Enhanced Lymphatic Delivery of Monoclonal Antibody Following OK432 Pretreatment

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Enhanced Lymphatic Delivery of Monoclonal Antibody Following OK432 Pretreatment. Tohoku J. Exp. Med., 1993, 169 (4), 319-323 — The effect of OK432 on the lymphatic delivery of monoclonal antibody (Mab) was investigated by injecting $^{[125]}$I-Mab A7 into BALB/c mice pre-treated with OK432, and measuring the radioactivity in the regional lymph nodes. The $^{[125]}$I-Mab A7, when administered subcutaneously to the foot pad, preferentially accumulated in the ipsilateral popliteal and para-aortic lymph nodes. Treatment with OK432 prior to antibody injection significantly enhanced the accumulation of Mab A7 in these regional lymph nodes. These findings suggest that pretreatment with OK432 may constitute a promising method of enhancing the lymphatic delivery of Mab, and improving the efficacy of therapy directed against lymphatic malignancies.

The sufficient supply of anticancer agents to the lymphatic system offers a promising means of preventing or eliminating lymph node metastasis, a situation which typically has a poor prognosis (Ariel et al. 1964; Takahashi et al. 1973; Hagiwara et al. 1988). The subcutaneous injection of an anticancer agent can deliver a relatively large amount of the anticancer agent to the lymph node, but the compounds with a relatively low molecular weight, such as conventional anticancer agents, appear to be absorbed from the interstitial space into the capillaries but not into the lymphatic vessels (Ballard 1968). IgG, however, passes into terminal lymphatic capillaries but does not pass readily into blood capillaries (Leak 1971). As such, a cancer-associated monoclonal antibody (Mab), when locally injected, will readily enter the lymphatics, migrate to the regional lymph nodes and bind existing cancer cells with a high affinity.

OK 432 (Okamoto 1966), a heat-and penicillin-treated lyophilized powdered form of an Su strain of streptococcus pyogenes, has been widely used as a potent immunomodulating agent, and has been demonstrated to have antitumor activity...
both in tumor bearing animals and in cancer patients. Furthermore, OK432 has been routinely administered to patients intradermally or intratumorally without major side effects (Ogita et al. 1987). This agent may accelerate the lymphatic delivery of Mab, presumably by inducing inflammation. These observations prompted us to apply OK432 in combination with Mab in the prevention and elimination of lymph node malignancies. This is the first report describing the enhanced lymphatic delivery of Mab through the use of the biological response modifier, OK432.

**Materials and Methods**

*Mab accumulation to the regional lymph nodes.* Mab A7 was prepared as described in the previous report (Kotanagi et al. 1986). Mab A7 is known to selectively react with human adenocarcinoma and belong to IgG1. Mab A7 was labeled with $^{125}$I by the chrolamin T method (Greenwood et al. 1963) with a specific activity of $1 \times 10^6$ cpm/$\mu$g. 2 $\times 10^5$ cpm of $[^{125}I]$-Mab A7 was administered i.v. via tail vein or s.c. to the foot pad of 5 mice, and 2hr later the lymph nodes (ipsilateral popliteal, contralateral popliteal and para-aortic) were resected and weighed. Radioactivity of the lymph nodes was measured in a gamma counter, and the data were expressed as cpm/gram of lymph node tissue.

*Mab accumulation to the regional lymph nodes after OK432 pretreatment.* Two mg of OK432 (Picibanil; Chugai Pharmaceutical Co., Tokyo), equivalent to 10 KE, was dissolved in 1 ml of saline. The optimal dose of OK432, which potentially activates lymph nodes as an immunologic modulator, was defined as 0.2 KE per mouse in a preliminary experiment (data not shown). Ten $\mu$l of the OK432 solution equivalent to 0.2 KE and 10 $\mu$l of saline was administered s.c. to the foot pad of BALB/c mice. Five days later, the mice were given i.v. or s.c. doses of $2 \times 10^6$ cpm of $[^{125}I]$-Mab A7. The lymph nodes were resected and weighed, and the radioactivity of the lymph nodes was measured in a gamma counter.

*Time course of Mab accumulation to the regional lymph nodes.* The effect of OK432 on lymph node accumulation of Mab was examined. Following pretreatment with OK432 or saline, $2 \times 10^6$ cpm of $[^{125}I]$-Mab A7 was administered s.c. to the foot pad of mice, and the popliteal lymph node was resected and weighed at various times following antibody injection. The radioactivity of the lymph node was measured in a gamma counter and compared between the two groups.

**Results and Discussion**

Using i.v injection, $[^{125}I]$-Mab A7 accumulated in similarly low quantities in all of the lymph nodes. Injecting s.c., however, resulted in high levels of radioactivity in the ipsilateral popliteal and para-aortic lymph nodes, but not in the contralateral popliteal lymph node (Table 1). Ipsilateral popliteal and para-aortic lymph nodes comprise the regional lymph nodes of the foot pad. An agent which enters the lymphatics, when locally injected into the foot pad, reaches the popliteal lymph node first, followed by movement into more distant, para-aortic nodes. Accordingly, our results indicate that Mab A7 preferentially accumulates in the regional lymph nodes following s.c. administration. In the extensive examination of the lymphatic delivery of Mab using s.c. injection, Weinstein et al. (1982) showed that Mabs entered the lymphatics, reached the regional lymph nodes, and then accumulated in the lymph node metastasis of cancer. This
accumulation was more notable following s.c. injection than following i.v. injection (Weinstein et al. 1983).

The present study did not directly show a high accumulation of Mab in lymph node metastasis, and may accordingly leave an open question of whether drug targeting could really be achieved with tumors by using Mab. However, a

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**Table 1. Lymph node accumulation 2hr after injection**

<table>
<thead>
<tr>
<th></th>
<th>Ipsilateral popliteal</th>
<th>Contralateral popliteal</th>
<th>Para-aortic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mab A7 i.v. saline</td>
<td>121 ± 25</td>
<td>105 ± 18</td>
<td>99 ± 11</td>
</tr>
<tr>
<td>Mab A7 i.v. OK432</td>
<td>382 ± 72</td>
<td>132 ± 22</td>
<td>250 ± 25</td>
</tr>
<tr>
<td>Mab A7 s.c. saline</td>
<td>3150 ± 379</td>
<td>115 ± 3</td>
<td>668 ± 167</td>
</tr>
<tr>
<td>Mab A7 s.c. OK432</td>
<td>6030 ± 785</td>
<td>135 ± 6</td>
<td>1917 ± 348</td>
</tr>
</tbody>
</table>

\( \times 10^5 \text{ cpm/g, mean ± s.e., } n=5 \)

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![Fig. 1. The effect of OK432 on lymphatic delivery of Mab: the time course study. \([^{125}\text{I}]\)-Mab A7 (3×10^5 cpm) was administered to the foot pad of mice pre-treated with OK432 (○) or saline (●). The popliteal lymph node was resected 1, 2, 4, 6 and 12 hr after the injection of Mab A7 and the radioactivity of the lymph node was measured by a gamma counter and compared between the two groups. Bars, ± s.e.; n=5.](image-url)
A high concentration of Mab in the lymph node should allow Mab to contact and bind metastatic cancer cells more efficiently, as a high circulating concentration of Mab is known to lead to high tumor localization. As such, Mab A7 should be able to target a metastatic region in the lymph node, and should constitute a good carrier in clinical immunotherapy directed at lymph node metastasis.

Following both i.v. and s.c. injection, the mice treated with OK432 exhibited a higher antibody accumulation to the regional lymph nodes than the corresponding saline control (Table 1). A higher Mab accumulation in OK432 pretreatment was also observed in the time-course experiment (Fig. 1). These observations are thought to be attributed to the accelerated immune function of the regional lymph nodes. An effect of OK432 administration is induction of inflammation at the injection site (Nagao 1982). This inflammatory change may be involved in the enhanced accumulation of Mab in the regional lymph node, since inflammation accelerates lymphatic flow.

The effect of OK432 on the lymphatic delivery of Mab may have several clinical applications, encompassing both radioimmunomaging and immunotherapy. It's potential utility in cancer therapies using Mab-drug, -toxin and-radiosotope conjugated, is particularly notable. The combined use of such a conjugate and OK432, as described in this study, may comprise a pharmacological method of eliminating cancer cells in the regional lymph nodes, and may improve the prognosis of certain types of cancer. This study will hopefully provoke further investigation of enhanced Mab lymphatic delivery.

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References


