Chapter 11

Myelosuppressive study

11.1 INTRODUCTION

Anticancer drugs generally affects all types of rapidly proliferating cells and hence toxicity is a major concern for these types of agents. These compounds present a narrow therapeutic index, with a small difference between the doses required for an antitumor effect and that responsible for unacceptable toxicity. Their recommended doses are determined according to the toxicity endpoint. Moreover, toxicity is observed earlier than the therapeutic effect, so, toxic effects represent a major endpoint for pharmacodynamic studies of cytotoxic drugs. Knowledge of toxicity patterns and main factors of toxicity of anticancer drugs is required before modelling data of these studies. Haematological toxicities represent the main toxicity of the anticancer drugs. Incidences of anaemia, thrombocytopenia, pancytopenia, leukopenia, and neutropenia have been reported frequently during chemotherapy of cancer.

The incorporation of anticancer drugs in the liposomal formulation is believed to reduce the toxicity of the drugs. The administration of liposome-associated chemotherapeutics has gained wide interest in the oncologic field as a means to improve the therapeutic index of anticancer drugs. Doxorubicin (DOX) in particular has been studied extensively in various liposomal formulations (Rahman *et al.,* 1990; Vaage *et. al.,* 1994). Indeed, experimental as well as clinical studies have indicated that the cardiotoxicity of DOX can be reduced by liposomal formulation (Rahman *et. al.,* 1990; Treat *et. al.,* 1990).

11.2 EXPERIMENTAL

11.2.1 Animals

Balb/c mice of either sex weighing 20-25gms were used for the study. The animals were fed *ad libitum* with standard pellet diet and had free access to water.

11.2.2 Myelosuppressive study of methotrexate and its liposomal formulations

The mice were divided into six groups of four animals each. Group I served as control. Group II received methotrexate (MTX) (10mg/kg, i.v.) for 14

days. Groups III, IV, V & VI received liposomal formulations (ML, SML5000-CC-PE, SML2000-CC-PE and SML5000-IC-PE) containing methotrexate (10mg/kg, i.v.) respectively for 10 days. After 24 hours of the last dose of methotrexate / its liposomal formulations, blood was collected from the retro orbital vein of the mice and was analysed for the total RBC, WBC and platelets count.

11.2.3 Myelosuppressive study of tamoxifen citrate and its liposomal formulations

The mice were divided into five groups of four animals each. Group I received tamoxifen citrate (TMX) (20mg/kg, i.v.) for 14 days. Groups II, III, IV & V received liposomal formulations (TL, STL5000-CC-PE, STL2000-CC-PE and STL5000-IC-PE) containing tamoxifen citrate (20mg/kg, i.v.) respectively for 10 days. After 24 hours of the last dose of tamoxifen citrate / its liposomal formulations, blood was collected from the retro orbital vein of the mice and was analysed for the total RBC, WBC and platelets count. The control used in the previous set for methotrexate was taken as control in this study also.

11.2.4 Myelosuppressive study of cytarabine and its liposomal formulations

The mice were divided into five groups of four animals each. Group I received cytarabine (CYT) (50mg/kg, i.v.) for 14 days. Groups II, III, IV & V received liposomal formulations (CL, SCL5000-CC-PE, SCL2000-CC-PE and SCL5000-IC-PE) containing cytarabine (50mg/kg, i.v.) respectively for 10 days. After 24 hours of the last dose of cytarabine / its liposomal formulations, blood was collected from the retro orbital vein of the mice and was analysed for the total RBC, WBC and platelets count. The control used in the previous set for methotrexate was taken as control in this study also.

Table 11.1 Haematological parameters of mice after treatment with methotrexate and its liposomes

Group	Mean Value* (S.E.)		
	WBC Count (x 10^3/µl)	**RBC Count (x 10^6/µl)**	**Platelet Count (x 10^3/µl)**
Control	7.1 (1.6)	9.3 (1.8)	780 (95)
MTX	2.8 (0.9)	3.7 (1.3)	314 (59)
ML	6.4 (1.1)	8.6 (0.9)	726 (88)
SML5000-CC-PE	6.7 (1.2)	8.8 (1.5)	742 (108)
SML2000-CC-PE	6.5 (0.8)	9.1 (1.6)	761 (72)
SML5000-IC-PE	6.4 (1.3)	8.7 (1.0)	758 (94)

*n = 4

Table 11.2 Haematological parameters of mice after treatment with tamoxifen citrate and its liposomes

Group	Mean Value* (S.E.)		
	WBC Count (x 10^3/µl)	**RBC Count (x 10^6/µl)**	**Platelet Count (x 10^3/µl)**
Control	7.1 (1.6)	9.3 (1.8)	780 (95)
TMX	4.7 (1.2)	7.6 (1.6)	334 (84)
TL	6.8 (1.5)	8.8 (1.3)	721 (86)
STL5000-CC-PE	6.9 (0.9)	9.1 (1.5)	746 (92)
STL2000-CC-PE	6.6 (1.2)	8.7 (1.2)	762 (89)
STL5000-IC-PE	7.0 (1.1)	8.5 (1.6)	755 (67)

*n = 4

Table 11.3 Haematological parameters of mice after treatment with cytarabine and its liposomes

Group	Mean Value* (S.E.)		
	WBC Count ($x\ 10^3/\mu l$)	**RBC Count ($x\ 10^6/\mu l$)**	**Platelet Count ($x\ 10^3/\mu l$)**
Control	7.1 (1.6)	9.3 (1.8)	780 (95)
CYT	3.2 (1.1)	3.5 (1.0)	337 (101)
CL	5.9 (1.0)	8.8 (1.2)	714 (75)
SCL5000-CC-PE	6.7 (0.9)	9.2 (0.9)	723 (86)
SCL2000-CC-PE	7.0 (0.6)	8.9 (1.0)	766 (79)
SCL5000-IC-PE	6.8 (1.3)	8.5 (0.8)	741 (82)

*n = 4

11.3 RESULTS AND DISCUSSION

The anticancer agents are generally potent myelosuppresants. They are known to cause haematological changes when administered even at therapeutic doses. So a comparison was made to find out the presence of any change in toxicity profile between the free drug and the liposomal formulations containing the drugs when it is given twice the therapeutic dose. Balb/c mice were used to analyse the changes in haematological parameters following administration of free and liposomal methotrexate, tamoxifen citrate and cytarabine. The mice were injected either with drugs / liposomal formulations containing drugs (MTX, TMX and CYT) with the dose equivalent to that of the twice the human therapeutic dose for a period of 10 days and after 24h of the final dose, the changes in haematological parameters like RBC, WBC and platelet count were analysed and tabulated in Table 11.1 to 11.3.

11.3.1 METHOTREXATE AND ITS LIPOSOMES

Examination of the haematological parameters presented in Table 11.1 showed the myelosuppresive activity of methotrexate and its liposomal formulations. After 10 days of dosing of double the quantity of the therapeutic dose of methotrexate and liposomal formulations containing methotrexate, the blood parameters of the treated mice were analysed. The results presented in the Table 11.1 showed very clearly that significant reduction in total RBC, WBC and platelet counts were noticed after administration of plain methotrexate drug (MTX). The RBC and platelet count was reduced to less than half and the WBC count was reduced to one third of the control. This indicates clearly the potential of the methotrexate to cause myelosuppression. The reduction of total RBC, WBC and platelet counts occurred after administration of methotrexate loaded conventional and sterically stabilized liposomes was not significant compared to the control as depicted in Table 11.1. This proves the reduction of myelosuppressive effect, (which is one of the major side effects) of methotrexate when encapsulated in liposomal formulations.

11.3.2 TAMOXIFEN CITRATE AND ITS LIPOSOMES

The myelosuppresive activity of tamoxifen citrate and its liposomal formulations are depicted in Table 11.2. Double the quantity of the therapeutic dose of tamoxifen citrate and liposomal formulations containing tamoxifen citrate were administered to the mice for a period of 10 days and the haematological changes were analysed. The results presented in the Table 11.2 illustrated that significant reduction in total WBC and platelet counts and a slight reduction in RBC count was found after administration of plain tamoxifen citrate drug (TMX). This indicates clearly the potential Myelosuppressive activity of tamoxifen citrate. The reduction of total RBC, WBC and platelet counts occurred after administration of tamoxifen citrate loaded conventional and sterically stabilized liposomes was not significant compared to the control as depicted in Table 11.2.

11.3.3 CYTARABINE AND ITS LIPOSOMES

Assessment of the haematological parameters shown in Table 11.3 illustrates the myelosuppresive activity of cytarabine and its liposomal formulations. After 10 days of dosing of double the quantity of the therapeutic dose of cytarabine and liposomal formulations containing cytarabine, the blood parameters of the treated mice were analysed. The results presented in the Table 11.3 showed that significant reduction in total RBC, WBC and platelet counts were noticed after administration of plain cytarabine drug (CYT). The WBC, RBC and platelet count was reduced to a very low level when compared to that of the control, which shows the potential myelosuppressive activity of the cytarabine. No significant reduction of total RBC, WBC and platelet counts occurred after administration of same dose of cytarabine in conventional and sterically stabilized liposomes as depicted in Table 11.3. Only a slight reduction as compared to the control was noticed. This proves the reduction of myelosuppressive effect of cytarabine when encapsulated in liposomal formulations.

11.4 REFERENCES

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