COMPARISON OF THE INHIBITORY EFFECT OF THE ANGIOGENESIS INHIBITOR, TNP-470, AND MITOMYCIN C ON THE GROWTH AND LIVER METASTASIS OF HUMAN COLON CANCER

Hiroyuki Konno\textsuperscript{1}, Tsuuo Tanaka, Iwao Matsuda, Toshikazu Kanai, Yuji Maruo, Nobuhiko Nishino, Satoshi Nakamura and Shozo Baba

Second Department of Surgery, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu, 431-31, Japan.

Angiogenesis inhibitors have attracted considerable interest. The anti-tumor and anti-metastatic effects of TNP-470, an angiogenesis inhibitor, and mitomycin C (MMC), a representative anti-neoplastic agent, were investigated using a xenotransplanted human colon cancer, TK-4. Suturing of small pieces of TK-4 tumors to the cecal wall in nude mice (orthotopic transplantation) induced liver metastasis. Mice were randomly divided into 3 groups: a control group given saline solution, a group receiving TNP-470 and a group receiving MMC. TNP-470 was given s.c. on alternate days for 5 weeks from day 10 after cecal transplantation and MMC was administered intraperitoneally (i.p.) once a week from day 10 after cecal transplantation. MMC significantly inhibited cecal tumor growth. In the control group, liver metastases developed in 9 out of 10 mice, including 3 with more than 20 metastatic foci. Liver metastasis also developed in 8 out of 10 mice receiving MMC, 2 of which had many metastases. In contrast, liver metastasis developed in only 2 out of 8 mice in the TNP-470 group and neither of these animals had numerous metastases.

MATERIAL AND METHODS

Preparation of TNP-470

The angiogenesis inhibitor TNP-470 was kindly provided by Takeda (Osaka, Japan). TNP-470 was suspended in a vehicle of 1% ethanol and 5% gum arabic in saline. MMC was purchased from Kyowa Hakko (Tokyo, Japan) and dissolved in saline.

Animals

Male BALB/c nu/nu mice, obtained at 4 weeks of age from Clea (Tokyo), were used for this study at 5 weeks of age.

Human colon cancer

The human colon cancer strain, TK-4, was established in our department from a metastatic liver lesion of a 50-year-old Japanese male with sigmoid colon cancer, and was maintained by passage in nude mice (BALB/c nu/nu males) for about 3 years. The tumor was a well-differentiated adenocarcinoma that maintained its original features after inoculation.

Experimental protocol

Small pieces of TK-4 tumor tissue (5 mm in diameter) were resected from s.c. lesions in the exponential growth phase. These tumor pieces were sutured to the wall of the cecum in nude mice with 6-0 Dexon (Davis-Geck, Manati, PR) after removal of the serosa. Mice were divided into 3 groups: control group (n = 10), TNP-470 group (n = 8) and MMC group (n = 10). Animals in the TNP-470 group received 0.2 ml of TNP-470 solution (30 mg/kg) s.c. and those in the control group received saline s.c., in each case on alternate days from day 10 after tumor transplantation. Animals in the MMC group received 0.2 ml of MMC (2 mg/kg) i.p. once a week from day 10 after transplantation. Treatment was continued until animals were killed at 6 weeks after tumor transplantation. Liver metastases were evaluated macroscopically and confirmed histologically. The transplanted tumors were also removed and weighed.

Statistical analysis

Student's t-test was used for comparison of the actual tumor weight and body weight. The \( \chi^2 \) test was used to compare the number of mice with liver metastasis in each group.

RESULTS

Primary tumor growth

Figure 1 shows the actual tumor weight at killing. The actual tumor weights were 499 ± 247 mg in the control group, 478 ± 95 mg in the MMC group, and 394 ± 202 mg in the TNP-470 group. These results were statistically significant (Students t-test, \( P < 0.05 \)).

\( ^{1} \)To whom correspondence and reprint requests should be sent. Fax: 053 435 2273.

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185 mg in the TNP-470 group and 276 ± 80 mg in the MMC group. TNP-470 had no effect on the primary tumors transplanted in the cecal wall, but MMC significantly inhibited primary tumor growth. The histological appearance of the primary tumors in the 3 groups is demonstrated in Figure 2. No histological evidence of a therapeutic effect of MMC or TNP-470, such as tumor-cell necrosis, was observed.

Liver metastasis

Table I demonstrates the effect of TNP-470 and MMC on liver metastasis. Administration of TNP-470 significantly inhibited liver metastasis. In contrast, MMC showed little anti-metastatic effect, although the number of metastatic foci was lower in the MMC group than in the control group (Table II). All the metastatic lesions were confirmed histologically. Figure 3 (a,b,c) shows the histological appearance of liver metastasis. Many metastases were observed in some mice of the control or MMC groups, but not in the TNP-470 group.

Body weight

The body weights at the time of killing were 20.37 ± 2.02 in the control group, 20.12 ± 1.24 in the MMC group and 19.41 ± 1.21 in the TNP-470 group. Mice treated with TNP-470 showed some weight loss, but this was not statistically significant.

DISCUSSION

The present orthotopic transplantation model employing nude mice has proved useful in clarifying the mechanism of metastasis and in developing anti-metastatic therapy. Metastasis occurs when human cancer strains of many kinds are transplanted orthotopically (Furukawa et al., 1993a,b) and we have already established 6 human colon cancers in this manner. Of these, TK-4 has the highest metastatic potential, and genetic analysis has demonstrated that it possesses mutants of p53, K-ras (data not shown).

The present study showed that TNP-470 had no effect on the primary tumor growth of TK-4, although a significant anti-metastatic effect was demonstrated. TNP-470 has also shown an anti-metastatic effect on other types of colon cancer transplanted in the cecum, despite having no inhibitory effect on the primary tumors (Tanaka et al., in press). On the other hand, TNP-470 shows an inhibitory effect on s.c. transplanted primary tumors in which the doubling time was about half of that of orthotopically transplanted tumors (data not shown). These results suggest that this anti-angiogenic agent may show its anti-proliferative effect on rapidly growing tumors. This is consistent with the hypothesis that rapidly proliferating tumors are more angiogenesis-dependent (Kim et al., 1993). It has been reported that the cytostatic action of TNP-470 is more important than its cytotoxic action in vivo, because TNP-470 exerts its anti-proliferative effect on endothelial cells through its cytostatic action (Yamaoka et al., 1993). This suggests that TNP-470 can exert its effect on any tumors that require
TABLE I  
INHIBITORY EFFECT OF TNP-470 AND MMC ON LIVER METASTASIS

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of mice with liver metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9/10</td>
</tr>
<tr>
<td>MMC</td>
<td>8/10</td>
</tr>
<tr>
<td>TNP-470</td>
<td>2/8*</td>
</tr>
</tbody>
</table>

*Significantly different from control ($p < 0.01$) and from MMC group ($p < 0.05$).

TABLE II  
NUMBER OF METASTATIC LIVER FOCI

<table>
<thead>
<tr>
<th>Group</th>
<th>0 &lt; foci &lt; 5</th>
<th>6 &lt; foci &lt; 20</th>
<th>≥ 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>MMC</td>
<td>2</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>TNP-470</td>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

neovascularization, even if they are resistant to TNP-470 in vitro. TNP-470 causes an increase in the proportion of endothelial cells in the G$_0$/G$_1$ phase and a decrease in the proportion of cells in the G$_2$/M and S phases, suggesting that this agent induces G$_0$/G$_1$ arrest (Kusaka et al., 1994). It has been also reported that the labeling index of endothelial cells is not decreased by treatment with chemotherapeutic agents, but is significantly decreased by TNP-470 (Yamamoto et al., 1994).

It is well known that MMC shows a strong cytotoxic effect on tumor cells both in vitro and in vivo by inducing G$_2$ arrest. MMC was selected as the chemotherapeutic agent for the present study because it is frequently used to treat liver metastasis of colon cancer and because Iigo et al. (1992) reported that it was more effective against liver metastasis induced by the intrasplenic injection of colon 26 cells than against s.c. tumors. In the present study, only a minimal anti-metastatic effect of MMC was demonstrated. In contrast, MMC significantly inhibited primary tumor growth to about half of that observed in the TNP-470 group. The orthotopic transplantation model used in the present study may reflect clinical liver metastasis more precisely than the model of Iigo et al., and our findings suggest that the anti-metastatic effect of MMC is limited, although its anti-proliferative effect on liver metastasis may be considerable. As an anti-metastatic therapy, anti-angiogenic agents are more likely than chemotherapeutic agents to be useful, because neovascularization is essential for tumor micro-metastases to become established and grow. The present study suggests that liver metastasis was prevented by TNP-470 because the growth of TK-4 tumor cells arriving in the liver from primary tumors was inhibited by its anti-angiogenic action.

The combination of TNP-470 with a chemotherapeutic agent which is strongly cytotoxic may be useful in treating solid tumors, because the anti-neoplastic actions of the 2 agents are clearly different. The efficacy of such combination therapy is currently under investigation.

Before clinical use, the toxicity of an anti-angiogenic agent also needs to be evaluated. In general, anti-angiogenic agents are less toxic than chemotherapeutic agents. The major adverse effect of TNP-470 appears to be weight loss. In the present study, 30 mg/kg of TNP-470 induced weight loss in the mice with cecal tumors, but the decrease was not significant.

FIGURE 3  
Histological appearance of the liver metastases ($\times 200$). (a) Control group; (b) MMC group; (c) TNP-470 group. Massive metastasis is observed in the control and MMC groups, but not in the TNP-470 group.

In conclusion, the anti-angiogenesis agent TNP-470 appears to be potentially useful for preventing liver metastasis of colorectal cancer.

REFERENCES


Furukawa, T., Kubota, T., Watanabe, M., Kitazima, M. and


