Chapter - 5  
Anti-tumor activity of PPC-Pr

Synopsis

Anti-tumor activity of PPC-Pr was evaluated using the Ehrlich’s ascites carcinoma (EAC) and Dalton’s lymphoma ascites (DLA) murine cell line. The PPC-Pr was found to be effective in increasing the life span of EAC cell line induced ascetic bearing mice. PPC-Pr administration at a dose of 25 and 50 mg/kg increased the life span of animals by 38.86% and 66.07% respectively. PPC-Pr was also found to have significant preventive and curative effects on solid tumor induced by DLA cell line. It was found that PPC-Pr at a dose of 50 mg/kg was able to prevent the tumor proliferation as effectively as the standard reference drug cisplatin. The experimental results thus, indicated that protein bound polysaccharide (PPC-Pr) isolated from P.rimosus possessed profound antitumor activity. The findings suggest the potential therapeutic use of this compound as an antitumor agent.

5.1. Introduction

Cancer is the second largest cause of death worldwide. The major modules for treating cancer till now include surgery, radiation and chemotherapy. Conventional cancer chemotherapy is used to kill or disable tumor cells while preserving the normal cells in the body by the application of synthetic compounds (Filder and Ellis 2000). These agents have a narrow margin of safety, and the therapy may fail due to drug resistance and dose-limiting toxicities. Hence attempts are being continued to develop new therapeutically useful anticancer agents.

The medicinal use of mushrooms has a very long tradition in the Asian countries; they have been utilized in China, Japan and other Asian countries for two thousand years for edible and medicinal uses. Recently, a number of bioactive molecules, including anti-tumor substances, have been identified in numerous mushroom species (Mizuno et al., 1995b). Polysaccharides, proteo-
polysaccharides and their derivatives from the mushroom have been recognized to be the potent immuno-stimulatory and anti-tumor active compounds (Kim et al., 2003d). Antitumor polysaccharides from mushrooms are considered as biological response modifiers (BRM) based on their action mechanism. They are known to have minimal adverse effects and drug-induced sufferings.

Ikekawa et al. (1969) published one of the first scientific reports on antitumor activities of essences obtained from fruiting bodies of mushrooms belonging to the family Polyporaceae (Aphyllophoromycetideae) and a few other families, against Sarcoma 180 transplanted tumors in animals (Ikekawa et al. 1982; Ikekawa et al. 1992; Ikekawa 2001). Soon thereafter the first three major drugs were developed from medicinal mushrooms. All three were polysaccharides, specifically β-glucans; Krestin from cultured mycelial biomass of *Tremetes versicolor* (Turkwey Tail), Lentinan from fruiting bodies of *Lentinus edodes* (Shiitake), and Schizophyllan from the liquid cultured broth of *Schizophyllum commune* (Split Gill).

Here we studied the effect of PPC-Pr on increasing the life span of Ehrlich’s ascites carcinoma (EAC) cell line induced mice and also its effect on preventing and curing the solid tumors induced by Dalton’s lymphoma ascites (DLA) cell line. PPC-Pr was found to have significant activity against both the murine tumor cell lines. The results are presented in this chapter.

### 5.2. Materials and methods

#### 5.2.1. Test animals

Female Swiss albino mice (6-8 weeks old) of weight 20 ± 5g were used for the anti-tumor studies.

#### 5.2.2. Cell lines

Ehrlich’s ascites carcinoma (EAC) and Dalton’s lymphoma ascites (DLA) cells were maintained by intraperitoneal (i.p.) passages in female Swiss albino mice. Ascetic fluid was aspirated on the 15th day after i.p. inoculation. The cells were washed thrice and the cell suspension was diluted to 1x10^6 viable cells/0.1 ml and used for the studies.
5.2.3. Isolation of PPC-Pr from *P. rimosus*

PPC-Pr was isolated from the hot water extract of *P. rimosus* as described in section 4.2.1.

5.2.4. Ascites tumor model

Animals were divided into four groups of six animals in each group. All animals were injected i.p. with \(1 \times 10^6\) viable EAC cells in PBS. Group1, maintained as control, was administered with the vehicle (sterile normal saline, i.p.) alone. Group2, maintained as standard, received the reference drug Cisplatin at a dose of 2 mg/kg body weight (i.p.). Group3 and 4 received the test compound PPC-Pr at a dose of 25 and 50 mg/kg body weight (i.p.). The drug administration was started 24h after the tumor implantation, continued for ten days with one dose in each alternate day and the total number of dose given was five. The mortality rate was noted in each group and the percent increase in life span (ILS) was calculated using the formula:

\[
\text{Percentage (\%)} \text{ ILS} = \left( \frac{T - C}{C} \right) \times 100
\]

where, \(T\) is mean survival time of treated group and \(C\) that of control group (Ajith and Janardhanan 2003).

5.2.5. Solid tumor model (Preventive effect)

Animals were divided into four groups of six animals in each group. Viable DLA cells (\(4 \times 10^6\)) in PBS were transplanted into the right groin of mice. Drug administration at 25 and 50 mg/kg body weight was started (i.p.) 24 h after tumor implantation and continued with one dose in each day for 10 consecutive days. Standard group received Cisplatin at a dose of 4 mg/kg body weight (i.p). The tumor development on animals in each group was determined by measuring the diameter of tumor growth in two perpendicular planes using Vernier calipers twice a week for 5 weeks. The tumor volume was calculated using the formula:

\[
V = \frac{4}{3} \pi a^2 b / 2
\]

where, \(a\) is minor diameter and \(b\) is major diameter. At the end of the fifth week, animals were sacrificed under anesthesia using diethyl ether, tumor expirated and
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weighed. The percent inhibition was calculated by the formula:

$$\left(1 - \frac{B}{A}\right) \times 100$$

where, $A$ is average tumor weight of the control group and $B$ that of the treated group (Ajith and Janardhanan 2003).

5.2.6. Solid tumor model (Curative effect)

Curative effect of PPC-Pr was tested after tumor development in mice (Ajith and Janardhanan 2003). Solid tumor in mice was induced as in above experiment. After 15 days, animals with tumor size around $1.1 \pm 0.1 \text{ cm}^3$ were divided into four groups as mentioned earlier. Drug was administered at 25 and 50 mg/kg body weight as i.p. once daily for 10 consecutive days. Tumor diameter was measured using Vernier calipers twice a week for a period of 3 weeks and volume was calculated. At the end of the experiment, animals were sacrificed, tumor expired and weighed. The percent inhibition was calculated as mentioned in the earlier experiment. The growth delay was determined as the difference in the number of days required for the control versus treated tumors to increase in volume twice. The tumor curative effect of PPC-Pr was determined by the reduction in tumor volume or weight.

5.3. Results

In the ascites tumor model, PPC-Pr significantly increased the life span of EAC induced ascetic mice. PPC-Pr administration at a dose of 25 and 50 mg/kg increased the life span of animals by 38.86% and 66.07% ($P<0.001$) respectively (Table 5.1). The PPC-Pr showed significant preventive activity against DLA induced solid tumor model in a dose-dependent manner (Table 5.2). The PPC-Pr at a dose of 25 mg/kg and 50 mg/kg could prevent 59.3% and 82.2% of solid tumor growth respectively in tumor induced mice. The weight and volume of tumor in PPC-Pr treated groups at the end of fifth week was significantly lower ($P<0.001$) than the control group. It was found that PPC-Pr at a dose of 50 mg/kg was able to prevent the tumor proliferation as effectively as the standard reference drug cisplatin (Figure 5.1).

The curative effect of PPC-Pr treatment on the growth of DLA induced solid tumor is shown in Table 5.3. The results showed that administration of
PPC-Pr significantly reduced the tumor weight in a dose-dependent manner, with inhibition rates of 54.21 and 60.30 % at doses of 25 and 50 mg/kg respectively, when the tumor weight was recorded on day 21. The inhibition rate of standard drug Cisplatin was 59.26%. PPC-Pr at a dose of 50 mg/kg was found to be as effective as the reference drug Cisplatin in curing the developed tumor in mice. PPC-Pr also showed a growth development delay of 8.85 and 13.12 days at doses of 25 and 50 mg/kg respectively (Figure 5.2).

Table 5.1. Effect of PPC-Pr on EAC induced Ascites tumor model

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatments (mg/kg)</th>
<th>Survival time (days)</th>
<th>% increase in life span</th>
<th>Mortality at 30th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal Saline</td>
<td>19.66±1.21</td>
<td></td>
<td>6/6</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>2</td>
<td>34.33±0.82*</td>
<td>78.03</td>
<td>0/6</td>
</tr>
<tr>
<td>PPC-Pr</td>
<td>25</td>
<td>27.30±1.37*</td>
<td>38.86</td>
<td>6/6</td>
</tr>
<tr>
<td>PPC-Pr</td>
<td>50</td>
<td>32.65±2.58*</td>
<td>66.07</td>
<td>2/6</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 6;  *P<0.001 significant with respect to control.

Table 5.2. Effect of PPC-Pr on DLA induced preventive solid tumor model

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatments (mg/kg)</th>
<th>Vol. on 5th week (cm³)</th>
<th>Weight of tumor (g)</th>
<th>% of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal Saline</td>
<td>4.75±0.38</td>
<td>4.67±0.21</td>
<td></td>
</tr>
<tr>
<td>Cisplatin</td>
<td>4</td>
<td>0.10±0.10</td>
<td>0.58±0.03*</td>
<td>87.6</td>
</tr>
<tr>
<td>PPC-Pr</td>
<td>25</td>
<td>1.88±0.40</td>
<td>1.90±0.14*</td>
<td>59.3</td>
</tr>
<tr>
<td>PPC-Pr</td>
<td>50</td>
<td>0.12±0.12</td>
<td>0.83±0.02*</td>
<td>82.2</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 6;  * P<0.001 significant with respect to control.
Table 5.3. Effect of PPC-Pr on DLA induced curative solid tumor model

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatments (mg/kg)</th>
<th>Growth delay (days)</th>
<th>Weight of tumor (g)</th>
<th>% of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal Saline</td>
<td></td>
<td>4.98±0.67</td>
<td></td>
</tr>
<tr>
<td>Cisplatin</td>
<td>4</td>
<td>12.75</td>
<td>2.03±0.19*</td>
<td>59.26</td>
</tr>
<tr>
<td>PPC-Pr</td>
<td>25</td>
<td>8.85</td>
<td>2.28±0.11*</td>
<td>54.21</td>
</tr>
<tr>
<td>PPC-Pr</td>
<td>50</td>
<td>13.12</td>
<td>1.98±0.16*</td>
<td>60.30</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 6;  * P<0.001 significant with respect to control.

Figure 5.1. Inhibition of tumor development by PPC-Pr at dose of 25 and 50 mg/kg in DLA induced preventive solid tumor model. Control group received vehicle (normal saline) only. Cisplatin at a dose of 4 mg/kg was used as standard. Drug was administered as i.p. for 10 consecutive days starting 24 h after tumor implantation. Values are mean ± SD, n=6.
Figure 5.2. Tumor growth development delay by PPC-Pr at dose of 25 and 50 mg/kg in DLA induced curative solid tumor model. Control group received vehicle (normal saline) only. Cisplatin at a dose of 4 mg/kg was used as standard. Drug was administered as i.p. for 10 consecutive days, when tumor size was around $1.1 \pm 0.1 \text{ cm}^3$. Values are mean ± SD, n=6.

5.4. Discussion

During the evolution of neoplastic diseases, the body’s natural defenses are normally lowered, while the activity of suppressor cells and the blocking antibodies are increased (Gazit and Kedar, 1994). This phenomenon facilitates tumor establishment and growth. Along with this imbalance in the tumor-host relationship, there is secondary immunodeficiency due to conventional cancer treatment, such as chemotherapy and radio therapy. Another mode of tumor therapy, immunotherapy, seeks to correct this immunological deterioration in the host’s favor, providing it with an efficient tumor immuno-surveillance capacity. Some polysaccharides or polysaccharide-protein complexes from mushrooms are able to stimulate the non-specific immune system and to exert antitumor activity through the stimulation of the host’s defence mechanism. The drugs activate
effector cells like macrophages, T lymphocytes and NK cells to secrete cytokines like TNF-α, IFN-γ, IL-β, etc., which are antiproliferative and induce apoptosis and differentiation in tumor cells (Wasser and Weis 1999b; Reshetnikov et al. 2001). β-D glucans are known to induce a biological response by binding to membrane complement receptor type 3 (CR3, alphaMβ2 integrin or CD 11b/CD 18) on immune effector cells. The ligand-receptor complex can be internalized. The intercellular events that occur after glucan-receptor binding have not been fully determined till now (Zhou and Gao, 2002).

Several species of *Phellinus* including *P. igniarius*, *P. hartigii*, *P. gilvus*, *P. pini*, etc. are reported to have different medicinal effects such as anti-tumor and immuno-stimulating activities (Ayer et al., 1996; Jung et al., 1997). The active polysaccharide purified from *Phellinus linteus* stimulates humoral and cell mediated immunity, and exhibits a wider range of immunostimulation and anti-tumor activity than other polysaccharides isolated from Basidiomycetes (Kim et al., 1996). Polysaccharide isolated from *P. linteus* was also suggested to be used in immunochemotherapy of cancer because of its effective activities on tumor growth and metastasis through the immunopotentiation of patients without toxicity (Han et al., 1999).

There is no available report on the anti-tumor activity of polysaccharides isolated from *P.rimosus*. The results of the current investigations show that D-glucan-protein complex isolated from *P.rimosus* significantly inhibited the tumor development and growth. The protein-linked glucans are found to be the better immunopotentiators than the corresponding glucans. Since PPC-*Pr* is a protein bound glucan, it can impart immunostimulation, which is supposed to be the main mechanism of action underlying the anti-tumor activity of polysaccharides. Higher anti-tumor activity seems to be correlated with higher molecular weight, lower level of branching and greater water solubility of β-glucans (Zjawiony, 2004). PPC-*Pr* which is completely soluble in water has a higher molecular weight of ~ 1,200,000. Although the mechanism of anti-tumor effect remains to be elucidated, the activity can be attributed to the greater solubility, high molecular weight and protein bound nature of PPC-*Pr*.