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Detection of Platinum in the Brain of Mice Treated with Cisplatin and Subjected to Short-term Hypoxia

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Abstract

Cisplatin is widely used for cancer treatment but has strong side-effects, including nephrotoxicity. Neurotoxicity has been thought to be limited to peripheral damage because the blood-brain barrier is thought to be impervious to hydrophilic substances such as cisplatin. Because anoxic ischaemia has been associated with lesions of the barrier, inductively coupled plasma mass spectrometry has been used to monitor the accumulation of platinum in the brains of mice treated with cisplatin and exposed to oxygen-deficient atmospheres.

Platinum was detected in the cerebral cortex of mice 24 h after the administration of cisplatin (3 mg kg^{-1}) followed by exposure for 60 s to an atmosphere containing 7% oxygen, but not in the cerebral cortex of mice exposed to normal atmospheres. Platinum was also observed in the cerebral cortex after exposure for 120 s to an atmosphere containing 14% oxygen, and platinum levels increased as the concentration of oxygen was reduced. The highest platinum levels were obtained 10 h after administration of cisplatin and exposure for 120 s to an atmosphere containing 7% oxygen. Platinum was still retained in the cerebral cortex one week after administration. In contrast, platinum levels in the blood and kidney decreased with time. Platinum levels were measured in seven regions of the brain: the right and left cerebral cortices, the basal ganglia, the thalamus and hypothalamus, the bulbus olfactorius, the cerebellum, and the mesencephalon. When cisplatin was administered to mice not subjected to hypoxia, platinum was not detected in the right and left cerebral cortices, basal ganglia or the thalamus and hypothalamus, but was detected in the bulbus olfactorius, cerebellum and mesencephalon. When such mice were exposed to low levels of oxygen, however, platinum was detected in the right and left cerebral cortices, the basal ganglia and the thalamus and hypothalamus. Platinum levels in the cerebellum and mesencephalon of mice exposed to low levels of oxygen were higher than those of mice exposed to normal air. In addition, platinum levels in the bulbus olfactorius were significantly higher than those in the other regions, although the platinum content of the bulbus olfactorius was not affected by hypoxia.

From these observations, it is concluded that platinum is easily accumulated in the bulbus olfactorius after the administration of cisplatin, and that after exposure to atmospheres containing low levels of oxygen, platinum easily passes through the blood-brain barrier and accumulates in all parts of the brain.

Cisplatin (*cis*-diamminedichloroplatinum (II)) has been widely used for ovary, bladder, head and neck tumour therapy (Hill et al 1975). The effect of cisplatin is a result of its ability to bind to DNA and to interfere with its replication (Lippert 1992; Bernges & Holler 1992; Brabec et al 1992; Hrubisko et al 1993). The drug has strong side-effects, however, such as nephrotoxicity (Brady et al 1993). Neurotoxicity has also been reported, but it was thought to be limited to peripheral damage, as the blood-brain barrier appeared to prevent the transportation of cisplatin to the brain (Thompson et al 1984). Because accurate measurements of platinum levels in the brain were not available, and because platinum was recently found in the vicinity of the spinal cord in patients treated with cisplatin (Minami et al 1994), we wondered if cisplatin can, in fact, pass through the blood-brain barrier and how this might occur when it is usually impervious to such hydrophilic substances. Rapoport et al (1971) reported that some hydrophilic substances can pass through the barrier when they are injected in a solution of high osmotic pressure. This is a rather unnatural process that would be unlikely to occur in patients treated only with cisplatin. Because lesions of the barrier appeared to be associated with anoxic ischaemia (Levine 1960), we wished to determine whether brain ischaemia would reduce barrier function, thus enabling passage of cisplatin. Trace levels of platinum in tissues have been measured using our recently developed method employing inductively coupled plasma mass spectrometry (ICPMS) (Minami et al 1995).

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Materials and Methods

Male ddY mice (6-weeks old) were obtained from Japan SLC Co. (Japan). Before the start of the experiments they were housed for one week during which time they were fed a standard mouse food (MF, Oriental Yeast Co., Japan) and had free access to tap water. All experiments were performed on groups of six mice.

Cisplatin was purchased from Nihon Kayaku (Japan). Randa (50 mg/100 mL), platinum standard solution (1000 $\mu g\ mL^{-1}$), nitric acid, and perchloric acid for the measurement of trace elements were purchased from Wako Pure Chemical Industries Ltd (Japan).

Mixtures of known oxygen content were prepared by mixing nitrogen gas and air supplied by an air compressor; oxygen levels were measured with an oxygen electrode (Kitagawa OM-4, Komyo Rikagaku Kogyo Co., Japan).

Effect of hypoxia on platinum level in the cerebral cortex

Cisplatin (3 mg kg^{-1}) was injected into mice through the tail vein. After 30 min, the mice were placed in plastic boxes (30 × 25 × 24 cm) and exposed for 30, 60, 90 or 120 s to an atmosphere containing 7% oxygen. In the control group, mice

were administered cisplatin followed by exposure to air (containing 21% oxygen). Twenty-four hours after administration of cisplatin, the mice were anaesthetized, blood collected, and brains removed. The cerebral cortex was obtained by the method of Gispen et al (1972).

Effect of oxygen concentration on the level of platinum in the cerebral cortex

Thirty minutes after administration of cisplatin (3 mg kg^{-1}), the effect of oxygen concentration was determined by exposing mice for 120 s to atmospheres containing 21 (air), 14, 10 or 7% oxygen, or for 30 s to an atmosphere containing 5% oxygen. The last exposure time was shorter because it was found that exposure to 5% oxygen for longer than 60 s caused decreases in systemic conditions. Twenty-four hours after cisplatin administration, the brain was removed and the cerebral cortex obtained.

Time-dependence of platinum levels in the cerebral cortex

Thirty minutes after administration of cisplatin (3 mg kg^{-1}), mice were exposed for 120 s to an atmosphere containing 7% oxygen. At various times after administration (2 h, 4 h, 10 h, 1 day, 2 days, 4 days and 1 week) the mice were anaesthetized, the blood was collected, and the brain and kidney were then removed. In the control group, mice were administered cisplatin and exposed to air (21% oxygen). Ten hours or one week after the administration, the blood was collected, and the brain and kidney removed.

Platinum levels in seven different regions of the brain

At various times (4 h, 10 h, 1 day, 2 days, 4 days and 1 week) after administration of cisplatin (3 mg kg^{-1}) mice were decapitated, and the brains were removed and separated into seven parts (right and left cerebral cortices, basal ganglia, thalamus and hypothalamus, bulbus olfactorius, cerebellum and mesencephalon) by the method of Gispen et al (1972).

Determination of the platinum content of tissues

Each brain locus and kidney was dried at 80°C for 16 h and weighed. After wet combustion with nitric acid (1.0 mL) and perchloric acid (0.5 mL), all samples were diluted to 10 mL with extra-pure water (Milli-Q, Nihon Millipore Kogyo, Japan). The sample solution was diluted 11-fold with extra-pure water, and platinum levels were measured by ICPMS (PIMS-3000, Shimadzu Co., Japan). The instrumental system and conditions used for measurement of platinum are described in our previous report (Minami et al 1995). The platinum level was taken as the number of counts s^{-1} under the peak at m/z 195. The background was estimated by nebulizing the same solution without tissue preparation. The absolute limit of detection of platinum was determined to be 0·03 ng mL^{-1} from the standard. All results are expressed as means.

Results

Table 1 shows the platinum level in the cerebral cortex after exposure for various periods to an atmosphere containing 7% oxygen. No platinum was detected in the cerebral cortex of the control group or the group exposed for 30 s. After 60 s exposure, platinum was observed in the cerebral cortex, and levels increased with increasing exposure to low levels of oxygen.

Table 2 shows the effect on the platinum content of the cerebral cortex of mice exposed to atmospheres containing various concentrations of oxygen. Platinum was not present in the cerebral cortex of mice exposed only to air (21% oxygen), but appeared in the group exposed to 14% oxygen; the level of platinum increased with decreasing concentrations of oxygen. A high level of platinum was observed in the cerebral cortex of mice exposed for only 30 s to an atmosphere containing 5% oxygen.

Table 3 shows how platinum levels varied with time after the administration of cisplatin followed by exposure for 120 s to an atmosphere containing 7% oxygen. Platinum was not detected in the cerebral cortex 2 h after cisplatin administration, was first detected after 4 h, and peaked at 10 h. Platinum was, moreover, still retained in the cerebral cortex one week after administration. In the control group, platinum was undetectable in the cerebral cortex. In contrast with the platinum levels in the cerebral cortex, those in the blood and kidney decreased

Table 1. Effect of the time of exposure to an atmosphere containing 7% oxygen on platinum levels ($\mu g\ g^{-1}$ dry weight) in the cerebral cortex. Cisplatin (3 mg kg^{-1}) was injected into the tail vein, and 30 min after administration, the mice were placed in a plastic box and exposed for 30, 60, 90 or 120 s to the low-oxygen atmosphere. Twenty-four hours after the cisplatin administration, the mice were anaesthetized, the blood was collected, and the brains removed. After wet combustion, the platinum content of the dried cerebral cortex was determined by ICPMS. Mice in the control group were also administered cisplatin, and exposed to air (21% oxygen).

	Control	Low oxygen group			
	120 s	30 s	60 s	90 s	120 s
Platinum content	ND	ND	0·12 ± 0·05	0·13 ± 0·07	0·30 ± 0·07

All experiments were performed on groups of six mice. Data show mean ± s.d.; ND, not detected.

Table 2. Effect of oxygen concentrations on platinum accumulation in the cerebral cortex. Thirty minutes after cisplatin administration (3 mg kg^{-1}), mice were exposed for 120 s to atmospheres containing oxygen concentrations of 21 (air), 14, 10 or 7%, or for 30 s to an atmosphere containing 5% oxygen. Twenty-four hours after administration, the platinum level of the cerebral cortex was measured ($\mu g\ g^{-1}$ dry weight).

	Control	Low oxygen group			
	21%	14%	10%	7%	5%
Platinum content	ND	0·15 ± 0·08	0·30 ± 0·04	0·31 ± 0·09	0·43 ± 0·11

All experiments were performed on groups of six mice. Data show mean ± s.d.; ND, not detected.

Table 3. Changes in platinum levels in the cerebral cortex, blood and kidney with time. Thirty minutes after cisplatin administration (3 mg kg^{-1}), mice were exposed for 120 s to an atmosphere containing 7% oxygen. At various times after administration, platinum levels were measured in the cerebral cortex, blood and kidney. In the control group, after the administration of cisplatin, mice were exposed only to air.

	Control		Low oxygen						
	10 h	1 week	2 h	4 h	10 h	1 day	2 days	4 days	1 week
Cerebral cortex platinum content ($\mu g\ g^{-1}$)	ND	ND	ND	0·23 ± 0·08	0·37 ± 0·05	0·30 ± 0·08	0·24 ± 0·08	0·16 ± 0·04	0·20 ± 0·04
Blood platinum content ($\mu g\ mL^{-1}$)	0·30 ± 0·06	0·11 ± 0·03	0·36 ± 0·06	0·35 ± 0·07	0·29 ± 0·03	0·26 ± 0·02	0·16 ± 0·03	0·12 ± 0·02	0·09 ± 0·03
Kidney platinum content ($\mu g\ g^{-1}$)	10·39 ± 0·42	2·86 ± 0·29	10·60 ± 0·79	9·15 ± 0·72	8·68 ± 1·20	6·83 ± 0·43	5·31 ± 0·37	3·79 ± 0·58	2·60 ± 0·12

All experiments were performed on groups of six mice. Data show mean ± s.d.; ND, not detected.

Table 4. Occurrence of platinum in different parts of the brains of mice injected with cisplatin. Values given are the numbers of mice in the control and low-oxygen groups, respectively, that had detectable levels of platinum in various parts of the brain. Thirty minutes after cisplatin administration (3 mg kg^{-1}) mice in the low oxygen group were exposed for 120 s to an atmosphere containing 7% oxygen, decapitated at the times indicated, and the brains were removed. The brain was dissected by the method of Gispen et al (1972), and platinum levels in each part were measured by ICPMS. In the control group, cisplatin was administered to mice followed by exposure to air instead of the low-oxygen atmosphere. The first number is for the control group, the second for the low-oxygen group. Each group contained six mice.

	4 h	10 h	1 day	2 days	1 week
Right cerebral cortex	0/2	0/6	0/5	0/4	0/2
Left cerebral cortex	0/6	0/6	0/5	0/4	0/2
Basal ganglia	0/6	0/6	0/5	0/5	0/5
Thalamus and hypothalamus	0/4	0/6	0/5	0/4	0/2
Bulbus olfactorius	6/5	6/6	6/6	6/6	6/6
Cerebellum	6/4	4/6	2/4	2/3	3/3
Mesencephalon	4/5	5/6	5/6	5/5	4/4

gradually during the week after administration, and there was no difference between platinum levels in the blood and kidney of mice exposed to low levels of oxygen and mice in control groups.

Table 4 shows the occurrence of platinum in each part of the brain at various times. After administration of cisplatin to the control group, on no occasion was platinum detected in the right or left cerebral cortices, basal ganglia or thalamus and hypothalamus. In the bulbus olfactorius of the control group, platinum was detectable at each sampling time, and in the mesencephalon, platinum was also detectable at almost all sampling times. Platinum was also detected in the cerebellum. When short-term hypoxia was induced 30 min after the administration of cisplatin, platinum was detected in all parts of the brain, especially 10 h after administration.

Table 5 shows platinum levels in each part of the brain after the administration of cisplatin followed by exposure for 120 s to an atmosphere containing 7% oxygen. Data are expressed as means ± s.d. of only those samples in which platinum was detected. Platinum was not detected in the right and left cerebral

Table 5. Platinum levels ($\mu g\ g^{-1}$) found in different parts of the brains of mice injected with cisplatin. Cisplatin was administered to the mice at a dose of 3 mg kg^{-1}. Thirty minutes after administration, mice in the low oxygen-group were exposed for 120 s to an atmosphere containing 7% oxygen, decapitated at the times specified, and the brains were removed. The brain was dissected by the method of Gispen et al (1972) and platinum levels in each region were measured by ICPMS. In the control group, cisplatin was administered to mice followed by exposure to air instead of the low-oxygen atmosphere.

Region	4 h	10 h	1 day	2 days	1 week
Right cerebral cortex					
Control	ND	ND	ND	ND	ND
Low oxygen	0·19 ± 0·04	0·13 ± 0·03	0·13 ± 0·02	0·13 ± 0·02	0·14 ± 0·05
Left cerebral cortex					
Control	ND	ND	ND	ND	ND
Low oxygen	0·14 ± 0·03	0·13 ± 0·03	0·14 ± 0·02	0·12 ± 0·02	0·16 ± 0·07
Basal ganglia					
Control	ND	ND	ND	ND	ND
Low oxygen	0·23 ± 0·09	0·19 ± 0·05	0·20 ± 0·02	0·26 ± 0·04	0·22 ± 0·08
Thalamus and hypothalamus					
Control	ND	ND	ND	ND	ND
Low oxygen	0·24 ± 0·06	0·16 ± 0·05	0·20 ± 0·02	0·16 ± 0·04	0·15 ± 0·05
Bulbus olfactorius					
Control	0·52 ± 0·07	0·44 ± 0·15	0·42 ± 0·06	0·40 ± 0·09	0·33 ± 0·07
Low oxygen	0·52 ± 0·11	0·47 ± 0·07	0·43 ± 0·07	0·43 ± 0·07	0·39 ± 0·09
Cerebellum					
Control	0·11 ± 0·03	0·12 ± 0·04	0·08 ± 0·01	0·08 ± 0·001	0·10 ± 0·01
Low oxygen	0·19 ± 0·07	0·11 ± 0·02	0·17 ± 0·02	0·16 ± 0·01	0·17 ± 0·05
Mesencephalon					
Control	0·11 ± 0·01	0·12 ± 0·04	0·11 ± 0·02	0·09 ± 0·01	0·14 ± 0·02
Low oxygen	0·19 ± 0·06	0·15 ± 0·01	0·16 ± 0·03	0·16 ± 0·02	0·14 ± 0·05

All groups consisted of six mice, but the data refer only to regions of the brain in which platinum levels were detectable. Data are expressed as means ± s.d.; ND, not detected.

cortices, basal ganglia or thalamus and hypothalamus in the control group but was detected in these parts in the low-oxygen group. Platinum levels in these parts in the low-oxygen group did not change with time. A high level of platinum was detected in the bulbus olfactorius, and there were no differences between the platinum levels measured in the control and low-oxygen groups. In the 24-h period following cisplatin administration, platinum levels in the cerebellum of the low-oxygen group were higher than those of the control group. Platinum levels in the mesencephalon were also higher in the low-oxygen group than in the control group 4 h, 1 day and 2 days after administration.

Discussion

The permeability of the blood-brain barrier increases under various conditions, such as high P_{CO_2}, presence of some toxins, exposure to dimethylsulphoxide, and administration of solutions of high osmotic pressure (Pardridge 1988). Because lipophilic substances readily pass through the barrier whereas hydrophilic substances do so only with difficulty, it has been necessary to use solutions of high osmotic pressure to effect such passage for the latter. Cisplatin is a hydrophilic substance and unable to pass through the barrier. Indeed, Ginos et al (1987), using labelled cisplatin, showed that intravenously injected cisplatin was not transported to the brain, and Thompson et al (1984) reported that platinum levels were 10 to 20 times lower in both the spinal cord and brain than in the peripheral nerves. On the basis of these studies, it is thought that the neurotoxic effects of cisplatin are negligible, although Gregg et al (1992) reported the detection of a high concentration of platinum in the dorsal root ganglion and Duffield et al (1976) detected platinum in the kidney, pancreas, liver and subcutaneous fat of persons exposed to environmental and occupational platinum pollution. It is well known that the major side-effects of cisplatin are observed in the kidney, because of the excretion of the compound (Brady et al 1993; Reznik et al 1993).

We recently determined that the absolute detection limits of platinum were 0·05 ng mL^{-2} by ICPMS, 50 ng mL^{-1} by ICP atomic emission spectroscopy (AES), and 200 ng mL^{-1} by atomic absorption spectrophotometry; the absolute detection limit of platinum by ICPMS could, furthermore, be reduced to 0·03 ng mL^{-1} (Minami et al 1995). As shown in Tables 1 and 2, platinum was not detected in the cerebral cortex after exposure of the mice to normal atmospheric oxygen concentrations, but after short-term hypoxia, platinum appeared in the cerebral cortex. Levine (1960) introduced a method for producing unilateral anoxic lesions in combination with anoxic-ischaemia of the rat brain. The results of our study suggest, however, that only slight ischaemia, not complicated brain injury, is needed to change the permeability of the barrier, and when hypoxia (7% oxygen, 120 s) was induced 30 min before injection of cisplatin, platinum levels in the cerebral cortex were similar to those in mice in which hypoxia was induced 30 min after injection (before: $0·243 \pm 0·048$; after: $0·223 \pm 0·050$ $\mu g\ g^{-1}$). It seems that the function of the blood-brain barrier is affected for a period of 30 min. In addition, as shown in Table 3, platinum could pass through the barrier for several hours after administration and remain in the cerebral cortex for many days if the mice were exposed to low-oxygen atmospheres. In contrast, the platinum level in both the blood and the kidney decreased with time. Recovery of the function of the barrier therefore takes some time, during which platinum readily accumulates in the brain. When the location of the platinum in the brain was investigated, it was found that the metal was transported to the bulbus olfactorius, cerebellum and mesencephalon, but not to the cerebral cortex, basal ganglia or thalamus and hypothalamus (Table 4). The bulbus olfactorius differs from the other parts of the brain, possibly because of the continuity of the olfactory nerve directly to that region from the nose. The cerebellum and mesencephalon might, on the other hand, be protected by the blood-brain barrier, but hormone-like substances might directly affect the thalamus and hypothalamus. Although it is thought that platinum is not detectable in the cerebellum and mesencephalon, but is detectable in the thalamus and hypothalamus, the opposite results were obtained in this study. The detection of platinum at different levels in each part of the brain is interesting and suggests that the permeabilities of these brain parts to platinum are different. Short-term hypoxia increased the permeability of the blood-brain barrier, enabling the accumulation of platinum in all parts of the brain. Zhang et al (1993) reported that hydroxyl-radical stress appeared to be higher in the cortex than in either the hippocampus or striatum. In contrast, these regions did not differ from other regions of the brain in the present study. Platinum levels in both the cerebellum and mesencephalon were higher in the group exposed to low-oxygen atmospheres than in the control group (Table 5), whereas platinum levels in the bulbus olfactorius, although significantly higher than in the other parts of the brain, were not changed by hypoxia.

Ladik et al (1980) reported that some pharmacists preparing cisplatin mixtures complained of light-headedness, dizziness and facial flushing, and peripheral sensory neuropathy is known to be a side-effect of cisplatin. (Roelofs et al 1984; Mollman et al 1988). Although no signs of neurotoxicity were observed in the present study, in which only a single dose was injected, the results indicate that platinum is easily accumulated in the bulbus olfactorius, and might be transferred to, and accumulate in, all parts of the brain after short-term hypoxia. Cisplatin is repeatedly administered to patients for tumour therapy; this raises the question of the effect of accumulation of platinum in the brains of the patients being treated. As platinum-containing substances are able to bind to DNA (Lippert 1992; Bernges & Holler 1992; Brabec et al 1992; Hrubisko et al 1993), some side-effects might occur.

In conclusion, cisplatin is unable to pass through the blood-brain barrier under conditions of normal oxygenation, but it does pass through the barrier under conditions of hypoxia and accumulates in all parts of the brain, even when the period of hypoxia is short. In addition, platinum easily accumulates in the bulbus olfactorius after the administration of cisplatin.

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