CHAPTER 7 PHASE IV STUDIES

EFFECT OF FERULIC ACID (FA) AND/OR CISPLATIN ON DLA INDUCED SOLID TUMOR STUDIES IN MICE

7.1. INTRODUCTION

The purpose of the phase III study is to find out the potential of ferulic acid in enhancing the effects of cisplatin in the treatment of solid tumor.

Addition of polyphenols with chemotherapy drugs was found to increase the antitumor activity in animal models. The antitumor efficacy of a drug or the potentiating affect of a drug in chemotherapy can be evaluated in a solid tumor model study. The antitumor efficacy of a particular compound would depend upon its ability to decrease the size and volume of a solid tumor (Garg et al. 2005).

Cisplatin is one of the most effective agents available for treating a variety of solid tumors. Solid tumors are tumors of body tissues other than blood, bone marrow, or the lymphatic system. Solid tumors can develop in virtually any tissue or organ, the most common sites being the lungs, breast, prostate and colon. Some of the solid tumors in which cisplatin is employed for treating them include cervical cancer, head and neck cancers, lung cancer, prostrate cancer, testicular cancer and ovarian cancer. Schrier (2002) stated that cisplatin is an active cytostatic agent that became successful in the treatment of several types of solid tumors after its nephrotoxic potential was controlled by hydration and diuresis.

The experimental model for solid tumors used in the Phase III study was Dalton’s lymphoma induced solid tumor Swiss albino mice. Experimental tumor models have a wide role in anticancer drug discovery. A Dalton’s
ascites lymphoma (DAL) tumorigenesis model in Balb/c / Swiss albino mice provides a convenient model system to study antitumor activity within a short time. Injecting tumor cells in rodents, usually mice, is an accepted experimental procedure for the purpose of either propagating a tumor line or for studying various cancers and cancer treatments. Dalton’s lymphoma induced solid tumor model was a widely accepted model most commonly used to test the anticancer potential of any chemical or plant products (Shanker et al. 2000).

In the Phase III study of the present research work, the potentiating effect of ferulic acid (FA) on cisplatin chemotherapy to control solid tumor growth was tested in Dalton’s lymphoma induced solid tumor in Swiss albino mice by measuring the solid tumor volume and growth.
7.2. MATERIALS AND METHODS

7.2.1. Chemicals

Ferulic acid (FA), cisplatin, trypan blue and cell culture chemicals such as heat inactivated fetal bovine serum (FBS), Dulbecco's modified Eagle's culture medium (DMEM), glutamine, penicillin - streptomycin and trypsin were purchased from Sigma Chemicals Co., St. Louis, USA. All other chemicals and solvents of analytical grade were obtained from S.D. fine chemicals, Mumbai, Fisher Inorganic and Aromatic Limited, Chennai and Central Drug House (P) Ltd, New Delhi, India.

7.2.2. Animal care

Healthy Inbred Swiss albino mice in the age group of 11–12 weeks, weighing 25–30 g were purchased from Small Animal Breading Station, Agricultural University (Mannuthy, Thrissur, Kerala, India). They were housed individually in polypropylene cages, maintained under normal laboratory conditions (temperature 24-28°C, relative humidity 60-70% and 1:1 dark and light cycle) and fed with commercially available food pellets (Hindustan Lever Ltd., Mumbai, India) and tap water *ad libitum*. All animal experiments were carried out according to the guidelines and approval of institutional animal ethics committee (IAEC), Government of India (Reg. No. KMCRET/Ph.D./2011).

7.2.3. Treatment schedule

The experimental animals were divided into 4 groups of 6 animals each group. Mice in all groups were induced with solid tumor by Dalton’s lymphoma (DLA) cells on day 0. Group 1 served as DLA - solid tumor control mice. The solid tumor induced group 2 mice were treated only FA alone from day 1 to day 14. The group 3 mice induced with solid tumor were treated with
a single injection of cisplatin on day 10. In group 4, the tumor induced mice were treated with both FA and cisplatin in the same way as in group 2 and 3. All the treatments were continued till the 14th day and the mice were kept for the observation of solid tumor volume till the day 35. The study design was given in the table 7.1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
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<tbody>
<tr>
<td>Solid tumor control</td>
<td>Solid tumor alone induced mice (untreated)</td>
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<tr>
<td>Solid tumor + FA</td>
<td>Solid tumor treated with FA from day 1 - day 14</td>
</tr>
<tr>
<td>Solid tumor + cisplatin</td>
<td>Solid tumor treated with cisplatin on day 10 alone</td>
</tr>
<tr>
<td>Solid tumor + FA + Cisplatin</td>
<td>Solid tumor treated with cisplatin on day 10 and FA from day 1 - day 14</td>
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Table 7.1 Experimental design of Phase IV study

7.2.4. Induction of solid tumor by Dalton’s lymphoma (DLA) cells

DLA cells were initially obtained from Cancer Institute, Chennai, South India and maintained in the peritoneal cavity of laboratory mice by serial transplantation. DLA cell suspension was diluted by 0.1 mL of PBS and about $1 \times 10^6$ cells were injected subcutaneously (s.c.) into the right hind limb of mice. The treatments were started after 24 hours of the tumor inoculation and continued till the end of experimental period of 35 days. The tumor volume was measured from the day 5 onwards. At the end of the experimental period, the animals were sacrificed and the average tumor weight of mice in all groups was calculated.
7.2.5. Dosage and preparation of ferulic acid

Ferulic acid (FA) was freshly prepared in tap water and administered thrice a week in alternative days by intra gastric intubation at a dose of 40 mg/kg body weight from the next day of tumor inoculation till the 14th day of the experimental period. The dosage of FA employed in the present study was adopted from the work of Alias et al. (2009).

7.2.6. Dosage and preparation of cisplatin

Cisplatin was dissolved in saline (0.9% NaCl) for administration. The therapeutic dose of cisplatin against malignant tumor has been established to be 8–10 mg/kg body weight (Khynriam and Prasad 2003). A single dose of cisplatin (8 mg/kg body weight) was administered intraperitoneally (i.p.) in tumor-bearing mice on the 10th day post-tumor transplantation when the tumor was in the log phase of growth.

7.2.7. Tumor Volume

From the 5th day onwards, initial diameter of the right hind limb was noted using Vernier calipers. Tumor diameter was measured every 5 days and recorded up to 35 days. The tumor volume was calculated by the following formula.

\[ V = \frac{4}{3} \pi r_1^2 r_2, \]

Where, \( r_1 \) and \( r_2 \) are the radii of tumors at two different planes.

The effect of FA plus cisplatin treatment was compared with DLA control and other treatments.
7.2.8. Statistical analysis

All values were expressed as means ± SD. The data were statically analyzed using one-way analysis of variance (ANOVA) and the significant difference among treatment groups were evaluated by Duncan’s Multiple Range Test (DMRT). The results were considered statistically significant at p<0.05. All statistical analysis were made using SPSS 17.0 (Statistical package for social sciences) software package (SPSS, Tokyo, Japan).
7.3. RESULTS

The effect of FA and/or cisplatin on Dalton’s lymphoma induced solid tumor was presented below.

The morphological changes in the size of Dalton’s lymphoma induced solid tumor treated with FA and/or cisplatin were shown in figure 7.1. Treatment with FA or cisplatin alone decreased the solid tumor size when compared to solid tumor control mice. Whereas, in solid tumor induced mice treated with both FA and cisplatin, the size of the solid tumor was further decreased when compared to other treated and untreated mice.
Figure 7.1. Effect of FA and/or cisplatin on morphological changes in tumor size of Dalton’s lymphoma induced solid tumor
Data are presented as the means ± SD; n=6

a–d p<0.05, the values not sharing a common superscript letter are significantly different

DLA – Dalton’s lymphoma; FA- Ferulic acid; CIS-Cisplatin

**Figure 7.2. Effect of FA and/or cisplatin on solid tumor weight in control and treated mice**
The effect of FA and/or cisplatin on tumor weight of DLA induced solid tumor in mice was shown in figure 7.2. The average weight of the solid tumor in tumor control mice was found to be around 3.03 g. Mice treated with either FA or cisplatin, decreased tumor weight significantly (p <0.05) to 1.55g and 0.97g respectively. Whereas, mice treated with both FA and cisplatin, significantly (p<0.05) decreased the tumor weight further to 0.50g, when compared to mice treated with FA or cisplatin alone.

Figure 7.3. showed the effect of FA and/or cisplatin on solid tumor volume in mice with DLA induced solid tumor. At the end of the experimental period of about 35 days, the volume of solid tumor was significantly (p<0.05) decrease in mice treated with either FA or cisplatin when compared to untreated control mice. A further significant (p<0.05) decrease in the tumor volume was observed in mice treated with both FA and cisplatin when compared to mice other groups.
Data are presented as the means ± SD; n=6

DLA – Dalton’s lymphoma; FA- Ferulic acid; CIS-Cisplatin

Figure 7.3. Effect of FA and/or cisplatin on solid tumor volume in control and treated mice
7.4. DISCUSSION

Cisplatin is one of the most widely used chemotherapeutic drugs but its response rate in the treatment of human cancers is very low. Combining other anticancer agents with cisplatin seems to be more effective in enhancing its anticancer activity. In the present study, the net anticancer effect of FA and cisplatin during treatment was tested in DLA induced solid tumor in mice.

Tumor volume is one of the important criteria for exploring the direct or indirect anticancer activity of any drug. In the present study, the DLA solid tumor size, volume and weight of FA and cisplatin treated mice were significantly (P<0.05) decreased when compared to the cisplatin alone treated mice and tumor control mice. The prophylactic treatment of FA and Cisplatin exhibited significant tumor reducing property. The cytotoxic action of FA and Cisplatin may be the reason for this.

Cisplatin is a well known chemotherapeutic drug effectively used to treat various solid tumors (Basu and Krishnamurthy 2010). FA showed antitumor effect against variety of cancers (Mori et al. 1999; Nyaradzo et al. 2009; Baskaran et al 2010; Prabhakar 2012; Burns et al. 2013). Moreover in the present study, it is found to induce cytotoxicity in DLA cells (Phase II study). But when solid tumor is treated with both FA and cisplatin, the inhibitory effect is great when compared to treatment with FA or cisplatin alone. The reason for the significant (p<0.05) enhancement in the reduction of solid tumor size and volume in mice treated with both FA as well as cisplatin may be due to the synergistic action of FA and cisplatin. In a study, theanine, an amino acid present in green tea and doxorubicin, a well known chemotherapy drug acted synergistically in mice with M5076 ovarian sarcoma, enhancing tumor concentration of chemotherapy, inhibiting tumor growth, and decreasing hepatic metastasis (Sugiyama and Sadzuka 1998; Sugiyama and Sadzuka 1999).
Reports have shown that cisplatin synergistically combined with many commercial drugs like germicidabine, lovostatin, trimidox, carboplatin, 5-fluorouracil, doxorubicin and Paclitaxel which were not from plant origin. (Feleszko et al. 1998; Novotny et al. 2006; Kathleen et al. 2007; El-Sayyad et al. 2009). But in all these combinations, despite the enhanced anticancer effect, dose-limiting side effects were predominant. Whereas, the present synergistic combination of FA and cisplatin may reduce the toxic effects due to the presence of the plant polyphenol FA.

Hence, the solid tumor model clearly demonstrated that FA does not interfere with the antitumor efficacy of cisplatin. At the same time administration of FA on cisplatin treated animals inhibited tumor growth synergistically.

7.5. CONCLUSION

From the results of the Phase III studies it was clear that FA increased the effect of cisplatin by decreasing the formation if Dalton’s lymphoma induced solid tumor in mice.