126 ± 0.031
20 mg/kg	8	8.04 ± 2.41	0.49 ± 0.09	0.085 ± 0.023**
MPP^+				
10 mg/kg	4	11.62 ± 4.84*	Not detected	–

[a] The ratio of brain extracellular to blood concentrations 3 h after administration. Paraquat dichloride (5, 10 and 20 mg/kg; equivalent to 15.8, 31.6, and 63.1 μmoles/kg, respectively) or MPP^+ iodide (10 mg/kg; equivalent to 33.1 μmoles/kg) was administered subcutaneously into the back of the neck. The extracellular concentration of paraquat 3 h after its administration was calculated by the liner regression analysis based on the data presented in Fig. 4. Data are expressed as means ± S.E.M.

* $p < 0.01$ vs paraquat 10 mg/kg and ** $p < 0.01$ vs paraquat 5 mg/kg.

Table 2

Effects of pre-treatment of L-valine and L-lysine on paraquat BBB penetration

	n	Ratio[a]	Striatal extracellular paraquat level			Serum paraquat
			0-1 h	1-2 h	2-3 h	3 h
Paraquat (control)	8	4.50 ± 1.06	2.03 ± 0.38	0.74 ± 0.13	8.04 ± 2.41	0.085 ± 0.023
Paraquat + L-valine	8	1.32 ± 0.29*	0.71 ± 0.13**, †	0.06 ± 0.23	11.74 ± 2.80	0.032 ± 0.006††
Paraquat + L-lysine	8	3.47 ± 0.90	1.90 ± 0.26	0.84 ± 0.26	7.59 ± 1.74	0.100 ± 0.028

L-Valine (200 mg/kg) or L-lysine (200 mg/kg) was intraperitoneally administered 30 min before a subcutaneous injection of paraquat dichloride (20 mg/kg; equivalent to 63.1 μmoles/kg). Data are expressed as means ± S.E.M. (nmoles/ml).

[a] The ratio of brain extracellular to blood concentrations 3 h after administration. The extracellular concentration of paraquat 3 h after its administration was calculated by the liner regression analysis based on the data presented in Fig. 4. * $p < 0.01$, ** $p < 0.05$ vs control rats and † $p < 0.01$, †† $p < 0.05$ vs L-lysine-treated rats.

Table 3

Paraquat and MPP^+ uptake into striatal cells

	n	Ipsi-lateral (pmoles)	Contra-lateral (pmoles)
Paraquat (50 µM)	5	172.5 ± 53.9	ND[a]
Paraquat (50 µM) with Na^+-free Ringer's solution	5	76.3 ± 6.8*	ND
Paraquat (50 µM) with Putrescine (50 µM)	3	169.1 ± 60.5	ND
MPP^+ (10 µM)	5	23.1 ± 8.2	ND

Accumulation of paraquat and MPP^+ into striatal cells after a 60-min perfusion with 50 µM or 10 µM solution, respectively, followed by a 180-min washout with Ringer's solution or Na^+-free Ringer's solution. The paraquat perfusion and the subsequent washout were also performed with Na^+-free Ringer's solution. Putrescine at 50 µM was co-perfused with 50 µM paraquat for 60-min followed by a 180-min washout with Ringer's solution.

[a] Not detected. * $p < 0.02$ vs accumulation of paraquat perfused under Ringer's solution.

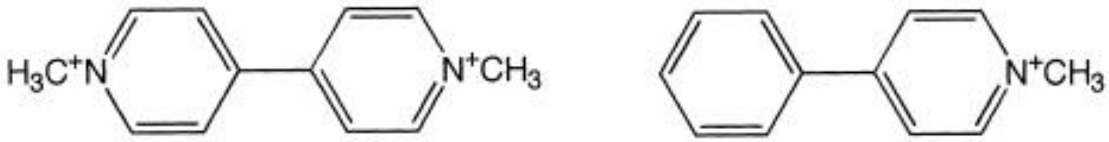
Legends for figures

Fig. 1. Chemical structures of paraquat and MPP^+.

Fig. 2. Typical HPLC chromatograms (A-D) of the dialysate samples before and after a paraquat injection. After 4 h pre-perfusion, the dialysate was collected for 60 min as a blank sample. Paraquat dichloride (20 mg/kg; equivalent to 63.1 μmoles/kg) was administered subcutaneously into the back of the neck. A: Before paraquat injection, B: 0-1 h, C: 1-2 h and D: 2-3 h after the administration. The chromatogram E was from the blood sample collected at 3 h after the paraquat administration. The peak with the retention time of ca. 12.5 min was paraquat. The peaks appeared within 10 min on the chromatograms (A - E) were derived from the solvent and unknown biological substances.

Fig. 3. Typical chromatograms (A-D) of the dialysate samples before and after a 10 mg/kg MPP^+ injection. MPP^+ iodide (10 mg/kg; equivalent to 33.1 μmoles/kg) was administered subcutaneously into the back of the neck. Subsequently, the dialysate collection with a 60-min interval was performed for 180 min. To clarify whether paraquat might injure endothelial cells in brain capillaries to destroy the BBB function, MPP^+ was administered 1 h after a paraquat injection (20 mg/kg; equivalent to 63.1 μmoles/kg). Then, MPP^+ in the dialysate was analyzed. A: Before MPP^+ injection, B: 0-1 h, C: 1-2 h and D: 2-3 h after the administration. Chromatogram E was from the dialysate sample 0-1 h after MPP^+ administration in the paraquat pre-administered rats. Chromatogram F was obtained from the blood sample collected 3 h after the MPP^+ administration. The peak with the retention time of ca. 11.1 min was MPP^+. The peaks appeared within 5 min on the chromatograms (A - F) were derived from the solvent and unknown biological substances.

Fig. 4. Extracellular concentrations of paraquat and MPP^+ in the striatum after subcutaneous injections. Paraquat dichloride (5, 10 and 20 mg/kg; equivalent to 15.8, 31.6, and 63.1 μmoles/kg, respectively) or MPP^+ iodide (10 mg/kg; equivalent to 33.1 μmoles/kg) was administered subcutaneously into the back of the neck. Drug concentrations were corrected by the recovery of the dialysis probe membrane. The data are expressed as means ± S.E.M.



Paraquat MPP^+

Fig. 1. Chemical structures of paraquat and MPP^+.

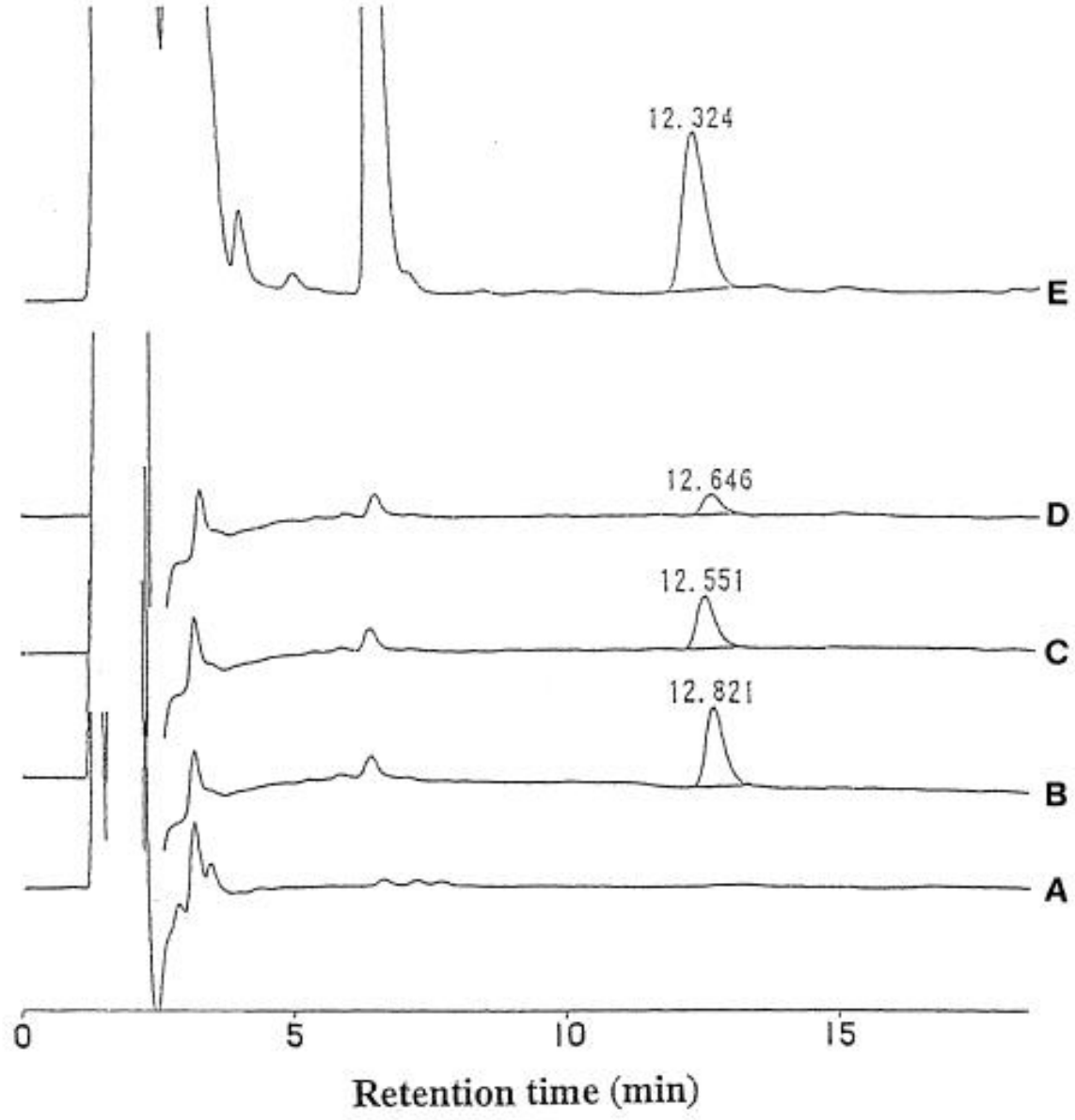


Fig. 2.

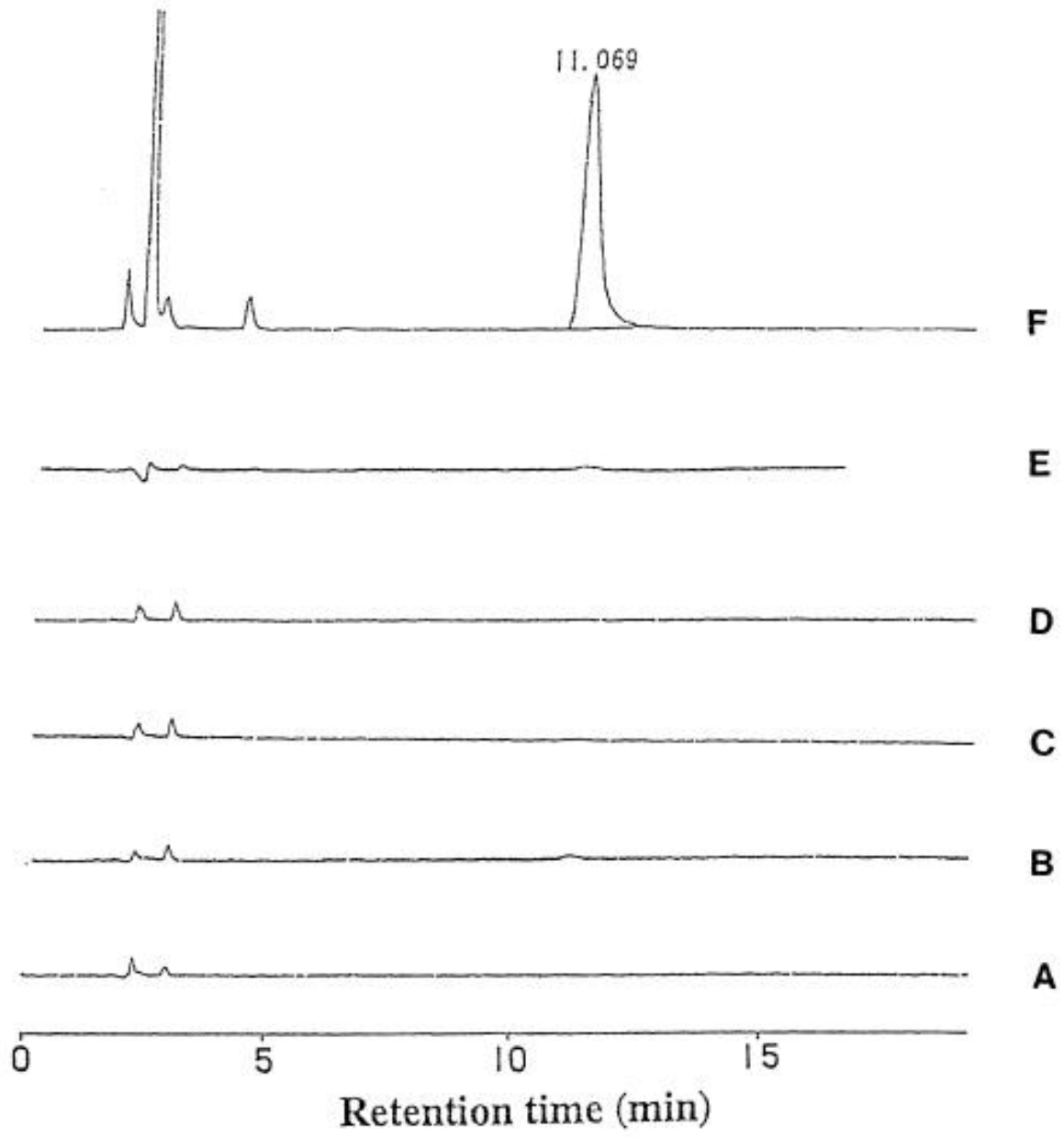


Fig. 3.

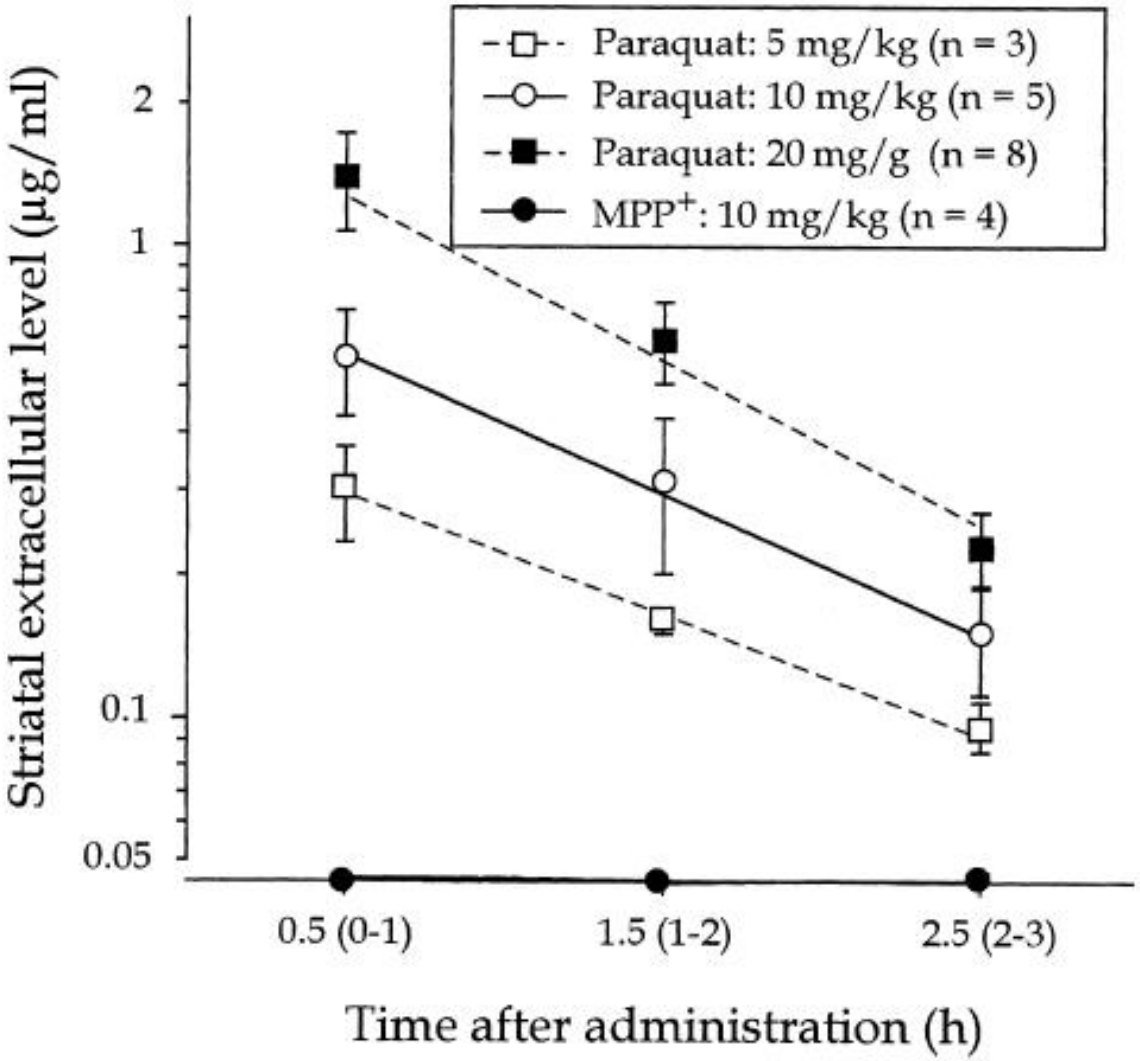

Paraquat: 5 mg/kg (n = 3)
Paraquat: 10 mg/kg (n = 5)
Paraquat: 20 mg/g (n = 8)
MPP+: 10 mg/kg (n = 4)
Striatal extracellular level (μg/ml)
2
1
0.1
0.05
0.5 (0-1)
1.5 (1-2)
2.5 (2-3)
Time after administration (h)

Fig. 4.