Prophylaxis of Regional Node Metastases with Liposomes

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Experiments on mice showed that local administration of cis-diaminodichloroplatinum incorporated into liposomes inhibited tumor metastases into regional lymph nodes with preserved draining function. When first-order nodes were replaced with tumor tissue, the liposome-incorporated drug inhibited metastasis formation in the second-order lymph nodes (inguinal): it reduced the number and size of metastases in these nodes.

Key Words: *lymph node metastases; liposomes; cis-diaminodichloroplatinum(II)*

Malignant tumors metastasizing via the lymphatic system primarily affect the first-order regional lymph nodes (LN). Further metastasizing involves LN of the second and higher orders. Radical surgery including resection of the primary tumor and regional LN is recommended irrespective on the presence of LN metastases, which are verified after surgery. The second-order LN are usually not removed because of low incidence of second-order LN metastases, difficult access, and undue extensive surgical intervention. However, these LN can also be involved in the metastasizing process.

Tumor cells passively penetrate lymphatic circulation due to high permeability of lymphatic vessels. Since liposomes can penetrate lymphatic vessels via the same ways, we assumed that drug-loaded liposomes can be used for prophylaxis of regional lymph node metastases. Being locally injected, these liposomes via the lymphatic pathways should reach LN and deliver the drug directly to metastatic tumor cells [2,3].

This assumption was confirmed by our previous studies of the distribution of radiolabeled liposomes in mice [4] and in therapeutic experiments where antitumor drug in a dose of one tenth the therapeutic dose incorporated into liposomes reduced the number of metastases in popliteal LN in mice after plantar tumor inoculation [2,3]. However, the delivery of the drug to inguinal LN and therapeutic effect of drug-loaded liposomes against inguinal metastases were not studied in this model.

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Here we present experimental data on the effect of cis-diaminodichloroplatinum (CDDP) incorporated into liposomes on the formation of metastases in second-order regional LN.

MATERIALS AND METHODS

Hepatoma A metastasizing via the lymphatic pathways [1] was inoculated into paw pad of A/He mice in a dose of 4×10^5 cells. When plantar tumors reached 5 mm in diameter and popliteal metastases appeared, the mice were divided into 3 groups (16 animals per group). Group 1 was control, groups 2 and 3 received free and liposome-incorporated CDDP (Platidiam, Lachema), respectively. Liposomes were prepared by ultrasonication of a phosphatidylcholine film in physiological saline containing 2 mg/ml CDDP [1]. Under these conditions 2.5% CDDP was incorporated into liposomes (30-50 μg/ml and a CDDP-lipid ratio of $0.4\text{-}0.7\times10^{-2}$ μM:1 μM). Free drug was removed by gel filtration on a Sephadex G-50 column. The drug was injected under the plantar aponeurosis proximally to the tumor (3 injections with 24-h intervals) in a total dose of 13.5 μg (about 0.5 mg/kg body weight). Twenty-four hours after the last injection, a tourniquet was placed around the limb above the heel and the foot was amputated. The mice were sacrificed by cervical dislocation 24 h after surgery and the popliteal and inguinal LN were excised and weighted. The number of mice with metastases and the weight of metastatic LN served as the measure of treatment efficacy. The data were analyzed using Student t and ϕ-tests (arc sin Fisher transformation).

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TABLE 1. Number of Mice with Metastases (%) and Mean Weight of Hepatoma A Metastases (mg) in Popliteal and Inguinal LN of Control Mice and Mice Treated with Free and Liposome-Incorporated CDDP ($M \pm m$)

Localization of metastases	Group					
	1		2		3	
	%	weight	%	weight	%	weight
Popliteal LN	100	333±41	100	273±44	93.8	210±33*
Inguinal LN	73	113±33	68.9	49±16	25	27±13*

Note. *$p<0.05$ compared with Group 1.

RESULTS

The incidence of popliteal LN metastases was practically the same in the control and experimental groups. Only a slight decrease in the weight of these LN was noted in group 3 (Table 1). This was not surprising, since the treatment was started after formation of metastases in the popliteal LN, and hence their filtration function was impaired. Therefore, liposomes passed by these metastatic LN and were captured by inguinal LN (second-order LN). In group 3 mice, the incidence of inguinal metastases was considerably reduced in comparison with the control (Table 1). The weight of popliteal metastatic LN was also decreased (Table 1). Free CDDP had no significant effect on the formation of metastases in popliteal and inguinal LN probably due to its rapid diffusion from the tumor into circulation demonstrated in previous studies [4].

Preserved barrier function of metastatic LN is essential for effective antitumor treatment with liposomes. Therefore, this method cannot be used for the treatment of large metastases, where lymphatic tissue is replaced by tumor cells [3], and compete with usual surgical and radiation treatment. However, being effective at the early stages of tumor process, this method can effectively supplement surgery and radiation therapy in preventing metastases in the second- and higher-order LN.

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