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Effect of Adriamycin Administered via Different Routes on Ehrlich Ascites Tumor Cells

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Abstract

Adriamycin (ADR) is an antineoplastic drug, isolated from *Streptomyces peucetius* var. *caesius*, and has cytotoxic features. It binds DNA by intercalation and inhibits replication and transcription. Clinically, ADR is used intravenously and intravesically. Ehrlich ascites tumor (EAT) was originated from breast carcinoma in a mouse. It grows in solid and ascitic forms. The aim of our study is to investigate the effectiveness of ADR on mice bearing EAT by comparing its administration routes. 0.01 $mg.g^{-1}$ ADR was administered mice bearing EAT via intraperitoneal, intravenous and subcutaneous. On day 0 each mouse in the experiment was inoculated with $3x10^5$ EAT cells. On the 2nd, 4th, 6th, 8th days after administration, number of viable cells and mitosis number were counted from each animal by drainage the ascites fluid from the peritoneal cavity of each mouse. Data were evaluated statistically. The most efficient in respect of curing properties was observed in group I in which ADR administered via intraperitoneal.

Keywords: Ehrlich Ascites Tumor, Adriamycin, i.p. administration, i.v. administration, s.c. administration.
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Abbreviations: ADR, Adriamycin; EAT, Ehrlich Ascites Tumor; i.p., intraperitoneal; i.v., intravenous; s.c., subcutaneous.

Introduction

In cancer therapy, chemotherapy has been used for a long time. Anthracyclins have antineoplastic activity against many types of cancer (Kayaalp 1996). Adriamycin (ADR), doxorubicin HCl, is a cytotoxic anthracycline antibiotic isolated from *Streptomyces peucetius* var. *caesius* (Arcamone 1969). It is given as a treatment for many cancer types such as leukemia, breast cancer, soft tissue sarcoma, lymphoma, neuroblastoma, ovarian cancer, Hodgkin's disease, osteogenic sarcoma, lung cancer (Ommaty 2001).

ADR damages DNA by intercalation of the anthracycline portion and inhibits topoisomerase II resulting in DNA strand breakage (Muggia et al. 1991; Mazzotta et al. 2001), generates free radicals (Myers et al. 1977; Rajagopalan et al. 1988; Olson et al. 1990). The drug is a cell cycle non-specific agent (Mazzotta et al. 2001), but cells in the S phase are the most sensitive to the cytotoxic action of ADR (Kim et al. 1972, Barranco et al. 1973) since it inhibits both DNA polymerase and RNA polymerase (Momparler et al. 1976).

Ehrlich Ascites Tumor (EAT) is a transplantable, poorly differentiated malignant tumor appeared originally as a spontaneous breast carcinoma in a mouse. When the ascites fluid with tumor cells is inoculated intraperitoneally and subcutaneously, EAT grows ascitic and solid forms respectively (Kaleoğlu et al. 1977).

The purpose of this paper is to investigate the effectiveness of ADR on mice bearing EAT by comparing its administration routes.

Material and Methods

Preparation of adriamycin

Adriamycin (Adriblastina) commercially available in flacon (powder for injection) purchased from Carlo Erba, Turkey. Stock solution was prepared 1 mg/ml concentration by dissolving in steril bidistilled water.

Ehrlich ascites tumor cells

EAT cells used in this study were hyperdiploid line. It was transplanted by weekly intraperitoneal inoculations about 2-5 x 10^6 saline - washed cells, the subsequent growth of which will be referred to as standard intraperitoneal growth. Hosts were BALB/C male mice weighed 20 – 25g or 2-3 months of age.

Experimental design

Animals in this study were divided into 4 groups as 1 control and 3 experimental (Group I, Group II, Group III) groups. Each group contained 12 mice. All animals were fed with standart laboratory mouse chow Chow and tap water ad libitum. They were kept in plastic cages under conditions of equal periods of light and dark in a room (12 h light and 12 h dark cycle).

$3x10^5$ EAT cells per mouse were inoculated intraperitoneally in both control and experimental groups on day 0. Experimental schedule was as follows:

Group I: $0.01 mg.g^{-1}$ ADR administered intraperitoneally on day 0, single dose.

Group II: $0.01 mg.g^{-1}$ ADR administered intravenously (into marginal tail vein) on day 0, single dose.

Group III: $0.01 mg.g^{-1}$ ADR administered subcutaneously on day 0, single dose.

On the 2nd, 4th, 6th and 8th days after administration, 3 mice randomly chosen from each control and experimental groups were sacrificed by cervical dislocation. Ascites fluid was collected from peritoneal cavity by washing with physiologic water, and viable EAT cells were determined by trypan blue and counted in using haemocytometer. Total of 3 slides per mouse were prepared for determine the mitotic index. All slides were fixed with Clark's fluid and stained using Feulgen and Giemsa (Bancroft et al. 1990). About 1000 cells were counted from each slide to determine the mitosis number.

Statistical evaluation

The results are presented as mean (±SE). Data were evaluated using Student's *t*-test. Statistically significant difference was obtained as $p<0.01$.

Results

In Group I treated with ADR via intraperitoneal and Group II in which intravenous administration of ADR the total viable cell count were significantly decreased when compared with Control Group ($p<0.01$). There was no significant effect in Group III ADR administered subcutaneous route ($p>0.01$). Group I showed more decrease in tumor growth rate when compared with Group II ($p<0.01$) (Table 1, Fig. 1).

Table 1. Total viable EAT cell count ($x10^3$). $3x10^5$ EAT cells per mouse were inoculated on day 0. The results are presented as mean (±SE).

	n	2nd day	4th day	6th day	8th day
Control Group	12	1325.0±50.5	17275.0±353.6	50200.0±57.7	50500.0±2823.3
Group I (i.p.)	12	2000.0±381.9	1833.3±166.7	2166.7±166.7	1000.0±57.7
Group II (i.v)	12	2800.0±500	3766.7±116.7	3800.0±256.6	8066.7±404.5
Group III (s.c.)	12	2783.3±179.3	5433.3±872.9	25726.7±1819.1	38586.7±3628.5

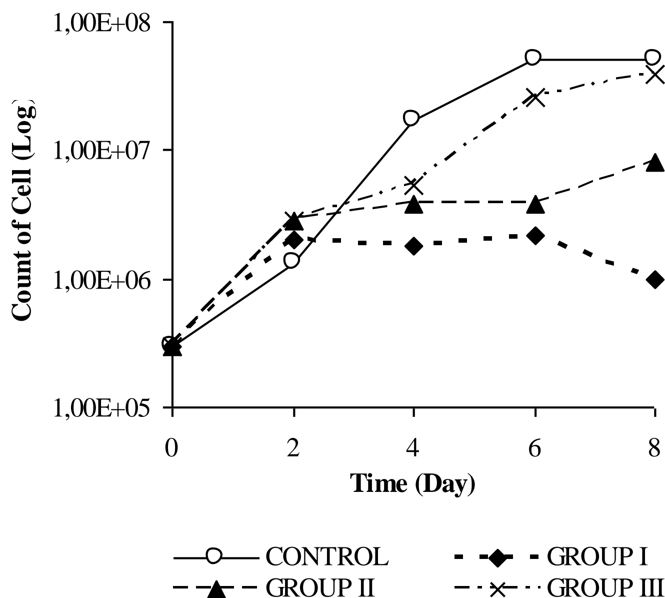

Figure 1. The growth rate of EAT in mice.

The number of mitosis reduced significantly in mice treated with ADR via intraperitoneal (in Group I) and intravenous (in Group II) on 2nd, 4th, 6th, 8th days ($p<0.01$) and 4th, 8th ($p<0.01$), 6th ($p<0.05$) days respectively. No difference between Group III in which subcutaneous administration of ADR and Control Group ($p>0.01$) has been observed (Table 2, Fig. 2).

Statistically, it was determined that intraperitoneal administration of ADR had more inhibition effect than intravenous route on the tumor growth rate and mitotic index of EAT. Intraperitoneal administration of ADR was observed to be the most effective route on cell proliferation of EAT.

Table 2. Mitotic index values of EAT cells.

	n	2nd day	4th day	6th day	8th day
Control Group	12	1.197±0.052	8.092±0.458	1.852±0.126	2.100±0.278
Group I (i.p.)	12	0.182±0.041	0.200±0.069	0.028±0.013	0.278±0.122
Group II (i.v.)	12	3.039±0.202	1.070±0.195	1.167±0.222	0.339±0.078
Group III (s.c.)	12	3.095±0.412	5.311±0.437	3.383±0.453	1.052±0.092

The results are presented as percentage of mitosis number in control and experimental groups (±SE).

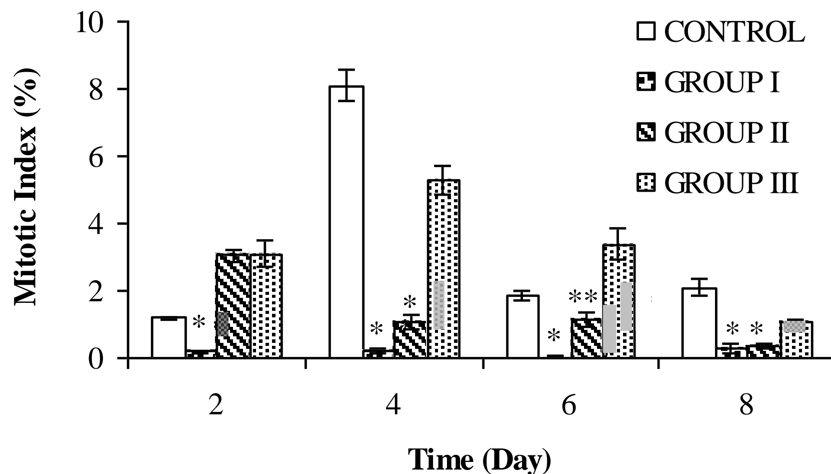

Figure 2. Mitotic index values of EAT cells (±SE)
(*): p<0.01 (**): p<0.05

Discussion

Adriamycin (ADR), an antineoplastic antibiotic, is used for treatment of many types of cancer, but its use limited due to highly toxic effects of the drug (Wang et al. 1971). The major dose limiting toxic effect is drug-induced cardiomyopathy (Lefrak et al. 1973). It produces free radicals (Myers et al. 1977) and inhibits both DNA and RNA synthesis in mammalian cells (Momparler et al. 1976), but it is a cell cycle non-specific agent (Mazzotta et al. 2001).

In a study in which atomic force microscopy was used, it was observed that DNA chains were broken and the chromatin structure was destroyed in ADR treated EAT cells although non - treated EAT cells exhibited intact chromatin structure *in vitro* (Ivic et al. 2005). Sugiyama et al. (1986) reported that ADR-induced changes in plasma membrane of EAT cells.

ADR is used intravenously and intravesically (Ommaty 2001), but not subcutaneous or intramuscular referred to its high irritant properties (Bowers et al. 1978; Luedke et al. 1979).

In some papers, it was reported that ADR - induced toxicity reduced when it combined with some antioxidants or several agents such as vitamin C, vitamin E. In an experimental study, it was observed that vitamin C significantly reduced tissue necrosis in ADR-induced extravasation (Şen 1999). Light and electron microscopy observations of the pathologic changes induced by chronic ADR administration indicate that vitamin E ameliorates the cardiotoxic effect of ADR in rabbits (Wang et al. 1980). Öz et al. (2006) showed that melatonin might provide protection against ADR-induced toxicity. It was indicated that combined administration of cepharantin and ADR-reduced growth of EAT cells compared to that of ADR alone, as well as cepharantin restored body weight loss caused by the treatment of ADR in mice bearing EAT (Asaumi et al. 1995).

Malviya et al (1990) showed that ADR provided therapeutic advantage when the drug was administered intraperitoneally in patients with ovarian carcinoma, but prohibitive local toxicity limited the use of ADR via the intraperitoneal route. In another study researchers found limited toxicity and promising clinical response when liposomal ADR was administered intraperitoneally in

patients with ovarian carcinoma (Booser et al. 1994).

Mimaki et al (1982), administered water-in-oil-in-water type ADR emulsion intraperitoneally to mice bearing Ehrlich solid tumor and they showed that the entrapment of ADR into emulsion reduced the subacute toxicity of the mouse and increased the antitumor effect of the drug against Ehrlich solid tumor.

Shimpo et al (1991), reported that ascorbic acid and its derivatives had no effect on the antitumor activity of ADR in mice inoculated leukaemia L1210 or Ehrlich Ascites Carcinoma, but they delayed general toxicity of ADR and also prevented the cardiac toxicity and the ascorbate derivatives significantly prolonged the life of animals treated with ADR.

In this study a significant decrease in proliferation of EAT cells was observed when ADR was administered via intraperitoneal and intravenous routes. There was no significant difference ($p>0.01$) between Group III (subcutaneous) and Control Group. The most efficient was shown in Group I in respect of curing properties. EAT develops into peritoneal cavity of mice. Consequently the most efficient might be shown in Group I since EAT encounters ADR directly when the drug is administered via intraperitoneal route. In another study we found that ethanolic extract of *Nigella sativa* seeds reduced tumor growth and increased the survival time of animals (Musa et al. 2004a). As mentioned above, some researchers reported that local toxicity of ADR limited the use of ADR via intraperitoneal route (Malviya et al. 1990). We also observed orally administration of ethanolic extract of *Nigella sativa* seeds ameliorated the toxicity in mice bearing EAT treated with ADR via intraperitoneal (Musa et al. 2004b).

In conclusion, further studies should be focused on intraperitoneal administration of ADR by combining with several antioxidants to Ehrlich Ascites Tumor cells.

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