Antitumor Effect of Ischemia-Reperfusion Injury Induced by Transient Embolization

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Abstract

The effect of ischemia-reperfusion, induced by the transient embolic agent degradable starch microspheres (DSMs) on tumor tissue was investigated from the standpoint of active oxygen species. Rabbits with VX2 carcinoma received regional infusion of DSMs by transcatheter angiography, and it was confirmed that DSMs occluded tumor vessels. Blood flow in the tumors decreased rapidly immediately after the DSM treatment and returned to the original level within 40 min. The size of tumors did not change after a single infusion of DSM, while five repeated DSM treatments led to a significant reduction in tumor size. This reduction in tumor size was prevented by the treatment of rabbits with superoxide dismutase and catalase, indicating that the generation of active oxygen species in the tumor was involved in the mechanism of action of DSMs. Thiobarbituric acid-reactive substances also increased in the tumors after DSM infusion, and this increase was also inhibited by treatment with superoxide dismutase and catalase.

In conclusion, the antitumor effect of the transient embolic agent DSM is secondary to the phenomenon of ischemia-reperfusion injury. In addition, active oxygen species and lipid peroxidation are possible causes of ischemia-reperfusion injury.

Introduction

We have previously combined DSMs2 (Pharmacia, Uppsala, Sweden), which are transient embolic agents, with chemotherapeutic agents, and have used them to perform embolization therapy for malignant tumors of the liver. When DSMs and chemotherapeutic agents are combined, DSMs form emboli at the arterio-capillary level. The antitumor effects of the chemotherapeutic agents are enhanced by high concentrations maintained at the local level for a fixed period of time. Because release of the chemotherapeutic agents into the general circulation is inhibited at the same time, DSMs may contribute to the reduction of systemic side effects (1, 2). DSMs, obtained from hydrolyzed potato starch, are easily digested by amylase in plasma (3). Arterial infusion of DSMs alone causes transient ischemia and subsequent reperfusion of tumor tissue. Transient occlusion of a blood vessel, followed by recanalization, is known to induce so-called ischemia-reperfusion injury to the tissue. Ischemia in itself causes tissue damage and eventual death of cells. While reperfusion is necessary and essential for the salvage of ischemic tissue, reperfusion is also thought to be accompanied by its own component of injury. Much evidence has accumulated implicating cytotoxic active oxygen species as mediators of at least part of this injury, based on the ability of free radical scavengers to attenuate injury in animal models of ische-

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1 To whom requests for reprints should be addressed.
2 The abbreviations used are: DSM, degradable starch microsphere; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substances.

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was calculated as:

\[(\text{longest diameter}) \times (\text{shortest diameter})^2 \times \frac{1}{2}\]

The ratio of the tumor volume on day 7 to day 1 was expressed as the growth ratio.

**TBARS.** The concentration of TBARS, an index of lipid peroxidation, was measured in the tumor tissue using the method of Ohkawa et al. (6). In brief, groups of the DSM-treated rabbits were killed 60 min after a single arterial infusion of DSM. Animals were killed by exsanguination via the abdominal aorta under i.v. sodium pentobarbital anesthesia (20 mg/kg). The tumor tissues were removed and homogenized with 1.5 ml of 10 mM potassium phosphate buffer (pH 7.8) containing 30 mM KCl in a Teflon Potter-Elvehjem homogenizer. The level of TBARS in the tumor tissue homogenates was expressed as nmol of malondialdehyde/mg protein using 1,1,3,3-tetramethoxyxylene as the standard. Total protein in the tissue homogenates was measured by the method of Lowry (7).

**Statistical Analysis.** Results are presented as the mean ± SE from 4–6 rabbits/group. The Kruskal-Wallis analysis was used to determine variances. The nonparametric Mann-Whitney test was used to compare differences between the DSM groups versus the control groups. The two-tailed nonparametric Dunnett’s test was used to compare groups treated with CuZnSOD and catalase versus controls. A level of \(P < 0.05\) was accepted as statistically significant.

**Results**

**Tumor Tissue Blood Flow after Arterial Infusion of DSM.** Tumor blood flow decreased to about 20% of pretreatment values immediately after injection of DSM and remained at this level for about 10 min. Tumor blood flow slowly recovered after 15 min, and had virtually returned to pretreatment levels at 40 min after injection (Fig. 1).

**Antitumor Effect.** The tumor growth ratio in the group given a single infusion of DSM was 3.59 ± 0.57 (SE), which was not significantly different from the control group (3.98 ± 0.61). The tumor growth ratio in the group given DSM once a day for 5 days was 0.96 ± 0.09; compared to the control group (4.39 ± 0.35), tumor growth was significantly inhibited (Table 1).

**Effect of Free Radical Scavengers on the Antitumor Effect.**

**Table 1 Antitumor effect after arterial infusion of DSM**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tumor growth ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.98 ± 0.61 (NS)</td>
</tr>
<tr>
<td>Single infusion of DSM</td>
<td>3.59 ± 0.57</td>
</tr>
<tr>
<td>Control 2*</td>
<td>4.39 ± 0.35</td>
</tr>
<tr>
<td>Repeated DSM for 5 times</td>
<td>0.96 ± 0.09</td>
</tr>
</tbody>
</table>

* The tumor size was measured by ultrasonic tomography on day 1 and day 7.
* The tumor growth ratio on day 7 to day 1.
* Control group 1 was given a single infusion of physiological saline.
* Mean ± SE.
* A single infusion of DSM 20 mg/kg was given on day 1.
* NS, not statistically significant.
* Control group 2 was given daily infusions of physiological saline for 5 days.
* Daily infusions of DSM (20 mg/kg) for 5 days.

**TBARS in the Tumor Tissue.** TBARS were measured in the tumor tissue 60 min after a single arterial infusion of DSM. The untreated group had TBARS of 0.25 ± 0.015 nmol/mg protein. The control group, which received DSM and heat-inactivated CuZnSOD and catalase, had TBARS of 0.37 ± 0.043 nmol/mg protein. Thus, there was a significant increase of TBARS in the tumor tissue after the infusion of DSM. In the presence of CuZnSOD plus catalase, TBARS decreased to 0.26 ± 0.010 nmol/mg protein. This strongly suggests that TBARS generated 60 min after an infusion of DSM are significantly suppressed by oxygen radical scavengers (Fig. 3).

**Discussion**

Because of their powerful reactivity, active oxygen species and free radicals attack biological structural components and are involved in the pathology of various disease states (8, 9). However, by effectively utilizing these potent actions, that is, by producing large quantities of active oxygen species at the site of a cancer, an antitumor effect could be induced. Chemotherapy and photochemical reactions are well known as cancer treatments that make use of the cytotoxicity of active oxygen species and free radicals (10, 11). In the present study, we studied the transient embolic agent DSM, which, as a drug delivery system, is combined with chemotherapeutic agents during chemical embolization for malignant tumors of the liver. We also investigated the effects DSM has on tumor tissues resulting from ischemia-reperfusion. Ischemia-reperfusion injury, which is induced by the generation of active oxygen species during the ischemic period, followed by an influx of oxygen during
A small intestine, have reported that the hypoxanthine-xanthine oxidase system was the principal source of active oxygen species in ischemia-reperfusion injury. The source of the active oxygen species in the tumor tissues was not investigated in the present study, but SOD and catalase, which are macromolecules, reportedly do not penetrate the cell membrane. It is therefore difficult to imagine that they inhibit the antitumor activity of DSM by scavenging active oxygen species produced by the hypoxanthine-xanthine oxidase system within the cell. Detailed studies of the involvement of polymorphonuclear leukocyte-derived active oxygen species and vascular endothelial cells will be needed in the future.

In summary, we have shown that the antitumor effect of DSM is due to the phenomenon of ischemia-reperfusion injury. In addition, the generation of active oxygen species and subsequent lipid peroxidation is the probable mechanism of action of DSM.

**References**


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