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Chemotherapy Targeting Regional Lymph Nodes by Gastric Submucosal Injection of Liposomal Adriamycin in Patients with Gastric Carcinoma

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We investigated the delivery of adriamycin (ADR) to the regional lymph nodes of the stomach following the gastric submucosal injection of liposomal adriamycin (Lipo-ADR) in 34 gastric carcinoma patients, as well as following intravenous administration of free ADR (F-ADR) in another 18 patients. Prior to radical gastrectomy, Lipo-ADR was endoscopically injected into the gastric submucosa adjacent to the primary tumor via a needle-tipped catheter. After Lipo-ADR injection, the ADR concentration in the primary and secondary drainage lymph nodes was higher than in the other regional lymph nodes. Thus, the regional nodes more susceptible to metastasis showed higher levels of ADR. In contrast, the intravenous administration of F-ADR produced a similar and far lower ADR concentration in all the nodes. Delivery of ADR to the primary drainage lymph nodes following injection of 5 ml of Lipo-ADR was compared with delivery to the left gastric artery lymph nodes after intravenous administration of an equal dose of F-ADR. The ADR levels (μg/g) after gastric submucosal injection were 15.1 ± 8.30 on day 1 ($n=4$); and 11.9 ± 4.80 on day 4 ($n=6$). Those after intravenous administration were 0.29 ± 0.10 on day 1 ($n=4$); and 0.36 ± 0.0 on day 4 ($n=2$). The differences between the two groups were significant ($P<0.05$). The ADR levels after the gastric submucosal injection were far higher than those after intravenous administration. These findings indicate that the gastric submucosal injection of Lipo-ADR can specifically deliver ADR to the regional lymph nodes at high concentrations. Such preoperative adjuvant chemotherapy targeting the regional lymph nodes may be useful for preventing the lymph node recurrence of gastric carcinoma.

Key words: Regional lymph node — Chemotherapy — Gastric carcinoma — Gastric submucosal injection — Liposomal adriamycin

The regional lymph nodes are a common site for the postoperative relapse of gastric carcinoma. Several investigators have administered regional chemotherapy in an attempt to improve the prognosis of this disease, and preoperative chemotherapy targeting the regional lymph nodes appears to show some promise of preventing recurrence after radical gastrectomy.[1-4]

ADR[4] is often used to treat patients with gastric carcinoma.[5,6] *In vitro* studies have demonstrated that the cytocidal effect of this drug depends on the concentration and the duration of exposure.[7] Unfortunately, the intravenous administration of high-dose ADR is associated with acute toxicity, such as myelosuppression and immunosuppression, as well as with cumulative dose-limiting cardiotoxicity.[8] The ability to deliver ADR to regional lymph nodes in a more appropriate manner could increase its therapeutic index, enhance its efficacy, and reduce its toxicity.

Gastric submucosal injection of anticancer agents allows the selective delivery of chemotherapy to the regional lymph nodes.[9-13] A previous study using F-ADR and Lipo-ADR in rabbits demonstrated that gastric submucosal injection could specifically deliver ADR to the regional lymph nodes of the stomach. However, it is not clinically appropriate to perform gastric submucosal injection of F-ADR because of local toxicity such as ulceration.[9] This toxicity can be reduced by encapsulation of ADR in liposomes and the feasibility of gastric submucosal injection of Lipo-ADR as preoperative adjuvant chemotherapy in gastric carcinoma patients has been suggested.[10]

In the present study, we injected Lipo-ADR into the gastric submucosa adjacent to the primary tumor in patients with gastric carcinoma prior to radical gastrectomy. The dose of Lipo-ADR was initially set at 5 ml (1 mg/ml of ADR). No signs of local toxicity such as ulceration or erosions were observed after the injection

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[4] Abbreviations: ADR, adriamycin; Lipo-ADR, liposomal adriamycin; F-ADR, free adriamycin; MLVs, multilamellar vesicles; AUC, the area under the concentration-time curve.

of 5 ml of Lipo-ADR, so the dose of Lipo-ADR was increased to 10 ml. In a pharmacokinetic study, ADR levels in the regional lymph nodes after the gastric submucosal injection of Lipo-ADR were compared with those after the intravenous injection of an equal dose of F-ADR. We also assessed the usefulness of gastric submucosal injection of Lipo-ADR as preoperative adjuvant chemotherapy targeting the regional lymph nodes.

MATERIALS AND METHODS

Patients and study design A total of 37 patients with biopsy-proven and potentially resectable gastric carcinoma were enrolled in this study from August 1989 to December 1990. The patients were assigned to receive either the gastric submucosal injection of 5 ml of Lipo-ADR or the intravenous administration of 5 mg of F-ADR as preoperative adjuvant chemotherapy according to a prospectively generated randomization schedule. From January to April 1991, another 15 patients received the gastric submucosal injection of 10 ml of Lipo-ADR as preoperative adjuvant chemotherapy. The study protocol was approved by our institutional review board as being in accordance with the Declaration of Helsinki and written informed consent was obtained from each patient before enrollment.

Lipo-ADR Liposomes containing ADR were prepared by the reverse-phase evaporation method.[14] The Lipo-ADR contained 10 mg of ADR, 100 mg of egg lecithin, 24 mg of cholesterol, and 100 mg of lactose. When stored at −20°C, Lipo-ADR remained stable for at least 3 months.[10] To reconstitute Lipo-ADR before administra-

Table I. Clinical Characteristics of the Patients

	Endoscopic injection of Lipo-ADR		Intravenous administration of F-ADR
Dose	5 ml	10 ml	5 mg
Number of patients	19	15	18
Median age (range), years	62.5 (40–77)	51.4 (37–67)	63.9 (36–83)
Sex (M:F)	12:7	9:6	10:8
Gastrectomy (distal:total:PD)	15:4:0	6:9:0	10:7:1
Curativity (curative:noncurative)	19:0	14:1	13:5
Time from administration to operation			
1 day	4	1	4
2 days	3	1	3
3 days	2	2	3
4 days	6	3	2
6 days	2	4	3
7 days	1	0	3
8–14 days	1	4	0
Stage			
stage I	6	5	7
stage II	2	4	1
stage III	10	3	7
stage IV	1	3	3
Macroscopic type:			
Type 0	3	4	7
Type 1	0	0	0
Type 2	2	3	2
Type 3	7	4	6
Type 4	2	2	1
Type 5	5	2	2
Histology:			
Pap	1	0	0
Tub_1	2	0	4
Tub_2	7	4	5
Por	4	7	2
Muc	2	1	0
Sig	3	3	7

All classifications are according to the General Rules for the Gastric Cancer Study published by the Japanese Research Society for Gastric Cancer.
Lipo-ADR, liposomal adriamycin; F-ADR, free adriamycin; PD, pancreatoduodenectomy.

tion, 1 ml of sterile saline was added to each vial and vortexing was performed for 10 min. The concentration of Lipo-ADR was then adjusted to an ADR potency of 1 mg/ml by adding another 8.7 ml of sterile saline. This procedure produced MLVs ranging from 1 to 10 μm in diameter. The median diameter was 3.85 μm and the mode diameter was 4.41 μm as measured by a laser diffraction particle size analyzer (SALD-1100, Shimadzu, Kyoto). The entrapment efficacy (ADR associated with the MLVs/total amount of ADR in the Lipo-ADR mixture) was 41.7$\pm$3.2% (mean$\pm$SD of four independent measurements). After reconstitution, the preparation retained this level of entrapment for 6 h at room temperature.[10]

Endoscopic injection of Lipo-ADR or intravenous injection of F-ADR Before radical gastrectomy, Lipo-ADR (5 or 10 ml) was injected into the gastric submucosa just adjacent to the primary tumor via a needle-tipped catheter passed through the gastroscope, or alternatively F-ADR (5 mg) was administered intravenously.

Regional lymph node studies After radical gastrectomy, the regional lymph nodes were classified according to the General Rules for the Gastric Cancer Study.[15] One lymph node from each group was immediately selected, rinsed in saline, weighed, and stored at -40°C. The lymph node ADR concentration was measured subsequently by standard high-performance liquid chromatography techniques.[16] Other nodes were examined histopathologically. A histological anticancer effect was evaluated according to the criteria established by the Japanese Research Society for Gastric Cancer.[15]

Statistical analysis Data are represented as the mean$\pm$SD. Results were tested for significant differences by Student's t test and $P<0.05$ was considered to indicate statistical significance.

RESULTS

Patients' characteristics From August 1989 to December 1990, 37 patients were enrolled in this study. Nineteen patients were randomly assigned to receive the gastric submucosal injection of 5 ml of Lipo-ADR as preoperative adjuvant chemotherapy and 18 patients were assigned to receive 5 mg of F-ADR intravenously. From January to April 1991, another 15 patients were enrolled and received the gastric submucosal injection of 10 ml of Lipo-ADR. The clinical characteristics of these 52 patients are presented in Table I. All of the patients had biopsy-proven gastric carcinoma that was potentially resectable. Radical gastrectomy and lymphadenectomy were performed from 1 to 14 days after treatment with ADR.

Case report A 53-year-old man was admitted with a Borrmann type III gastric carcinoma on the lesser curva-

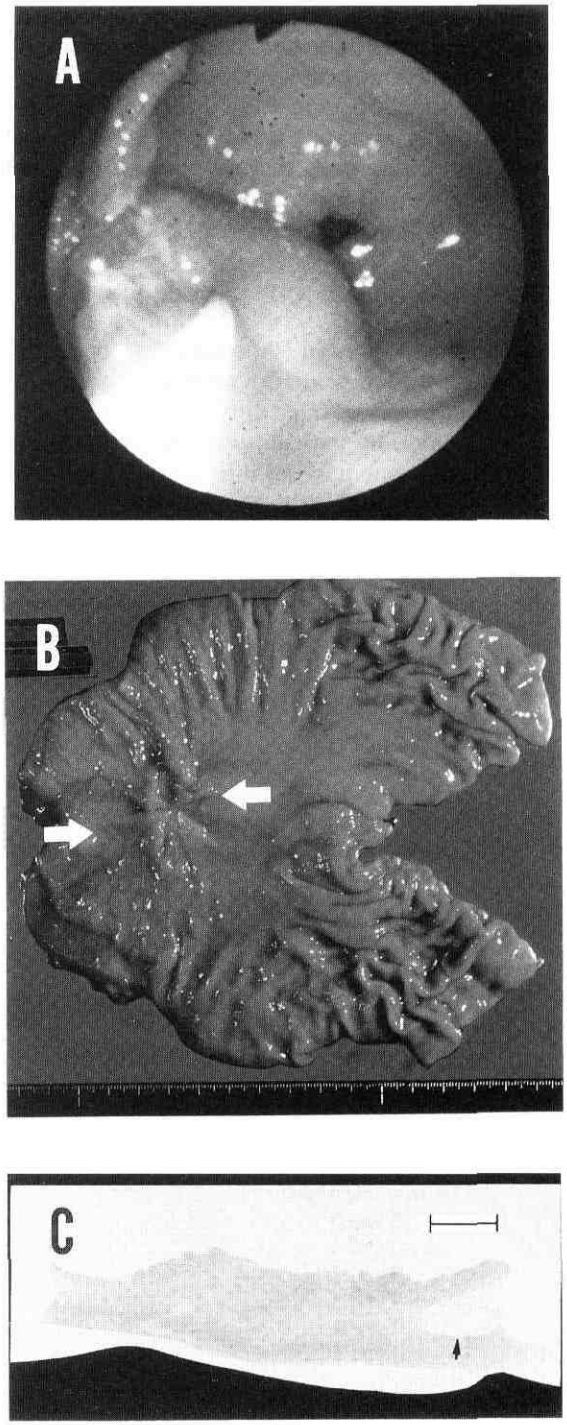

Fig. 1. The gastric submucosal injection of Lipo-ADR. A: Lipo-ADR is being injected adjacent to the primary tumor via a needle-tipped catheter passed through a gastroscope. B: The resected stomach shows small mucosal bulges at the injection sites (arrows). There are no erosions or ulcers. C: A histological specimen stained with hematoxylin and eosin shows partial retention of the Lipo-ADR (arrow) that was injected into the submucosa 2 days previously. Bar: 5 mm.

ture in the middle third of the stomach. Two days after the gastric submucosal injection of 5 ml of Lipo-ADR, distal subtotal gastrectomy was performed and the regional lymph nodes were removed (Fig. 1).

Among the regional lymph nodes, the primary drainage nodes (the infrapyloric, suprapyloric, and lesser curvature nodes) and the secondary drainage nodes (the left gastric, common hepatic, and celiac artery nodes) were found to have high levels of ADR. One of the suprapyloric lymph nodes was determined to be positive for tumor involvement. The regional lymph nodes that were more susceptible to metastasis had higher ADR levels than the other regional nodes that were further from the primary tumor, and a high dose of ADR was delivered to the regional lymph nodes which were susceptible to metastasis (Fig. 2).

The ADR concentrations in the regional lymph nodes of this patient were compared with the mean values in three patients given intravenous F-ADR. The primary and secondary drainage lymph nodes had higher tissue ADR concentrations after the submucosal injection of Lipo-ADR than after intravenous administration of F-ADR. Following intravenous administration, all the lymph nodes had similar concentrations of ADR (0.22 μg/g to 0.72 μg/g), which were much lower than those in the primary and secondary nodes following submucosal injection of Lipo-ADR (Fig. 3).

Time course of ADR levels The time course of changes of ADR level in the primary drainage lymph nodes following the gastric submucosal injection of 5 ml of Lipo-ADR was compared with that in the left gastric artery lymph nodes following the intravenous administration of an equal dose of F-ADR. In the patients receiving gastric submucosal injection of Lipo-ADR, the mean values of the highest ADR concentrations among the regional lymph nodes calculated, The ADR concentration was 15.1 ± 8.30 μg/g on day 1 (n=4), 22.5 ± 4.95 μg/g on day 2 (n=3), 11.9 ± 4.80 μg/g on day 4 (n=6), and 3.6 μg/g on day 7 (n=1) after Lipo-ADR injection. Following intravenous F-ADR, all the lymph nodes were found to have about the same ADR concentrations. Therefore, the ADR concentrations in the lymph nodes around the left gastric artery were determined, and the levels were 0.29 ± 0.10 μg/g on day 1 (n=4), 0.60 ± 0.12 μg/g on day 2 (n=3), 0.36 ± 0.0 μg/g on day 4 (n=2), and 0.29 ± 0.12 μg/g on day 7 (n=3) after F-ADR injection. The differences between the two groups were significant on day 1, day 2 and day 4 ($P<0.05$). Thus, ADR concentrations in the patients receiving gastric submucosal Lipo-ADR were much higher than those in patients receiving intravenous F-ADR for at least 7 days after treatment (Fig. 4).

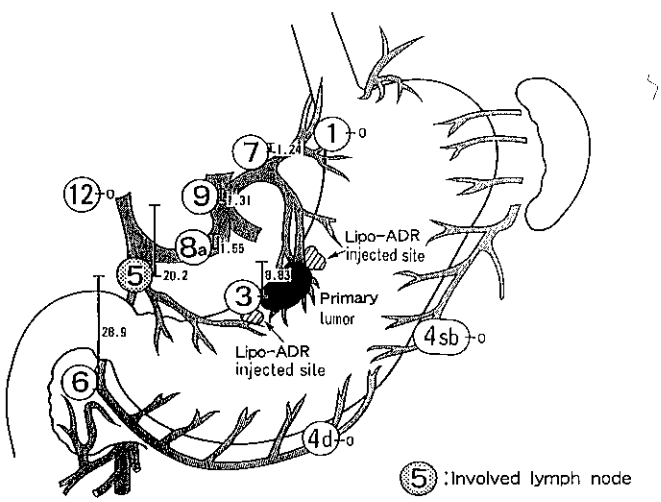

Fig. 2. Concentrations of ADR in the regional lymph nodes of the patient shown in Fig. 1 at 2 days after the injection of 5 ml of Lipo-ADR. Bars represent the ADR concentrations (μg/g) in the regional lymph nodes. 1: right paracardial lymph nodes, 3: lesser curvature lymph nodes, 4sb: lower left great curvature lymph nodes, 4d: right great curvature lymph nodes, 5: suprapyloric lymph nodes, 6: infrapyloric lymph nodes, 7: left gastric artery lymph nodes, 8a: common hepatic artery lymph nodes, 9: celiac artery lymph nodes, 12: hepatic pedicle lymph nodes. Lymph node designations are according to the Japanese General Rules for Gastric Cancer Study.

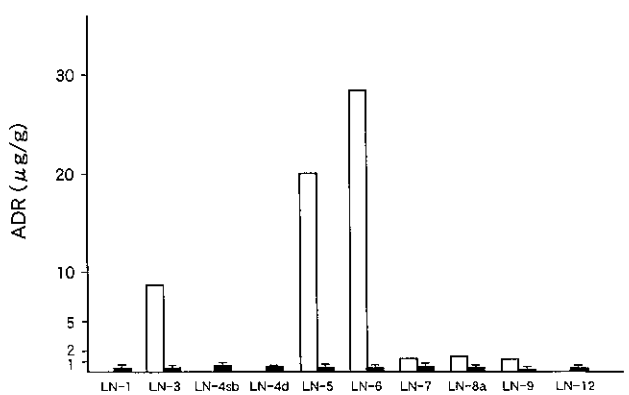

Fig. 3. ADR concentrations in the regional lymph nodes of the patient shown in Figs. 1 and 2 following Lipo-ADR injection. A comparison is made with the ADR levels following intravenous administration of F-ADR. Open bars (□) represent the ADR concentrations after Lipo-ADR injection. Closed bars (■) represent the mean value for 3 patients operated on 2 days after the intravenous administration of 5 mg of F-ADR. Bars show the SD.

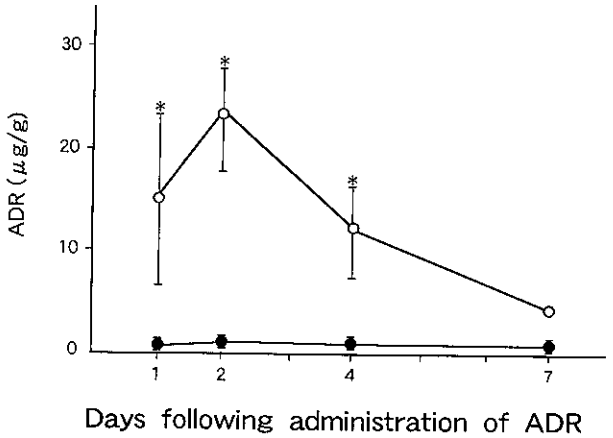

Fig. 4. ADR concentrations in the primary drainage lymph nodes following the gastric submucosal injection of Lipo-ADR and in the left gastric artery lymph nodes following intravenous administration of F-ADR. Open circles (○) represent the mean of the highest ADR concentrations found in the regional lymph nodes after submucosal Lipo-ADR. Closed circles (●) represent the mean values for the lymph nodes around the left gastric artery after intravenous administration of F-ADR. Bars show the SD. * $P<0.05$ for gastric submucosal injection of Lipo-ADR vs. intravenous F-ADR.

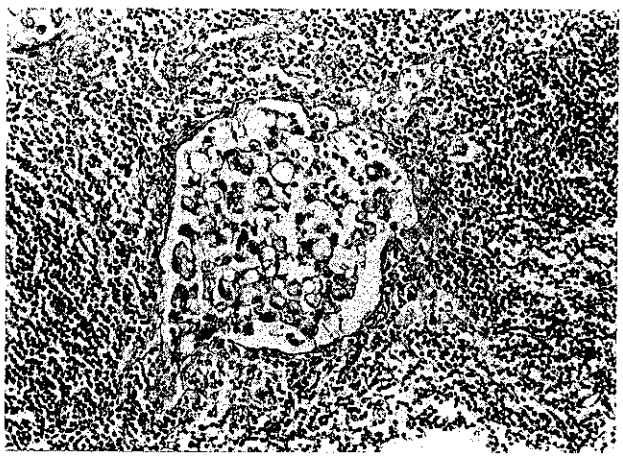

Fig. 5. The involved regional lymph node of a patient who was operated on at 4 days after the injection of 10 ml of Lipo-ADR. The carcinoma cells show marked degeneration (H & E, original magnification ×200).

Histological anticancer effect of gastric submucosal Lipo-ADR Nineteen of the 34 patients had lymph node involvement. A histological anticancer effect showing degeneration of the carcinoma was seen at the metastatic foci in the involved regional lymph nodes of 11 patients (Fig. 5). Ten of the 18 patients receiving intravenous F-ADR also had involved lymph nodes, but no histological anticancer effect of more than grade I was found in this group.

Local toxicity No evidence of local toxicity such as ulceration or erosion of the gastric mucosa was observed in this study.

DISCUSSION

There are three important sites of metastasis in patients with gastric carcinoma, and these are the lymph nodes, the liver, and the peritoneum. Regional lymph nodes are often involved in advanced gastric carcinoma and the regional nodes are one of the common sites of recurrence following curative resection.[1,2] To improve the prognosis, we have attempted to eradicate lymph node metastasis as well as the primary tumor. Several investigators have used regional chemotherapy targeting the lymph nodes for this purpose.[3,4,17-19] In the present study, we used targeting chemotherapy by the endoscopic gastric submucosal injection of Lipo-ADR just adjacent to the primary tumor. This form of chemotherapy is based on the premise that Lipo-ADR injected into the gastric submucosal space will be delivered specifically and continuously to the regional lymph nodes via the lymphatic circulation, thus maintaining a high ADR level at the sites likely to be affected by metastasis.

We chose ADR because this drug is commonly used for gastric carcinoma,[5,6] liposomal encapsulation may reduce the toxicity of ADR for the gastric wall, which often limits the dose administered,[10,20] and liposomal encapsulation may enhance the delivery of ADR to the regional lymph nodes, when compared with injection of F-ADR.[10] The net result might be a greater anticancer efficacy.

Before clinically administering this form of chemotherapy, we investigated the ability of gastric submucosal injection of Lipo-ADR to provide efficient and selective delivery of the drug to the regional lymph nodes in a rabbit model.[10] We evaluated the distribution of ADR in rabbits for up to 7 days after Lipo-ADR injection in comparison with the gastric submucosal injection or intravenous administration of F-ADR. After the gastric submucosal injection of Lipo-ADR, the regional lymph node ADR concentration was significantly higher than after intravenous administration of F-ADR. The area under the ADR concentration-time curve (AUC) was about 10 times higher with Lipo-ADR. In addition, the AUC of ADR for the bone marrow, spleen, and heart was significantly lower after gastric submucosal injection than after intravenous administration.[10] The AUC of the tissue ADR concentration correlates well with the

anticancer efficacy and toxicity of this drug.[21,22] Mild ulceration limited to the submucosa was noted at the injection sites at 7 days after the submucosal injection of Lipo-ADR. Thus, the severe ulceration caused by gastric submucosal injection of F-ADR was attenuated by encapsulating ADR in liposomes.[10] These data demonstrated that the gastric submucosal injection of Lipo-ADR was well suited for the specific delivery of ADR to the regional lymph nodes.[10]

In the present study, the dose of Lipo-ADR was initially set at 5 ml (1 mg/ml of ADR). In the pharmacokinetic study, ADR levels in the regional lymph nodes after the gastric submucosal injection of Lipo-ADR were compared with those after the intravenous injection of an equal dose of F-ADR. The dose of Lipo-ADR was then increased to 10 ml, and still no signs of severe local toxicity such as ulceration or erosions were observed. Mild local toxicity at the injection site is not a sufficient problem to contraindicate the clinical application of this chemotherapy to patients with resectable gastric carcinoma, because the injection site is adjacent to the primary tumor and is resected during gastrectomy. Therefore, further increase of the dose of Lipo-ADR would be possible in the future.

After the gastric submucosal injection of Lipo-ADR, the primary and secondary drainage lymph nodes had higher ADR levels than the other regional lymph nodes. The lymph nodes that were more susceptible to metastasis received higher levels of ADR than the nodes further from the primary tumor. In contrast, intravenous administration of F-ADR produced similar ADR levels in all the lymph nodes.

We compared the delivery of ADR to the primary drainage lymph nodes following the injection of 5 ml of Lipo-ADR and that to the left gastric artery lymph nodes following intravenous administration of an equal dose of F-ADR. ADR concentrations in the regional lymph nodes were much higher after gastric submucosal injection than after intravenous administration, and this was noted for more than 7 days.

We also observed the histological anticancer effect on involved regional lymph nodes in some patients of the Lipo-ADR-injection group. The anticancer effect on the metastatic foci in the involved lymph nodes was due to the delivery of a high dose of ADR by gastric submucosal injection.

Our results demonstrated that the gastric submucosal injection of Lipo-ADR adjacent to the primary tumor is suitable for the specific delivery of ADR to the regional lymph nodes susceptible to metastasis. Targeting such lymph nodes may prevent the recurrence of gastric carcinoma.

As preoperative adjuvant chemotherapy, gastric submucosal injection of Lipo-ADR may perhaps improve the prognosis after curative resection of gastric carcinoma. We are currently conducting a study of the patients who received this chemotherapy to evaluate the prophylactic effect on the lymph node relapse of gastric carcinoma.

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