Development of Miriplatin, a Novel Antitumor Platinum for Hepatocellular Carcinoma

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Miriplatin was approved for lipiodolization for the treatment of hepatocellular carcinoma in 2009. It is a lipophilic platinum complex containing myristates as leaving groups, and can be easily suspended in ethyl esters of iodized fatty acids obtained from poppy seed oil. Miriplatin suspension was active and was retained selectively in rat hepatic tumors after intra-hepatic arterial administration with reduced toxicities in normal livers and the whole body. In this review, we summarize the characteristic formulation, preclinical studies, and clinical studies of miriplatin.

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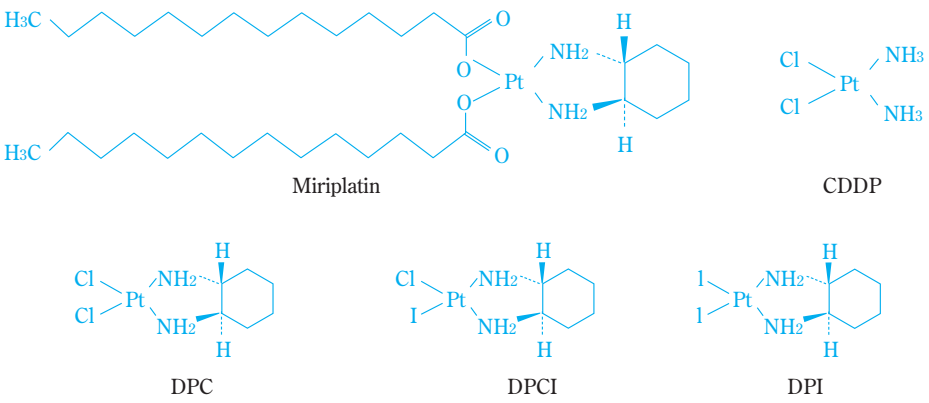
Introduction

More than 90% of hepatocellular carcinoma (HCC) cases develop from virus-associated chronic hepatitis and cirrhosis, and such carcinoma recurs multicentrically and heterochronically at a high percentage even after treatment. Therefore, controlling the tumor progression while at the same time maintaining the residual hepatic function can improve prognosis.[1), 2)] Based on these facts, the current HCC treatment algorithm is structured using the three factors as the treatment selection criteria: the level of liver damage, the number of tumors, and the tumor diameter.[3)]

If the level of liver damage ranges from low to medium and the number of tumors smaller than 3 cm in diameter is three or less, such a case will be subject to surgical resection or local ablation therapy (radiofrequency ablation, microwave coagulation, percutaneous ethanol injection therapy). Moreover, for an advanced HCC case to which surgical resection or internal local therapy cannot be applied, transcatheter arterial embolization (TAE) such as transarterial chemoembolization (TACE) or lipiodolization is widely performed. In Japan approximately 30% of all HCC cases are subject to those treatments.[4)] The theory of the treatments is based on the fact that HCC is fed exclusively by the hepatic artery, while the normal liver tissues receive a dual blood supply – approximately 75% is from the portal vein, and approximately 25% is from the hepatic artery – and that oily lymphographic agents, i.e., the ethyl esters of iodized fatty acids obtained from poppy-seed oil (iodinated poppy seed oil) administered into the hepatic artery are selectively retained in tumors. It is thought that in lipiodolization the antitumor drug released from the suspension retained in HCC is involved in the antitumor effect. It is also thought that TACE, which utilizes embolic materials as well as the antitumor drug, has additional effect due to the blockage of hepatic arterial blood flow. The antitumor drugs used for TAE include cisplatin (CDDP), epirubicin, doxorubicin, mitomycin C and zinostatin stimalamer (ZS). Of these substances, to date only ZS has been approved as an antitumor drug for administration via intrahepatic arterial injection after being suspended in iodinated poppy seed oil. Other antitumor drugs have not been permitted for the usage and dosage suitable for intrahepatic arterial injection after being suspended in iodinated poppy seed oil. Additionally, because those drugs are water-soluble, no optimum suspension method has been standardized. It has therefore been assumed that the benefit of those antitumor drugs that have generally been used for TAE up till the present time (i.e., they become selectively retained in tumors along with iodinated poppy seed oil) is not sufficiently used. Therefore, in the hope that the systemic



Chemical structures of miriplatin (left upper), CDDP (right upper), DPC (left lower), DPCI (middle lower), DPI (right lower).

Fig. 1 Chemical structures

effects and the adverse effect to the normal liver will be reduced while increasing the antitumor effect against the tumor regions during TAE by formulating an antitumor drug having high affinity to iodinated poppy seed oil, we initiated the process of research and development for a lipophilic antitumor platinum complex.

Miriplatin (Miripla®, (*SP*-4-2)-[(1*R*, 2*R*)-cyclohexane-1,2-diamine-*N*,*N*'] bis (tetradecanoato-*O*) platinum) discovered by Maeda, et al., of the National Cancer Center, is a divalent antitumor platinum complex having myristate in the leaving group and the diaminocyclohexane (DACH) carrier ligand (**Fig. 1**).[5)]

First, several platinum complexes having various types of fatty acids in the leaving group were synthesized. The present drug was then selected from those synthetic compounds based on the following considerations: suspensibility in iodinated poppy seed oil; platinum release from the suspension to physiological saline; and *in vivo* antitumor activity using the survival benefit on a tumor-bearing mouse as an index. In Japan, Miriplatin was approved in 2009 for lipiodolization in HCC, and it has been marketed since January 2010. According to the approved usage and dosage, 70 mg of the drug is suspended in 3.5 mL of iodinated poppy seed oil, which is then administered to the patient once a day through a catheter inserted into the hepatic artery. The administration of the drug must be discontinued when the tumor vessels are completely filled with suspension. The maximum dosage is 6 mL per administration (120 mg as miriplatin). When administering the drug repeatedly, it is necessary to have an observation period of at least four weeks between each administration.

Miriplatin's drug characteristics and the results of the nonclinical and clinical trials are summarized below.

Drug Characteristics

The freeze-dried miriplatin drug product has the following two characteristics: It is easily suspended into iodinated poppy seed oil, and it keeps its dispersed state for a long duration. The freeze-drying method is applied to the formulation process in order to achieve those characteristics.

Because crystalline particles of the miriplatin drug product had cohesiveness with each other, unless special processes such as sonication were added, it was difficult to uniformly disperse them into iodinated poppy seed oil. However, the miriplatin drug product obtained via the freeze-drying method formed fine, spherical particles with an average size of approximately 10 μm (**Fig. 2**), and the specific surface area of each particle became enlarged. The increase of miriplatin's wettability toward iodinated poppy seed oil through particle-property modification has enabled us to prepare a uniform suspension simply by shaking a vial by hand immediately after adding iodinated poppy seed oil (**Fig. 3**). Furthermore, it has been confirmed that the suspended particles remain dispersed for a long period of time (over 24 hours) at room temperature after the suspension operation.

It was assumed that if miriplatin possessed a high affinity toward iodinated poppy seed oil, the platinum components should be released slowly from miriplatin suspended in iodinated poppy seed oil (miriplatin suspension) prepared through the above procedures. We therefore created an *in vitro* release test system using a gyrotary shaker in order to evaluate the characteristics of miriplatin in terms of the platinum release from the suspension. First, physiological saline was selected as the test solution and then the platinum concentration in

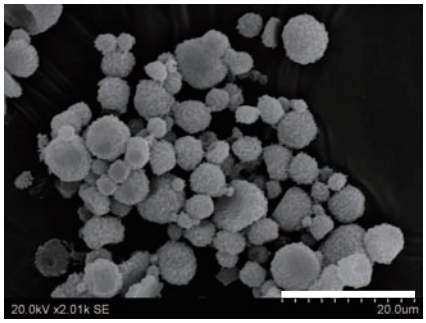


Fig. 2 Scanning electron micrograph of freeze-dried particle of miriplatin

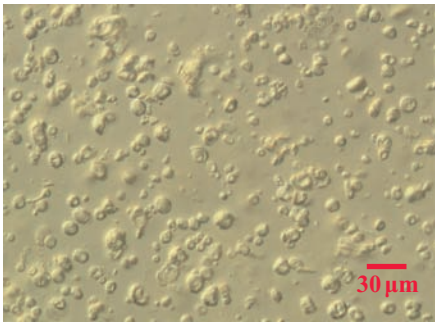
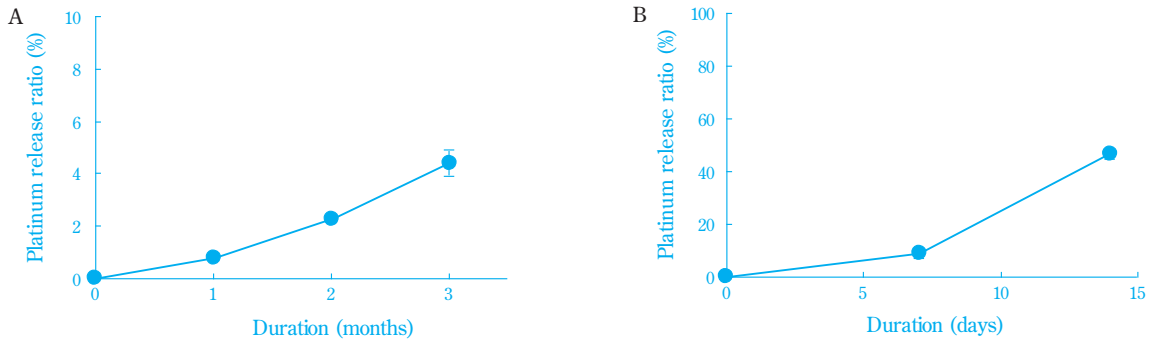
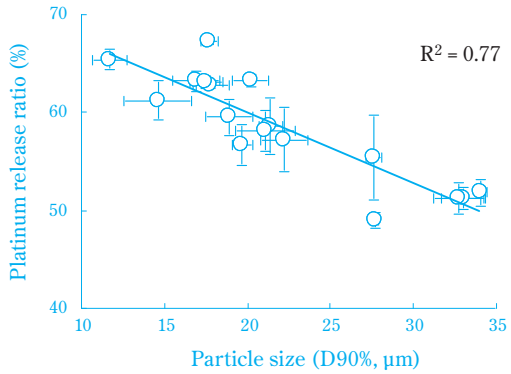


Fig. 3 Differential interference contrast micrograph of miriplatin suspended in ethyl ester of iodized fatty acids obtained from poppy seed oil



A) 0.1 mL of miriplatin suspension (20 mg/mL) was layered over 10 mL of physiological saline in test tubes. Test tubes were rotated vertically for 1, 2, and 3 months at 5 rpm in an incubator at 37°C.
B) 0.5 mL of miriplatin suspension (20 mg/mL) was layered over 10 mL of rat serum containing 1 % polysorbate 80 in test tubes. Test tubes were rotated vertically for 7 and 14 days at 5 rpm in an incubator at 37°C.
The amounts of platinum in the aqueous phase were quantitatively analyzed by atomic absorption spectrometry. Platinum release ratio (%) was calculated with the following formula: A / B × 100, where A is the amounts of platinum released in the aqueous phase, and B is the amounts of platinum in miriplatin suspension added into test tubes. All results are given as the mean ± SD (n = 3).

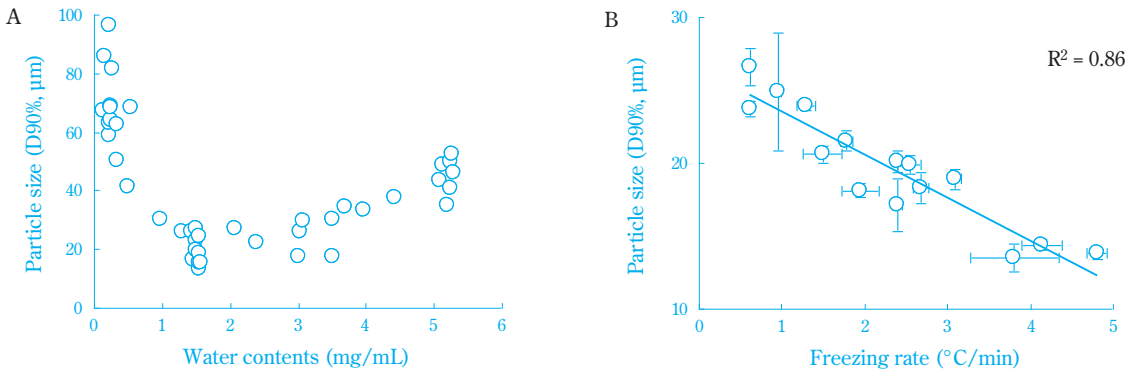
Fig. 4 Platinum release profile from miriplatin suspension to physiological saline (A) and rat serum (B)



17 profiles of miriplatin with indicated particle size were suspended in ethyl ester of iodized fatty acids obtained from poppy seed oil (iodinated poppy seed oil). 0.1 mL of miriplatin suspension (20 mg/mL) was layered over 50 mL of 0.5 M HCl in test tubes. Test tubes were rotated vertically for 6 days at 15 rpm in an incubator at 25°C. All results are given as the mean ± SD (n = 3).

Fig. 5 Dependence of particle size on platinum release ratio

the physiological saline was measured chronologically. As a result, the fact that the platinum release ratios one and three months after the initiation of the test were just approximately 1% and 4%, respectively, suggested that the release of the platinum components from the miriplatin suspension is extremely slow (**Fig. 4**-A). Next, a similar evaluation was conducted on the system using rat serum containing 0.1% of polysorbate 80 as the test solution, which reflected the biogenic environment better than the aforementioned system. Although the result showed a faster release rate than that of physiological saline, the platinum release ratio reached approximately 50% in two weeks (**Fig. 4**-B), thus suggesting that the miriplatin suspension possesses a long-term sustained release property. Furthermore, the acceleration evaluation was conducted by replacing the test solution with 0.5M hydrochloric acid. The result revealed that the



Miriplatin prepared under the indicated conditions was suspended in isopropyl myristate, and analyzed for particle size distribution. D90% represents a value on the distribution such that 90% of the particles have a volume of this value or less. All results are given as the mean in A, and the mean ± SD (n = 2 or 3) in B, respectively.

Fig. 6 Dependence of water contents in bulk solution (A), and freezing rates in freeze drying process (B) on particle size distribution

larger the particle size is, the slower the release rate will be. Investigating the relationship between the 90% D value (the particle size that makes up 90% of the total volume distribution of the particles of smaller size) and the platinum release ratio on the sixth day after the initiation of the test, a high correlation was recognized (**Fig. 5**).

One could therefore assume that, in order to control the release of the platinum components from the miriplatin suspension, it was important to define the drug particle size. Based on the above findings, the relationship between various formulation parameters and drug particle size were thoroughly investigated. Consequently, it was recognized that a trace amount of moisture content in the *tert*-butyl alcohol bulk solution prepared before the freeze-drying process and the freezing rate in the freeze-drying process (**Fig. 6**) were crucial. In other words, in order to achieve the appropriate drug particle size it was necessary to maintain the moisture content in the *tert*-butyl alcohol solution at a constant level as well as to hasten the freezing speed during the freeze-drying process. Based on the knowledge obtained from those examinations, the most appropriate process-management system for commercial production of the drug product has been established. This system has enabled the steady production of the drug product with the target particle size.

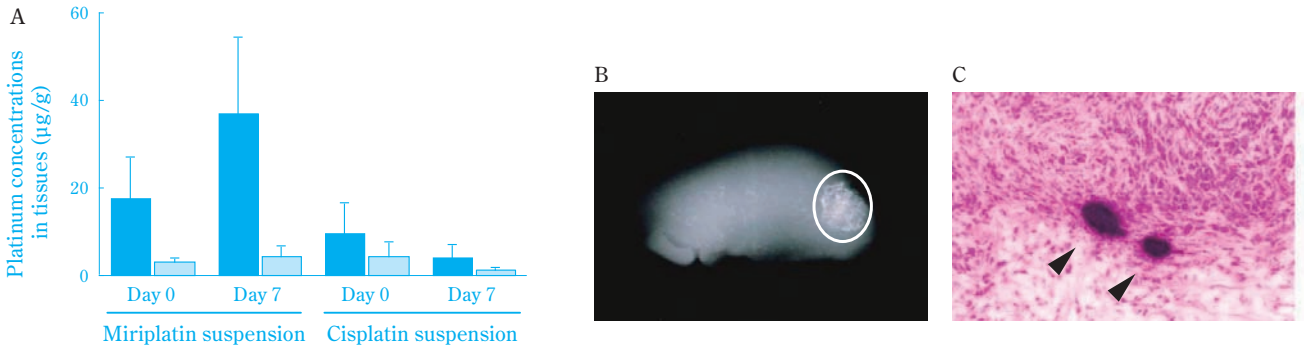
Nonclinical Trial Results

1. Pharmacokinetics

(1) Tumor Retention

Because miriplatin is a lipophilic compound and its drug form and particle size are controlled, the platinum components were gradually released from the miriplatin suspension to the serum and the physiological saline. Thus it was expected that if the miriplatin suspension possessed the sustained-release property, the tumor retention would have become heightened. Based on this assumption, the platinum concentrations in tissues were compared after intrahepatic arterial injection of the miriplatin suspension and CDDP suspended in iodinated poppy seed oil (CDDP suspension) using a rat model for implanted hepatoma (**Fig. 7**-A).[6)]

Consequently, although both drugs were distributed at higher levels in the tumor tissues than in the normal, in the tumor tissues the miriplatin suspension showed a higher level of platinum concentration than the CDDP suspension. Regarding the change over time, while the platinum concentrations in tissues decreased to approximately one-third in the CDDP suspension seven days after the administration, only a slight decrease was observed in the miriplatin suspension until the seventh day. As well, the result of soft x-ray photography performed on the liver seven days after the administration of the miriplatin suspension revealed that iodinated poppy seed oil was selectively retained in tumor tissues (**Fig. 7**-B). Furthermore, as a result of the investigation through microautoradiography for the drug distribution in the liver seven days after the administration of the miriplatin suspension labeled with [^{14}C], the histopathology showing that radioactivity was distributed in the tumor vessels was observed (**Fig. 7**-C). In the meantime, the image showing that radioactivity distributed in the normal liver was taken into macrophage-like cells was observed, thus suggesting that the miriplatin sus-



A) Platinum concentrations in tumor and normal liver tissues.
Miriplatin suspension (400 μg/head) and CDDP suspension (400 μg/head) were injected into the hepatic artery of AH109A tumor-bearing rats at the volume of 0.02 mL/head. Immediately and 7 days after the administration, resected livers were divided into tumor tissues (■) and normal liver tissues (□). Tissue homogenates incinerated using nitric acid and hydrogen peroxide were dissolved in 4% aqua regia and introduced to flameless atomic absorption spectrometry. All results are given as the mean ± SD (n = 7).

B) Distribution of iodinated poppy seed oil.
Miriplatin suspension (400 μg/head) was injected into the hepatic artery of tumor-bearing rats at the volume of 0.02 mL/head. Seven days after the administration, resected livers were radiographed with a soft X-ray machine. White circle indicates tumor site.

C) Distribution of ^{14}C-labeled miriplatin.
^{14}C-labeled miriplatin suspension (400 μg/head) was injected into the hepatic artery of tumor-bearing rats at the volume of 0.02 mL/head. Seven days after the administration, resected livers were frozen, sectioned to 5 μm slices, and subjected to microautoradiography. Arrow heads indicate tumor vessels filled with miriplatin suspension.

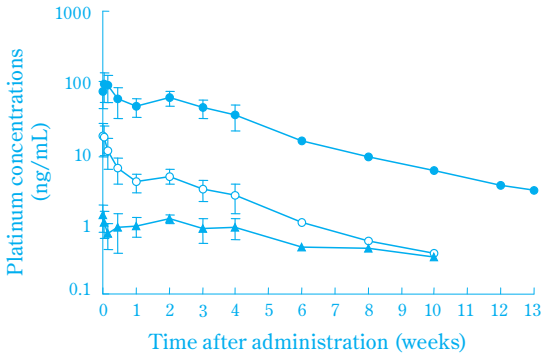
Fig. 7 Platinum concentrations in rat livers, distribution of iodinated poppy seed oil, and ^{14}C-labeled miriplatin

pension did not distribute widely in the hepatic parenchymal cells. The above findings showed that the miriplatin suspension was selectively distributed in the tumor over a longer period of time and with a higher concentration than the CDDP suspension.

(2) Pharmacokinetics in Dogs

The miriplatin suspension was also administered to a normal dog in the same clinical dosing procedure, i.e., a single intrahepatic arterial injection through a catheter inserted from the femoral artery for the purpose of investigating the pharmacokinetics of miriplatin at the whole-body level. The plasma concentration, tissue distribution and excretion rate were then measured until 13 weeks after the administration.

The total platinum concentration in the plasma showed gradual change at a constant low concentration, thus suggesting that the transfer of the platinum components into the circulating blood was slow (**Fig. 8**). Because one could assume that the platinum components released into the blood irreversibly bonded to proteins in the blood as with the related drugs such as CDDP and oxaliplatin,[7] methanol extractable fraction concentration (including the reversible protein binding components and the non-protein binding components) and the ultrafiltrate concentration (including the non-protein binding components) were measured in addition to the total platinum concentration. The methanol extractable fraction concentration and the ultrafiltrate concentration were both low at approximately 10% and several percent of the total platinum concentration respectively, and they changed mostly in parallel to the total platinum concentration. Those findings suggested that most of the platinum components released into the blood after the intrahepatic arterial injection existed in the form of the protein-bound types, while the majority of them were bound to proteins irreversibly and could not be extracted by an organic solvent.



Total platinum (●), methanol extractable platinum (○), ultrafilterable platinum (▲). All results are given as the mean ± SD of six (-day 1)or four (day 3-week 4) animals, or the mean of two (week 6–13) animals.

Fig. 8 Plasma concentrations of platinum after single intra-hepatic arterial administration of miriplatin suspension to normal dogs at a dose of 2.4 mg/kg

Table 1 Tissue distribution and excretion of platinum after single intra-hepatic arterial administration of miriplatin suspension to normal dogs at a dose of 2.4 mg/kg

		Time after administration / Animal number					
		Day 1		Day 28		Week 13	
Samples		No.1	No.2	No.3	No.4	No.5	No.6
Brain		< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Heart		0.02	0.02	0.01	0.01	0.01	0.01
Lung		0.11	0.14	0.17	0.09	0.03	0.04
Liver (total)		82.50	81.95	44.85	40.79	15.38	16.32
Gall bladder		0.01	0.02	0.02	< 0.01	< 0.01	0.01
Spleen		0.05	0.06	0.12	0.06	0.04	0.05
Pancreas		0.03	0.09	0.97	< 0.01	0.20	< 0.01
Kidney (cortex)		0.06	0.09	0.54	0.41	0.39	0.31
Kidney (medulla)		< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Excretion[a]	Urine	–	–	18.36	17.67	66.27	41.75
	Feces	–	–	4.56	3.46	10.81	9.75
	Total	–	–	22.92	21.14	77.08	51.51

Results are expressed as % of dose.
a) Cumulative excretion until the sacrifice.

Regarding tissue distribution, most of the platinum components were distributed in the liver one day after the administration (**Table 1**). In tissues other than the liver tissue, although higher distribution was observed in the lung, gallbladder, spleen, pancreas and kidney cortical tissues than in other organs, the percentage dose was less than 1% in any of the aforementioned tissues. Based on those findings, it has been confirmed that the miriplatin suspension is selectively delivered into the liver through intrahepatic arterial injection. Although the platinum amount remaining in the body decreased as time passed, most of it was distributed in the liver as seen one day after the administration, while the amounts distributed to other tissues were minimal. Moreover, up until 13 weeks after the administration 66.27% and 41.75% were excreted into urine, 10.81% and 9.75% were excreted into feces, and the total of 77.08% and 51.51% were excreted, thereby showing that the primary excretory route was urine. The above results have revealed that the miriplatin suspension, which has been selectively delivered into the liver, is retained in the liver for a long period of time, and that during this retention period the platinum components are gradually released into the circulating blood and eventually excreted into the urine.

(3) Production of Active Form DPC

Generally, it is thought that platinum-based antitumor drugs exert their antitumor effects through the binding to DNA after the release of the leaving group (platinum-DNA adduct formation). The nucleophilic substitution reaction – in which the leaving group of a platinum-based antitumor drug is displaced by the nucleophilic factor of the organism – is a non-enzymatic physiochemical reaction, and it has been reported that when using a platinum-based antitumor drug having DACH as the carrier ligand such as oxaliplatin and tetraplatin, dichloro[(1*R*, 2*R*)-1,2-cyclohexanedamine-*N*,*N*'] platinum (DPC) is the common active form.[7), 8)]

Consequently, one could predict that the active form released from the miriplatin suspension was DPC. In fact, as a result of analysis of the platinum components released into the aqueous phase when the miriplatin suspension was mixed with Earle's balanced salt solution (a solution having a composition similar to that of serum or culture medium except that it does not contain amino acid or protein), it was found that DPC, chloro [(1*R*, 2*R*)-

Table 2 Formation of DPC after incubation of miriplatin suspension with Earle's balanced salt solution for 7 days

		Concentrations in the buffer phase
Total platinum[a] (μg/mL)		0.85 ± 0.09
Component[b] (μg eq. Pt/mL)	DPC	0.42 ± 0.03
	DPI	0.044 ± 0.003
	DPCI	0.18 ± 0.02
	Total	0.65 ± 0.02

3 mL of miriplatin suspension (20 mg/mL) was layered over 6 mL of Earle's balanced salt solution in test tubes. Test tubes were rotated vertically for 7 days at 5 rpm in an incubator at 37°C. Buffer phase was analyzed by a) atomic absorption spectrometry or b) inductively coupled plasma-mass spectrometry combined with liquid chromatography. Results are given as the mean ± SD of triplicates.

1,2-cyclohexanediamine-*N*,*N*'] iodo platinum (DPCI) or [(1*R*, 2*R*)-1,2-cyclohexanediamine-*N*,*N*'] diiodo platinum (DPI) was the primary component. Of those principle components, DPC concentration was the greatest (**Table 2**). Moreover, after mixing the miriplatin suspension with the rat, dog and human serums, DPC was detected in addition to protein conjugates and amino-acid ligands.

However, as described above, most of the platinum components were present in the form of protein conjugate in the *in vivo* plasma samples, and DPC was not detected. One could assume that this was because the DPC concentration in the circulating blood remained at extremely low levels that were under the detection limit, given the fact that DPC disappeared immediately after binding with protein or amino acid as well as the slow release of the platinum components from the miriplatin suspension.

2. Safety

Because intrahepatic arterial injection is applied to the miriplatin suspension, thus targeting the vessels in the vicinity of the tumor (administered as close to the periphery of the intrinsic hepatic artery as possible), when evaluating the safety of the miriplatin suspension it was necessary to reveal the effects upon the liver (i.e., the target tissue) as well as the systemic effects of miriplatin after the suspension was transferred to the circulating blood. Thus the effects upon the liver were evaluated using a test system in which the miriplatin suspension was administered to dogs by intrahepatic arterial injection through the same procedure used for the clinical administration. The drug's systemic effects were evaluated through the following methods: For the evaluation of general toxicity and reproductive toxicity, a subcutaneous administration was conducted; and for a safety pharmacology evaluation and a genotoxicity evaluation, an intravenous administration was conducted. Furthermore, a safety evaluation was conducted on the active form of DPC.

(1) Safety Evaluation of Miriplatin

(i) General Toxicity Evaluation

(a) Evaluation of Safety in the Liver

The miriplatin suspension was administered to dogs at the maximum suspendable concentration (20 mg/mL) by a single or repeated intrahepatic arterial injections (a total of three or six administrations at a frequency of once every four weeks) with the maximum clinical dose volume or the dose volume exceeding such a clinical dose volume. Consequently the changes, which were deemed to be caused by the embolization of the suspension administered into the hepatic vessels by a single administration were observed. In the repeated administration studies, neither exacerbation of the toxicity nor any new finding was observed. Also, there were no findings that suggested damage to the blood vessel or at the injection site.

Because many hepatocellular carcinoma patients develop the complication of hepatic cirrhosis, a single intrahepatic arterial injection of the miriplatin suspension was given to the dogs with liver damage similar to chronic hepatitis or hepatic cirrhosis induced by thioacetamide, in order to investigate the safety of this present drug when the complication occurred. Consequently, although the changes that were deemed to be caused by the embolization of the suspension administered into the hepatic vessels as with the test conducted on the normal dog were observed, neither the exacerbation of liver damage nor any new finding was observed.

(b) Systemic Safety Evaluation

The toxicity studies for one month or six months in rats by repeated subcutaneous administration were conducted to achieve high systemic exposure. Consequently, only changes in the plasma protein and the increases in the total plasma cholesterol and in the liver weight were observed. The systemic toxicity was extremely weak, and there was no exacerbation of the toxicity after the administration period was extended. The no-observed-adverse-effect level (NOAEL) in the six-month toxicity study was 25 mg/kg for males and 12.5 mg/kg for females, which was equivalent to exposure approximately eleven times greater than the clinically defined maximum exposure.

Additionally, for a safety pharmacology evaluation, the effects of miriplatin on the function of various organ systems were evaluated in the *in vitro* and *in vivo* studies. Through a single intravenous administration of miriplatin (emulsion drug) to mice, rats, cats and dogs, the effects of miriplatin on the following systems were investigated: the central nervous system; the respiratory and cardiovascular systems; the autonomic nervous system and smooth muscle; the gastrointestinal system; the urinary system; and the blood systems. In the *in vitro* studies, the effects on the smooth muscle and platelet aggregation function were investigated. Consequently, there were no effects associated with miriplatin.

(ii) Other Safety Evaluations

(a) Evaluations of Genotoxicity and Reproductive and Developmental Toxicity

Regarding the genotoxicity, while the result of the reverse mutation test using bacteria was positive, the results of the chromosomal aberration test in cultured cells and the micronucleus test using miriplatin (emulsion drug) in mice turned out to be negative.

The reproductive and developmental toxicity studies were conducted by subcutaneous administration using rats and rabbits. The following studies were conducted: the study for effects on fertility and embryo-fetal development in rats; the study for effects on pre- and postnatal development, including maternal function; and the study for effects on embryo-fetal development in rabbits. Consequently, although a decrease in viability of offspring due to effects on maternal reproductive function was observed at the dosage of 25 mg/kg or higher in rats, miriplatin did not show an embryonic/fetal lethal effect or teratogenic effect, and there was no effect on fetal growth.

(b) Antigenicity Studies and Investigations on Increase in the Eosinophil Count

In the clinical trial, the transient increase of the eosinophil ratio (percentage), which reached its peak one to three weeks after the administration, was observed as an adverse event unique to miriplatin. Therefore, several types of antigenicity studies were conducted in order to evaluate the antigenicity of miriplatin upon its administration and several investigations were conducted for the purpose of elucidating the mechanism. The results of the studies of the active systemic anaphylaxis (ASA) reaction in rabbits, the passive cutaneous anaphylaxis (PCA) reaction in rabbits and guinea pigs, ASA and PCA reactions in guinea pigs, and PCA reaction in mice and rats were all negative. Furthermore, as a result of intravenous administration studies in rabbits and intravenous administration studies and intrahepatic arterial injection studies in rats (all of which were conducted for the purpose of elucidating the mechanism of the increase in eosinophil count), a transient increase in the eosinophil count was observed in the blood. While the anti-miriplatin antibody (IgG) concentration in blood was increased in rabbits after intravenous administration, no antibody production was observed in intravenous administration studies or intrahepatic arterial injection studies in rats. These results suggested the low possibility that miriplatin causes serious immune reactions such as anaphylaxis. In the intrahepatic arterial injection studies in rats, the following changes were observed: eosinophilic infiltration into the liver three days after administration; and the increase of eosinophil count in the blood and bone marrow, as well as the increase of IL-5 positive cells (T cells) in the bone marrow seven days after administration. Furthermore, as a result of the investigation on the chemotaxis ability of human eosinophils using miriplatin, it has been revealed that miriplatin does not affect the chemotaxis ability of eosinophils. These results suggested that the transient increase of the eosinophil count observed during the clinical trial may be caused by eosinophil chemotaxis to the liver due to the indirect action of the miriplatin suspension as well as the increased eosinophil count in the bone marrow accompanied by the increase in the IL-5 positive cells (T cells), which occurred immediately after the eosinophil chemotaxis.

However, eosinophilic infiltration into the liver occurred transiently and there were no findings suggesting hepatotoxicity in the hematology and biochemical examinations. These results suggested the low possibility that miriplatin caused serious liver damage.

(2) Safety Evaluation of Active Form DPC

In order to evaluate the toxicity of the active form DPC under systemic exposure, DPC was administered to rats by subcutaneous administration. Consequently, the injury changes at the injection site and the various changes that were deemed to be caused by DPC's cytostatic activity were observed, and the NOAEL was 0.03 mg/kg in repeated administration studies. Furthermore, as described previously, DPC immediately disappears after the administration of the miriplatin suspension due to the reaction with protein and other substances, and therefore it is not detected in the circulating blood. Additionally, for the safety pharmacology evaluation of DPC, the effects on the following systems were investigated: the central nervous system; the respiratory and cardiovascular systems; smooth muscles; and the blood systems. As a result of the investigation, although DPC affected the respiratory and cardiovascular systems in the anesthetized dogs, those changes were transient, mild and observed only at the high dosage (3 mg/kg, intravenous administration). Following the above-described procedures, the miriplatin suspension was administered to dogs by intrahepatic arterial injection. Although changes that were deemed to be caused by

Table 3 *In vitro* antitumor activities of miriplatin, DPC, DPI, CDDP, and ZS against rat and human liver cancer cell lines

Cell Lines	IC_{50} (μg/mL)				
	Miriplatin	DPC	DPI	CDDP	ZS
AH109A	> 20	0.14 ± 0.07	0.83 ± 0.32	0.30 ± 0.07	0.13 ± 0.00
HepG2	> 20	0.26 ± 0.24	2.3 ± 1.0	0.96 ± 0.27	0.32 ± 0.19
HuH-7	> 20	1.9 ± 1.8	6.5 ± 2.4	1.2 ± 0.3	0.69 ± 0.18
Li-7	> 20	0.31 ± 0.02	2.1 ± 0.2	0.42 ± 0.03	0.22 ± 0.05

One day after the plating of cells into microplates, miriplatin, DPC, DPI, CDDP, and ZS were added as aqueous solutions. Cells were exposed to agents for three days at 37°C in 5 % CO_2. The IC_{50} value was defined as the concentration inhibiting cell growth by 50 % compared with control. All results are given as the mean ± SD of triplicates.

the embolization of the liver vessels were observed, no exacerbation was observed in repeated administration studies, nor was the liver toxicity increased in the animal model with liver damage. Furthermore, the systemic toxicity was investigated by subcutaneous administration, which revealed that the systemic toxicity was extremely weak. Additionally, as a result of the safety pharmacology studies, miriplatin did not demonstrate any functional change that would predict serious side effects to various organ systems. It was suggested that the increased eosinophil count observed in the clinical trial may have been caused by eosinophil chemotaxis to the liver due to the indirect action of miriplatin, as well as by the increased eosinophil count in the bone marrow accompanied by the increase in IL-5 positive cells (T cells), which occurred immediately after the eosinophil chemotaxis. It was also suggested that there was only a low possibility that miriplatin would demonstrate serious immune reactions such as anaphylaxis.

3. Efficacy and Pharmacology

(1) *In vitro* Antitumor Activity of Active Form DPC

The *in vitro* antitumor activities of miriplatin, DPC, DPI, CDDP and ZS on rat or human hepatoma cell lines were investigated.[6), 9)] As shown in **Table 3**, using the IC_{50} value (the drug concentration inhibiting cell growth by 50% compared with controls) as the index, *in vitro* antitumor activity of DPC was stronger than that of DPI and was similar to that of CDDP and ZS. Furthermore, when iodinated poppy seed oil was not used as the carrier, no activity was observed in water-insoluble miriplatin. Therefore, based on the strength of the *in vitro* antitumor activity as well as the aforementioned results of the investigation on the *in vitro* and *in vivo* metabolic pathways, one could surmise that the major active form of miriplatin was DPC.

(2) *In vivo* Antitomor Activity of Miriplatin Suspension

In various animal models where rat and rabbit tumors were implanted in the liver and tumors were induced by chemical carcinogens, the miriplatin suspension demonstrated *in vivo* antitumor activity upon single administration by intrahepatic arterial injection.[6), 9)–11)] Of all those models, **Table 4** shows the results of the comparison of the efficacies of miriplatin and other drugs using the rat tumor model.

Table 4 Antitumor activities of miriplatin suspension, CDDP suspension, and ZS suspension at the therapeutic dose after intra-hepatic arterial administration

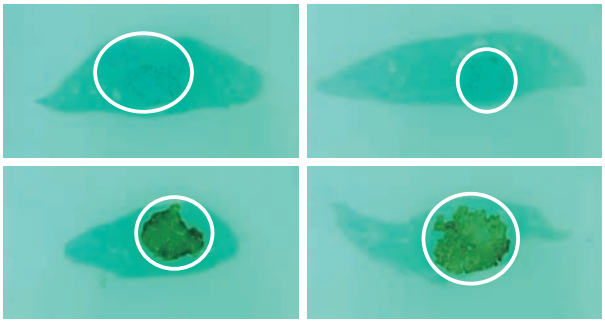
Treatment	Dose (μg/head)	Tumor growth rate (%)		Body weight change (%)
Untreated		213 ± 29		–2.8 ± 7.3
Sham-operated		202 ± 106		–1.2 ± 2.1
Iodinated poppy seed oil		185 ± 37		–3.1 ± 3.2
Miriplatin suspension	400	67 ± 24	*	–2.9 ± 1.9
CDDP suspension	400	33 ± 23	*	–4.7 ± 4.3
ZS suspension	20	175 ± 31		–1.9 ± 1.7

Miriplatin suspension (400μg/head), CDDP suspension (400μg/head), ZS suspension (20μg/head), and iodinated poppy seed oil alone were injected into the hepatic artery of AH109A tumor-bearing rats at the volume of 0.02 mL/head. Seven days later, tumor growth rates and changes in body weight were evaluated. No agent was administered in the untreated group (only measurement of tumor size) or sham-operated group (measurement of tumor size and occlusion of the gastroduodenal artery). All results are given as the mean ± SD (n = 7). A Dunnett test of the tumor growth rate or change in body weight at day 7 demonstrated a significant difference, $*p < 0.01$, comparing the group treated with iodinated poppy seed oil alone to those treated with agents.

Because the dosage is individually adjusted according to the tumor size, the condition of the tumor vessels and the degree of liver dysfunction for TACE and lipiodolization, one can assume that the drug exposure level (dose), which is the basis of the local effectiveness of the drug, is determined by the drug concentration in iodinated poppy seed oil. Accordingly, the efficacies of the three drugs of miriplatin, CDDP and ZS were compared by setting each drug concentration in iodinated poppy seed oil at the level equivalent to that used in the actual clinical situation. Using as an index the tumor growth rate one week after the administration, ZS suspended in iodinated poppy seed oil (ZS suspension, concentration of 1 mg/mL) did not show efficacy as compared to the iodinated poppy seed oil, and the tumor growth rate decreased significantly in both the miriplatin suspension and the CDDP suspension (the concentration was 20 mg/mL in both suspensions). Moreover, no significant reduction in body weight was observed in any of the models. Additionally, in this rat model the iodinated poppy seed oil alone showed no effect on tumor growth. These results have revealed the fact that, under the condition in which each drug concentration in iodinated poppy seed oil is set at the level equivalent to that used in the actual clinical situation, the miriplatin suspension demonstrates stronger *in vivo* antitumor activity than the ZS suspension does.

(3) Mode of Action

It is considered that the platinum-DNA adduct formation and the induction of apoptosis are important mechanisms of action for antitumor platinum complexes.[12] These actions were investigated using the rat hepatoma cell line AH109A and the human hepatoma cell line Li-7. The results indicated that the miriplatin suspension induced the platinum-DNA adduct formation and apoptosis *in vitro* as with the CDDP suspension.[6), 9)] Moreover, the fact that in the tumor tissues of the rat tumor model (to which the miriplatin suspension was administered by intrahepatic artery injection) 61±52 pg of platinum bound to DNA was detected per 1 μg of DNA, and the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL)-positive cells were observed (**Fig. 9**), indicated that miriplatin also induced the platinum-DNA adduct formation and apoptosis *in vivo*.[6)]



Miriplatin suspension (400 μg/head), CDDP suspension (400 μg/head), and iodinated poppy seed oil alone were injected into the hepatic artery of tumor-bearing rats at the volume of 0.02 mL/head. Three days after the administration, livers were resected and fixed in formalin. Paraffin sections of livers were stained by the TUNEL method and counter-stained with hematoxylin. Left upper) untreated, right upper) iodinated poppy seed oil alone, left lower) miriplatin suspension, right lower) cisplatin suspension. White circles indicate tumor sites.

Fig. 9 TUNEL staining of rat livers bearing tumors

Clinical Trial Results

A phase I clinical trial of miriplatin was conducted on the CDDP-resistant HCC patients with the maximum dosage of 6 mL per administration by gradually increasing the miriplatin concentration in iodinated poppy seed oil.[13)] Consequently, the maximum allowable dose of miriplatin used for intrahepatic arterial injection was estimated at 20 mg/mL or greater. Based on this estimation, the recommended dose of this present drug was concluded to be 20 mg/mL (120 mg as miriplatin). The maximum blood concentration of platinum after the administration of miriplatin was extremely low, ranging from 5.3 ng/mL to 14.2 ng/mL. Moreover, the half-life was extremely long, ranging from 18 days to 707 days. As a result of the early phase II clinical trial conducted on 15 HCC patients with the above recommended dose, nine cases (60.0%) achieved CR (tumor reduction of 100% or a necrosis effect of 100%) according to the Criteria for the Evaluation of Direct Efficacy in Treatment of Hepatocellular Carcinoma.[14)] With regard to safety, no serious side effects classified as Grade 4 or higher were observed, and most of the side effects were gone within four to six weeks after the administration. Furthermore, in one patient in which surgical resection was performed three months after the second administration of the drug, the platinum concentrations in the tumor were nine times higher than that in the nontumorous liver tissues.

In the late phase II clinical trial, the efficacy and safety of the miriplatin suspension (20 mg/mL) were investi-

gated through a parallel group comparison using the ZS suspension (1 mg/mL) as a reference arm.[15] As a result of the evaluation of the antitumor effect conducted in compliance with the Criteria for the Evaluation of Direct Efficacy in Treatment of Hepatocellular Carcinoma, the percentages of the primary endopoint TEV (a necrosis effect of 100% or tumor reduction of 100%) were 26.5% and 17.9% in the miriplatin suspension group (n=83) and the ZS suspension group (n=39) respectively. Moreover, the two-year survival rates were 75.9% and 70.3% in the miriplatin suspension group and the ZS suspension group respectively, and the three-year survival rates were 58.4% and 48.7% in the miriplatin suspension group and the ZS suspension group respectively. The incidence rates of adverse events classified as Grade 3 or higher were 59.0% (49/83) and 64.1% (25/39) in the miriplatin suspension group and the ZS suspension group respectively. The incidence rates of serious adverse events (including death) were 10.8% (9/83) and 7.7% (3/39) in the miriplatin suspension group and the ZS suspension group respectively. Although one fatal case was recognized in each group, the causal relationship of the incident with miriplatin was denied for the miriplatin suspension group. Other serious adverse events were seen in eight cases and two cases in the miriplatin group and the ZS suspension group respectively. Although the second administration was canceled for four of the eight cases in the miriplatin group due to the incidence of serious adverse events, all eight patients recovered with no treatment or the appropriate treatment, and in two cases a causal relationship with miriplatin was denied. Furthermore, the hepatic vascular injury expressed in the ZS suspension group at the high percentage was not observed in the miriplatin suspension group. Based on those results, we have conclude that miriplatin demonstrates an antitumor effect comparable to that of ZS (which is the only existing approved pharmaceutical agent used in the form of suspension through intrahepatic arterial injection), and that it is a safer, more useful drug.

Generally, while it can be expected that the embolic materials used for TAE will enhance the antitumor effect due to the blockage of hepatic arterial blood flow, there is also concern that it may strengthen the impact on the nontumorous liver. As previously described, all the miriplatin clinical trials were conducted through lipiodolization without any embolic materials. Therefore, a pilot study was conducted on a small number of patients for the purpose of investigating the safety and efficacy of miriplatin in combination with embolic materials.[16] The result of the pilot study suggested that miriplatin combined with embolic materials might enhance the antitumor effect. Regarding safety, as compared to studies conducted with lipiodolization, no new adverse event that could cause clinical problems, or other adverse effect that could cause serious problems to the liver were recognized. Based on the results of these studies, a phase III clinical trial is now in progress to compare miriplatin and epirubicin in combination with embolic materials.

Conclusion

Although CDDP for intravenous injection is an antitumor agent used for the standard combination chemotherapy for various types of cancers, the efficacy against advanced HCC is not high when administered systemically. Meanwhile, although a tumor-selective drug distribution and a promising antitumor effect have been reported regarding the CDDP suspension used for lipiodolization for the treatment of HCC, [17] the method for the preparation of the CDDP suspension has not been standardized yet. Contrary to the actual situation in which TAE is widely performed for advanced HCC, there is insufficient clinical evidence for the antitumor agent used for this therapy, and thus no drug has been established as the standard therapeutic agent. Therefore, we have developed miriplatin with the concept of a lipophilic antitumor platinum complex having a high affinity to iodinated poppy seed oil intending to achieve an antitumor agent that is easy to use in an outstanding local therapy such as TACE and lipiodolization (i.e., whose methods for the preparation of their suspensions have been standardized and optimized). Miriplatin is the first antitumor platinum complex approved for lipiodolization for the treatment of HCC.

The HCC morbidity is particularly high in East Asia, including Japan. According to the “Cancer Statistics in Japan 2010” released by the Foundation for Promotion of Cancer Research, the incidence of liver cancer in Japan in 2005 was the fifth highest, following those of stomach, lung, colon and breast carcinomas. Moreover, HCC accounts for approximately 90% of primary liver cancer. For advanced HCC, although it is believed that controlling the disease progression while maintaining the liver residual function will improve the prognosis, in most cases a complete cure cannot be expected. Moreover, although molecular target drugs have been

introduced recently, the actual situation in which HCC is extremely intractable remains unchanged. Under such a treatment environment, we hope the new HCC treatment agent miriplatin will bring good news to many patients.

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