Improvement of Intraperitoneal Chemotherapy for Rat Ovarian Cancer Using Cisplatin-containing Microspheres

Seisuke Kumagai, Toru Sugiyama, Takashi Nishida, Kimio Ushijima and Michiaki Yakushiji

Department of Obstetrics and Gynecology, Kurume University School of Medicine, 67 Asahimachi, Kurume, Fukuoka 830

Microspheres consisting of L-lactic acid and glycolic acid copolymer containing cisplatin (CDDP-PLGA) were developed to improve the delivery of cisplatin. We evaluated the effects of intra-peritoneal administration of cisplatin prepared as CDDP-PLGA in rats with ovarian cancer. The toxicity, platinum distribution, and therapeutic effects of CDDP-PLGA were evaluated as compared with those in the case of cisplatin aqueous solution. The LD_{50} of CDDP-PLGA was almost four-fold higher than that of cisplatin aqueous solution. CDDP-PLGA released cisplatin slowly and achieved a higher concentration in the peritoneal cavity and in peritoneal tumors for prolonged periods, while the tissue concentration of cisplatin was reduced elsewhere in the body, as compared with the case of cisplatin aqueous solution. The survival of rats with peritoneal carcinomatosis was increased by this delivery system relative to cisplatin aqueous solution. CDDP-PLGA thus allows a higher dose to be given without increasing systemic toxicity, enhancing the therapeutic effect of cisplatin.

Key words: Drug delivery system — CDDP-PLGA — Intraperitoneal administration — Ovarian cancer — Rat

Despite the progress in the development of chemotherapy for ovarian cancer, no improvement in the long-term prognosis of this disease has resulted. Recently, along with dose-intensified chemotherapy, new drug delivery systems (DDS) have been investigated.[1–9] Intraperitoneal (IP) administration of an anticancer drug is a popular method of chemotherapy for ovarian cancer and has the advantage of directly exposing the peritoneal carcinomatosis to the anticancer drug.[10–14] However, because most anticancer drugs are small water-soluble molecules, they are readily absorbed through the capillaries and reach the circulation. Thus, it is difficult to sustain a high drug concentration for a long time in the peritoneal cavity and surrounding tissues.[12–15] Conversely, corpuscular particles are absorbed slowly into the lymphatic system without being absorbed into the capillaries, and thus, can be retained in the peritoneal cavity for long periods of time.[15] To address this issue, a new delivery system of cisplatin was devised. L-Lactic acid and glycolic acid copolymer (PLGA) microspheres, which are corpuscular particles, were prepared, and microspheres containing cisplatin (CDDP-PLGA) were newly developed. CDDP-PLGA was absorbed *in vivo* and released the drug in a slow, predictable manner.[16, 17]

We examined the IP administration of CDDP-PLGA in rats, focusing on drug-targeting and controlled release, in order to maintain a high cisplatin concentration for a long period in the peritoneal cavity and to reduce systematic toxicity, thus allowing the administration of higher doses of cisplatin.

MATERIALS AND METHODS

Drug preparation Microspheres of CDDP-PLGA were prepared from L-lactic acid and glycolic acid copolymer (molecular weight 13,000) and cisplatin by the oil-in-oil emulsion method. The microspheres averaged approximately 100 μm in diameter and contained a cisplatin load of 5%. In *in vitro* experiments, cisplatin was released slowly during a 3-week period.[16]

Toxicity Sixty female Wistar rats, 2 weeks old and weighing approximately 40 g, were bred in the Animal Experimental Center of Kurume University and used for this experiment. CDDP-PLGA and aqueous cisplatin solution (CDDP-solution, Briplatin: Bristol-Myers Squibb Co. Ltd., NY) were used for IP administration. The standard cisplatin dose was decided 2.4 mg/kg, equivalent to a clinical dose of 100–120 mg/body of cisplatin IP. Sixty rats were divided into two groups of thirty rats. An IP dose of 2.4 mg/kg of cisplatin, either CDDP-PLGA or CDDP-solution, was administered to each group in 1 ml of normal saline with a 22-gauge needle. The CDDP-PLGA group and the CDDP-solution group were each subdivided into six groups of five rats each. At 0.5, 1, 3, 24 and 48 h, and 7 days after treatment, rats of one subgroup were killed under general anesthesia. Sera, kidneys, livers, spleens, and hearts were collected. The total cisplatin concentration in each tissue sample, along with the free (non-protein-bound) cisplatin concentration in the serum samples, was measured by atomic absorption spectrophotometry.

Following this experiment, 192 female Wistar rats, 4 weeks old and weighing approximately 80 g, were obtained and divided into 32 groups of six rats each. Twenty groups were given CDDP-PLGA, eleven groups were given CDDP-solution, and one control group was given normal saline. In the twenty CDDP-PLGA groups, 7.5–106.1 mg cisplatin/kg was given at twenty doses, increasing in a ratio of 1:1.15. In the eleven CDDP-solution groups, 2.8–38.6 mg cisplatin/kg was given at eleven doses, increasing in a ratio of 1:1.30. In the single control group, animals received 4 ml of normal saline. Each drug was directly administered by laparotomy, in 4 ml of normal saline, under general anesthesia. The rats were observed for 3 weeks after drug administration and the date of death was recorded. The lethal dose was evaluated, and the LD_{50} was calculated by the Lichfield-Wilcoxon method.

Distribution of platinum Eighty female Wistar rats, 2 weeks old and weighing approximately 40 g, with peritoneal carcinomatosis and malignant ascites were used in this experiment. These rats were prepared as neonates by intraperitoneal transplanting of 2×10^6 tumor cells of 7,12-dimethylbenz[a]anthracene (DMBA)-induced ovarian cancer.[18, 19] The eighty rats were divided into four groups of twenty rats each. A dose of 2.4, 4.8, or 9.6 mg cisplatin/kg was administered IP as CDDP-PLGA, or a dose of 2.4 mg cisplatin/kg as CDDP-solution, in 1 ml of normal saline with a 22-gauge needle. Each of the four groups was further subdivided into four groups of five rats each. At 0.5, 1, 3, and 24 h after treatment, rats of one subgroup were killed under general anesthesia, and sera, ascites, omenta, and tumors were removed for evaluation. The total cisplatin concentration in each tissue sample, along with the free cisplatin concentration in serum and ascites, was measured by atomic absorption spectrophotometry.

Therapeutic effects Forty-five female Wistar rats, 30 days old and weighing approximately 75 g, with peritoneal carcinomatosis were used in this experiment. Tumor cells (1×10^7) from DMBA-induced ovarian cancers were intraperitoneally transplanted when the rats were 20 days old. These 45 rats were divided into four groups of ten rats each and one group of five rats. The first 4 groups received 2.4, 4.8, or 7.2 mg/kg cisplatin as CDDP-PLGA, or 2.4 mg/kg cisplatin as CDDP-solution. Each drug was directly administered by laparotomy, in 2 ml of normal saline, under general anesthesia. The control group was given 2 ml of normal saline by the same method. Rats were observed and their duration of survival was recorded. Ninety days after drug administration, the survivors were killed under general anesthesia and autopsied. Macroscopic examination was performed to detect rats with or without carcinomatosis.

RESULTS

Toxicity Total cisplatin in the spleen and heart was not measured in every group. Total cisplatin in the kidney and liver was measured in every group, and cisplatin levels were significantly lower in the CDDP-PLGA-treated group at all times, as compared with the CDDP-solution group (Fig. 1). The serum concentrations of total and free cisplatin were determined only in the CDDP-solution group.

The lethal dose of CDDP-PLGA was higher than that of CDDP-solution. The LD_{50} value for the CDDP-PLGA group was 53.0 mg/kg (95% confidence interval range: 46.09–60.95), and that for the CDDP-solution group was 14.0 mg/kg (95% confidence interval range: 11.00–17.80). Thus, the LD_{50} value in the CDDP-PLGA group was almost four-fold higher than that in the CDDP-solution group (Table I).

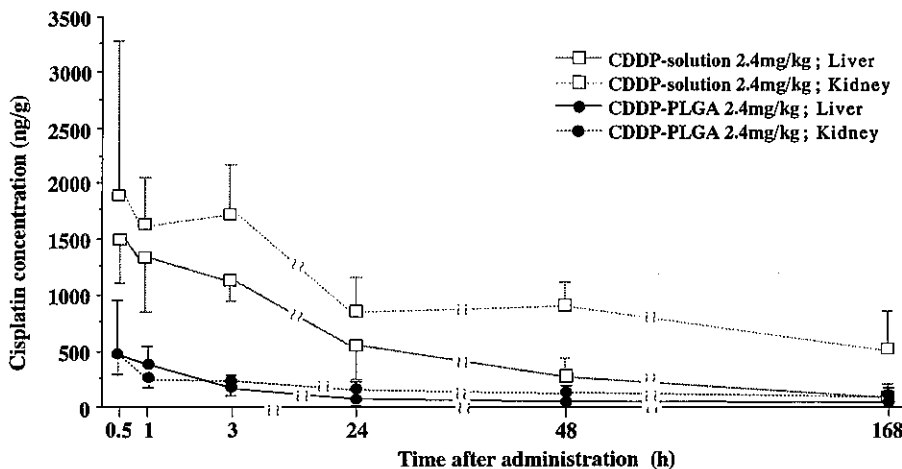Fig. 1. Total cisplatin concentrations (mean±SD) in kidney and liver.

Distribution of platinum Total and free cisplatin in the serum was not measured in the CDDP-PLGA 2.4 mg/kg group. In the remaining groups, the serum total and free cisplatin concentrations in the CDDP-solution group exceeded those in the CDDP-PLGA 9.6 mg/kg group at 0.5 h after drug administration. Conversely, after 1 h, the cisplatin levels in the CDDP-solution group decreased to a level between those in the CDDP-PLGA 4.8 mg/kg and 9.6 mg/kg groups. After 3 h, free cisplatin was barely detectable in any group (Fig. 2). In the ascites, the total and free cisplatin levels in the CDDP-PLGA 2.4

Table I. Survival of Rats and 50 % Lethal Dose of CDDP-PLGA and CDDP Solution

Dosage form	Dose of cisplatin (mg/kg)	$n^{a)}$	Number of deaths	Day of death
CDDP-PLGA	$7.5^{b)}$	6	0/6	—
	8.6	6	0/6	—
	9.9	6	0/6	—
	11.3	6	0/6	—
	13.0	6	0/6	—
	15.0	6	0/6	—
	17.3	6	0/6	—
	19.8	6	0/6	—
	22.8	6	0/6	—
	26.2	6	0/6	—
	30.1	6	0/6	—
	34.7	6	0/6	—
	39.9	6	0/6	—
	45.9	6	1/6	7
	52.8	6	4/6	5,5,7,8
	60.7	6	5/6	6,6,7,7,7
	69.8	6	5/6	6,6,6,6,6
	80.3	6	6/6	5,5,6,6,6,7
	92.3	6	6/6	5,5,5,5,6,6
	106.1	6	6/6	5,5,5,5,6,6

LD$_{50}$ (95% confidence interval) 53.0 mg/kg (46.09–60.95)

CDDP-solution	$2.8^{c)}$	6	0/6	—
	3.6	6	0/6	—
	4.7	6	0/6	—
	6.2	6	0/6	—
	8.0	6	0/6	—
	10.4	6	2/6	6,7
	13.5	6	4/6	6,6,6,7
	17.5	6	5/6	4,5,6,7,7
	22.8	6	6/6	4,4,4,5,6,6
	29.7	6	6/6	4,4,4,5,5,5
	38.6	6	6/6	3,3,3,4,4,5

LD$_{50}$ (95% confidence interval) 14.0 mg/kg (11.00–17.80)

a) Number of rats given each dose of CDDP-PLGA or CDDP solution.
b) CDDP-PLGA 7.5–106.1 mg cisplatin/kg; dose ratio 1:1.15.
c) CDDP solution 2.8–38.6 mg cisplatin/kg; dose ratio 1:1.30.

mg/kg group were lower than those in the CDDP-solution group at all times. Among the other groups, the total cisplatin levels in the CDDP-solution group were higher than that in the CDDP-PLGA 9.6 mg/kg group at 0.5 h after the drug administration. After 1 h, the total cisplatin levels in the CDDP-solution group decreased to levels between those of the CDDP-PLGA 4.8 mg/kg and 9.6 mg/kg groups. During the first hour of analysis, free cisplatin concentrations in the CDDP-solution group attained values between those of the CDDP-PLGA 4.8 mg/kg and 9.6 mg/kg groups. These levels continued to decrease to levels between those of the CDDP-PLGA 2.4 mg/kg and 4.8 mg/kg groups after 3 h (Fig. 2). In contrast, in the omentum, the total cisplatin levels in all CDDP-PLGA-treated groups were higher than those in the CDDP-solution group (Fig. 3). In the tumors, the total cisplatin levels in the CDDP-PLGA 2.4 and 4.8 mg/kg groups were lower than those in the CDDP-solution group immediately after drug administration. However, after 3h, the levels exceeded those in the CDDP-solution group (Fig. 3).

Therapeutic effects Regarding the analysis of the therapeutic effects, every group that received CDDP-PLGA or CDDP-solution exhibited an increase in the mean survival time relative to the control group. In addition, the CDDP-PLGA groups showed a greater increase in their mean survival time compared with the CDDP-solution group. Moreover, in the CDDP-PLGA groups, the mean survival time increased with increasing cisplatin dosage (Table II). A total of twelve rats that survived for 90 days after drug administration were considered free of carcinomatosis upon macroscopic autopsy examination.

DISCUSSION

In the chemotherapy of ovarian cancer, drug-targeting is important about DDS,[1–3] and the targets are primary carcinomas, disseminated peritoneal carcinomatose, and lymphatic metastasis. Intraperitoneal chemotherapy is generally used for peritoneal carcinomatosis.[4–14] However, most of the available anticancer drugs, including cisplatin, are small water-soluble molecules, and it is difficult to maintain an adequate drug concentration in the peritoneal cavity for long periods.[12–14] Various modified dosage forms, including high-molecular-weight anticancer drugs, are being developed to mitigate these shortcomings. With regard to cisplatin-containing preparations, it has been difficult to prepare a stable cisplatin-releasing dosage form because of the burst phenomenon,[17, 20] and the paucity of experimental data.[21–23] CDDP-PLGA was developed as a delivery system for cisplatin because of its stable cisplatin-releasing characteristics. For proper IP administration of CDDP-PLGA, the CDDP-PLGA must not be absorbed into the capillaries, but should be
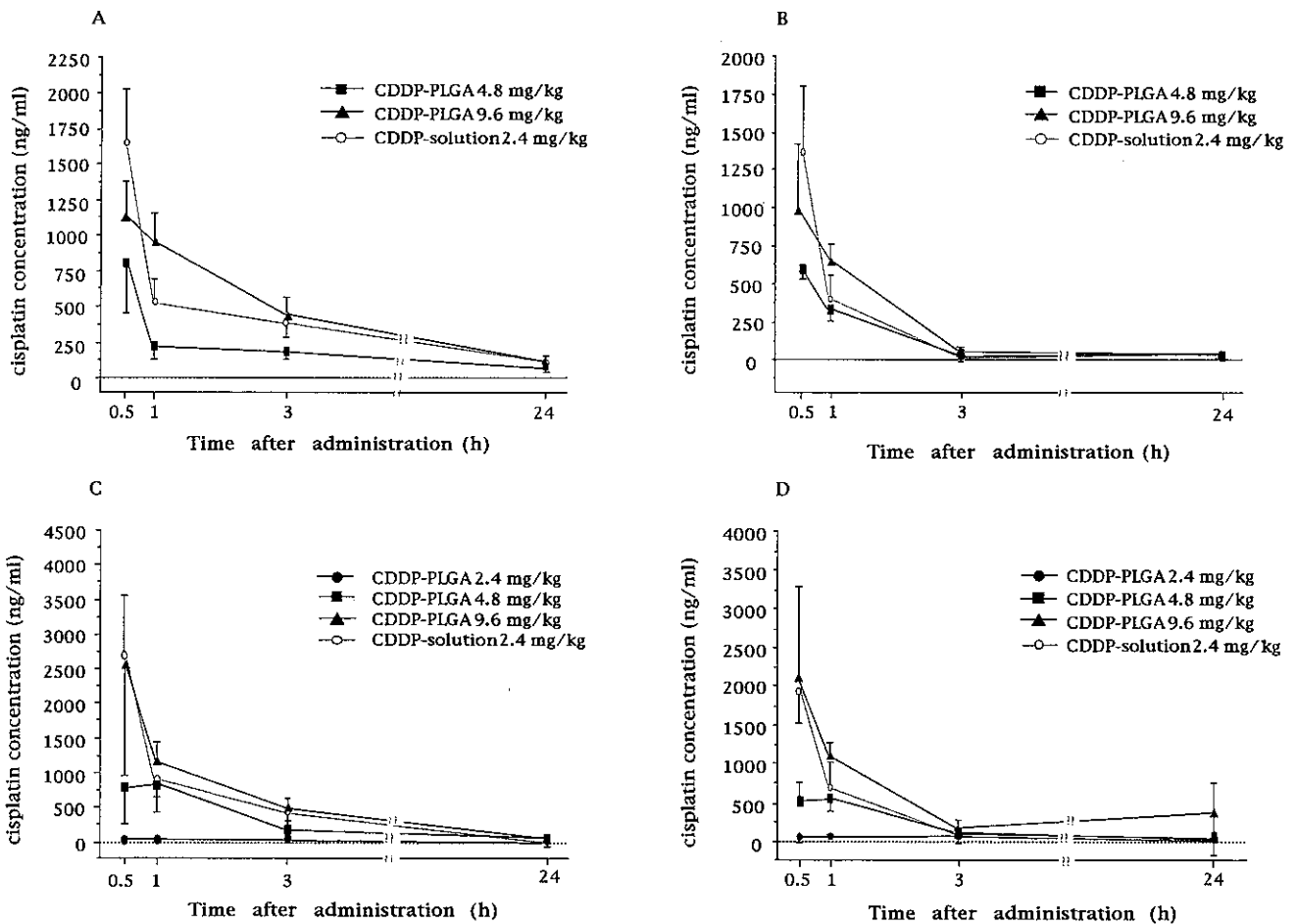
Fig. 2. Total and free cisplatin concentrations (mean±SD) in serum and ascites. A: Total cisplatin concentration in serum. B: Free cisplatin concentration in serum. C: Total cisplatin concentration in ascites. D: Free cisplatin concentration in ascites.
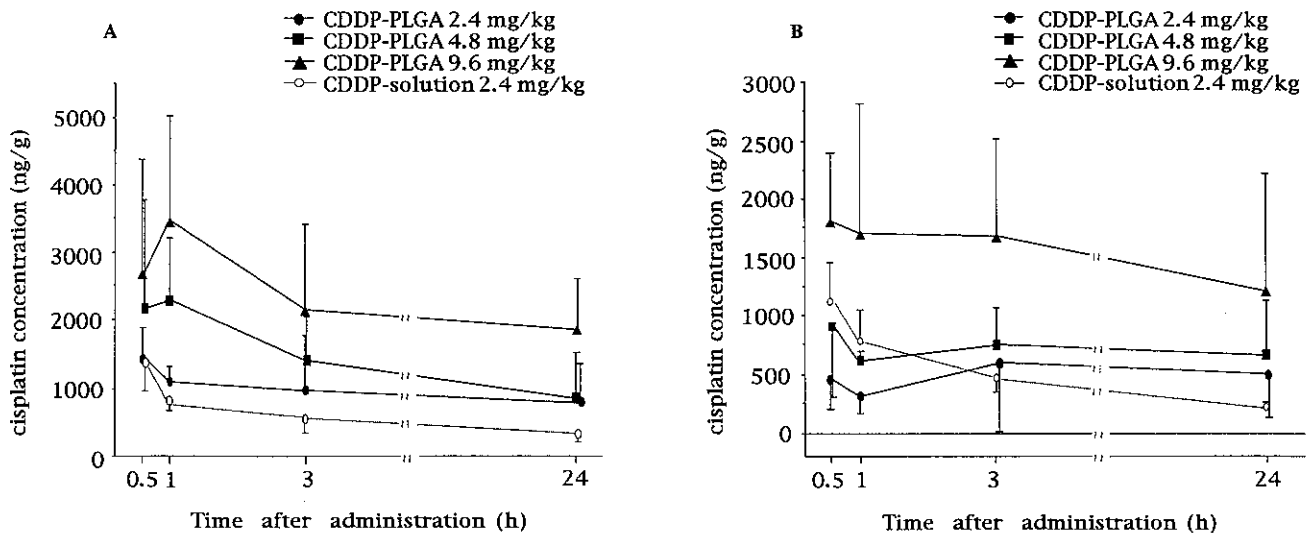

Fig. 3. Total cisplatin concentrations (mean±SD) in omentum (A) and tumor (B).

Table II. Therapeutic Effects of CDDP-PLGA and CDDP-solution in Rats with Peritoneal Carcinomatosis

Dosage form (mg of cisplatin/kg)	$n^{a)}$	Mean survival time$\pm$SD (day)	Number of survivors[b]
CDDP-PLGA 2.4	10	52.0 ± 32.9	3
CDDP-PLGA 4.8	10	62.6 ± 31.5	3
CDDP-PLGA 7.2	10	74.0 ± 18.0	4
CDDP-solution 2.4	10	48.4 ± 22.8	2
Control (normal saline 2 ml)	5	9.4 ± 2.9	0

a) Number of rats in group.
b) Survival for 90 days after drug administration.

slowly absorbed into the lymphatic system. These characteristics result in the particles remaining for a long period of time in the peritoneal cavity, where they release cisplatin slowly. This produces a higher cisplatin concentration in the peritoneal cavity for a long period and also results in a lower cisplatin concentration in the circulatory system. Therefore, we examined this dosage form.

In the toxicity experiments, cisplatin concentrations in the sera, kidneys and livers of the CDDP-PLGA group were lower than those in the CDDP-solution group. Concentrations in these tissues, in addition to the spleen and heart, can be considered as indicative of those in the rest of the body. Thus, it is plausible to infer that CDDP-PLGA slowly releases cisplatin into the peritoneal cavity, leading to lower drug concentrations in the circulation and extraperitoneal tissues. The LD_{50} of the CDDP-PLGA group was approximately four times that in the CDDP-solution group. These results suggested that high doses of cisplatin can be used without systemic toxicity, provided that stable, controlled cisplatin release is achieved.

In the experiments on the distribution of platinum, the cisplatin concentration in the ascites of the CDDP-PLGA group was lower than that of the CDDP-solution group that received the same drug dose (2.4 mg/kg). Further, the cisplatin concentrations in the serum of the CDDP-PLGA group were lower than those of the CDDP-solution group at the same drug dose (2.4 mg/kg). These results also suggested that CDDP-PLGA released cisplatin slowly into the peritoneal cavity, thereby minimizing its concentration in the circulation. In the omentum, which can be considered as an indicator of the tissues contiguous with the peritoneal cavity, the CDDP-PLGA groups showed higher cisplatin concentrations than the CDDP-solution group. CDDP-PLGA thus maintained a high concentration of cisplatin in the peritoneal cavity. This preparation may therefore be effective in treating the lymphatic metastasis common in patients with ovarian cancer. In the tumors, similar trends to the omentum were observed. The AUC (area under the curve; μg h/g) values of the total cisplatin concentration in all tumor groups were calculated. The values were as follows: CDDP-solution group, 9.8; the CDDP-PLGA 2.4 mg/kg group, 12.7; the CDDP-PLGA 4.8 mg/kg group, 15.3; and the CDDP-PLGA 9.6 mg/kg group, 32.8. These values indicate that the CDDP-PLGA preparation of cisplatin would provide a superior antitumor effect.

In the therapeutic effect experiments, the CDDP-PLGA treatment groups, especially the 4.8 and 9.6 mg/kg groups, clearly exhibited prolonged survival compared with the CDDP-solution group. Overall, the results indicated that CDDP-PLGA can be administered a higher dose of cisplatin than CDDP solution and can offer a superior therapeutic effect.

In conclusion, the CDDP-PLGA preparation of cisplatin administered intraperitoneally to rats released the drug slowly over a long period in the peritoneal cavity. This new delivery system appeared to slow the loss of cisplatin into the circulation. This effect should reduce systemic toxicity, and it may be possible to administer almost three times higher doses of cisplatin with less toxicity, while obtaining a greater therapeutic effect than that observed with aqueous solution. This new cisplatin delivery system may be useful in treating patients with disseminated peritoneal carcinomatosis and lymphatic metastasis. Its safety and efficacy should be evaluated clinically.

ACKNOWLEDGMENTS

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