A Sequential Immunosuppressive Treatment with Mizoribin (Bredinin) plus Cyclosporin A on the Subrenal Capsule Assay

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Summary: To minimize immunological interferences on the subrenal capsule (SRC) assay, a new immunosuppressor, mizoribin (MZB : Bredinin) alone or combined with cyclosporin A (CsA) was evaluated by an experimental SRC assay system using a rat ovarian cancer tissue. Daily applications of MZB (200 mg/kg) for 7 days following the xenograft of cancer tissue were insufficient to suppress immunological reactions of the recipient mice, and all the grafted cancer tissues were rejected. Although CsA monotreatment (60 mg/kg of CsA given daily for 7 days) successfully suppressed the host immune reaction, enhanced toxicities of CsA in combination with anticancer agents caused high lethal rate of host mice during the experimental chemotherapy. Sequential use of CsA on day 0 to day 2 followed by MZB on day 3, 5 and 7 brought the most favorable results with minimal host reactions and toxicities. An anticancer screening test using the modified SRCA accurately reflected the results of experimental chemotherapy against the rat ovarian cancer. The results suggest that the sequential treatment of MZB and CsA is a feasible immunosuppressive treatment which minimizes immunological interferences with SRC assay chemoscreening test.

Key words: subrenal capsule assay — immunosuppressive treatment — cyclosporine A — mizoribin (Bredinin) — experimental chemotherapy

Introduction

Subrenal capsule (SRC) assay using normal immunocompetent mice is now becoming a convenient method of in vivo chemosensitivity test. According to Bogden's original introduction of the assay (Bogden et al. 1979), the results could be obtained at the 6th day without any consideration to immunological reactions in the recipient mice. However, Edelstein et al. (1983) demonstrated that there were significant host immune cell infiltrations surrounding the xenografted tumor under the renal capsular space, and they warned that such immunological interferences could lead us a misinterpretation of the results of the SRC assay.

Bennedet et al. (1985) applied cyclosporin A (CsA) to the SRC assay system, and suggested that such immunosuppressive treatments were required to suppress the host immune reaction against the grafted tissue. However, since CsA was proved to have calcium channel blocker-action (Osieka et al. 1986), it can possibly enhance cytotoxicity of anticancer agents via this effect. In a previous study, an occasional death of the recipient mouse was also experienced be-
cause of unexpected toxicities of CsA when combined with cytotoxic agents (Uslijima, 1989). The enhanced toxic effects prompted us to search for alternative, less toxic immunosuppressive methods.

Mizoribin (MZB, C9H13N306; Bredinin, Toyo Jyozo KK, Shizuoka) is a substance isolated from the culture filtrate of Eupenicillium Brefeldianum; Uchida et al. (1980) found a strong immunosuppressive activity of MZB. In the present study, the utility of MZB was examined with respect to the SRC assay chemosensitivity test and the effect of MZB was compared to that of CsA used alone or in combination with MZB.

Materials and Methods

Female BDF1 mice were used in this experimental SRCA system. As a tumor material, a transplantable ovarian cancer tissue which had been originally induced by direct application of 7, 12 dimethylbenz (a) anthracene (DMBA) to the ovary of Wistar strain rat (Nishida et al. 1986) and maintained through 40 generations on the back of the same strain of rats (Sugiyama et al. 1986), was used. Under pentobarbital anesthesia, 1 mm$^3$ of ovarian cancer tissues was implanted beneath the kidney capsule of mice following the method of Bogden et al. (1979), and the initial tumor sizes were measured by ocular micrometer.

The mice were devided into 5 groups of 10 mice each according to the differences in the immunosuppressive treatments they received. The first 10 mice received daily subcutaneous injection of 200 mg/kg MZB from day 0 to day 7. Group 2 mice was treated with intraperitoneal administration of 60 mg/kg CsA from the day of implantation to the 7th day. The third group received 30 mg/kg of CsA from day 0 to 7. The fourth group was administered concomitantly 30 mg/kg of CsA plus 100 mg/kg of MZB from day 0 to day 7. The fifth group received sequential administration 60 mg/kg of CsA from day 0 to day 2 followed by 200 mg/kg of MZB on day 3, 5 and 7. The effects of these immunosuppressive treatments were judged in terms of $\Delta$TS (difference between the initial and final tumor size) and histological findings of the grafted tumor including mitotic index and host immune cell infiltration.

Cisplatin (8 mg/kg), doxorubicin (7 mg/kg), etoposide (17 mg/kg) and 5-fluorouracil (60 mg/kg) were tested using the mice in which the host responses were favorably controlled. The drug sensitivities were evaluated in terms of histological change of the grafted tumor and $\Delta$TS criteria described by Mäenpää et al. (1985). The results were interpreted as indicating tumor sensitivity if the mean tumor size had decreased by 1 omu (ocular micrometer unit: 10 omu = 1 mm) or more, and tumor resistance if the mean tumor size increased by 1/3 $\Delta$TS or more.

Results

Monotreatment with MZB failed to control the immunoreaction in the host mice. All of the grafted tumors in group 1 mice were rejected or replaced by granulomatous tumor showing fibroblast proliferations and marked host immune cell infiltration. While an increase in the tumor size ($\Delta$TS) was observed in group 3 and 4 mice, a degenerative area including a considerable amount of immune cell infiltrations and fibroblastic proliferations were also found (Fig. 1), suggesting the insufficient immunosuppressive effects of 30 mg/kg of CsA whether with or without MZB. Similar results were obtained in the mice of group 2 and group 5. In both groups, the maximal tumor growth was observed on the 8th day and
Fig. 1. A transplanted tumor in the subrenal capsular space in the group 3 of mice. Note the large central necrotic space surrounded by thick fibrous capsule.

Fig. 2. A histology of grafted tumor in a mouse treated with CsA followed by MZB. Cancer cell proliferation with marked mitotic activity was found. (HE ×100)

TABLE 1

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Cisplatin (10)</th>
<th>5-Fluorouracil (8)</th>
<th>Etoposide (6)</th>
<th>Doxorubicin (7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Results from tumor size</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Mitotic activity of residual tumor</td>
<td>none</td>
<td>plus</td>
<td>none</td>
<td>none</td>
</tr>
</tbody>
</table>

S: sensitive, R: resistant

the averaged tumor size was 18 mm in the maximal diameter at that time, showing numerous mitotic activities and minimal inflammatory cell infiltration (Fig. 2).

Experimental chemosensitivity test was performed using groups 2 and 4 of mice. Severe toxicity was noted when cytotoxic agent and CsA were concomitantly used, and it caused an occasional death of the experimental mice. Because of the CsA-induced enhanced effect of cytotoxicity of anticancer agents, group 1 mice were omitted from the test. Results of chemosensitivity test using the experimental SRC assay were summarized in Table 1. The tumors were considered as sensitive to cisplatin, doxorubicin and etoposide, while the tumors were thought to be resistant to 5-fluorouracil.

Discussion

The evaluation of chemosensitivity using a xenografted tumor is based on the premise that the transplanted tumor cells retain their original characteristics in the transplanted site. In the SRC assay, the fundamental question which still remains is whether or not the host immune reaction against grafted tumor can influence the results. Since the host cells occupied only 15-20% of the total grafted volume, Aamdal et al. (1984) concluded that the influence of mouse cell infil-
tration on the growth of grafted tumor was slight and that it is unnecessary to use athymic mice. In the previous work (Ushijima, 1989), however, a significant host immune cell reaction against the grafted rat ovarian cancer tissue was observed, and the host cell infiltration could modify the size of grafted tumor and occasionally brought a complete rejection of the grafts.

Although sufficient immunosuppressive effects of CsA were observed in this study, unexpected toxicity was also experienced when an anticancer agent was concomitantly used. There are some explanations for the enhanced cytotoxic effects of CsA: i) it decreases the hepatic drug metabolizing enzyme activity (Edelstein et al. 1983); ii) CsA itself is cytotoxic and nephrotoxic, and it can induce a focal necrosis in the mice tubular cell of the kidney (Ushijima, 1989); and finally, iii) CsA is a chemosensitizer as a calcium channel blocker (Osieka et al. 1986). In this content, a remarkable cytotoxic effects of concomitant use of CsA and an anticancer agent was noted. When CsA was combined with etoposide, most of the mice could not tolerate against the toxicity. Hence, these results suggest that CsA treatment should be ceased before the initiation of experimental chemotherapy.

This study dose not yield any conclusive results of the immunosuppressive effect of MZB monotherapy on the experimental SRC assay system. However, the immunosuppressive effect of 3 days of applications of 200 mg/kg of MZB following 3 days of CsA treatments were comparable to that of 7 days CsA monotherapy. Although the optimal method to avoid immunological interference in the SRC assay remains to be resolved, the sequential treatment of CsA and MZB seems to be feasible without modification of cytotoxic effects of anticancer agents.

References


