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Effect of Cremophor-EL on Cisplatin-Induced Organ Toxicity in Normal Rat

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ABSTRACT

Cisplatin in an inorganic platinum compound with a broad spectrum antineoplastic activity against different types of tumours. Pharmacological and cytogenetic evaluations of the possible protective effects of cremophor-EL (cremophor) against cisplatin-induced nephro-, hepato- and bone marrow toxicity in normal rats were carried out. A single dose of cisplatin (7.5 mg/kg i.v.) caused acute nephrotoxicity biochemically manifested as an increase in serum urea nitrogen (528%) and serum creatinine (1307%). Serum alanine and aspartate aminotransferase levels were also increased. In the liver tissue, glutathione level (GSH) decreased significantly 7 days after cisplatin treatment. Cisplatin caused a significant increase in the frequency of micronuclei (MN) of polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs), as well as the ratio of PCEs/NCEs. Moreover, a significant delay in the mitotic activity was observed after cisplatin treatment. Administration of cremophor at a dose of 50 µl/kg intravenously 5 minutes before cisplatin protected the kidney and liver as indicated by restoration of urea and creatinine concentrations, as well as aminotransferase activities. Pretreatment with cremophor resulted in a significant recovery in the frequency of cisplatin-induced increase in MV, in the ratio of PCEs/NCEs, as well as in the MI of bone marrow. Administration of cremophor before cisplatin did not interfere with the antitumour activity of cisplatin in EAC-bearing mice. These results suggest that cremophor administration may play a role to prevent cisplatin-induced damage to the kidney, liver and bone marrow without interfering with its antitumour activity.

Key Words: *Cisplatin - Cremophore - Organ toxicity - Micronucleus.*

INTRODUCTION

Cisplatin cis-dichlorodiammine platinum (II) represents a class of antineoplastic agents containing a heavy metal; platinum. It shows a broad spectrum of anticancer activity in animals **[29,32]** and in certain human cancers **[15]**. Cisplatin was also found to have radiosensitizing properties **[11]**. A growing number of in vivo and in vitro studies have demonstrated that cisplatin induced greater than additive cell kill when used in combination with radiation **[12,24,26]**. Yapp et al. **[48]** found that cisplatin was effective in potentiating the effect of both acute and fractionated radiation doses. Unfortunately, the optimal use of cisplatin has been hampered by its dose-limiting nephrotoxicity **[14]**. Different approaches have been tried to ameliorate cisplatin toxicity aiming at increasing its excretion, decreasing its systemic peak concentration, or co-administrating it with certain antidotes **[10,22,28,40]**.

In a recent study by Antunes et al. **[3]** to investigate the modulatory effects of MN (curcumin as a dietary antioxidant) on cisplatin-induced chromosomal aberration in Wistar rat bone marrow cells, a great reduction of the clastogenic effect of cisplatin in vivo has been reported.

Cremophor-EL is a polyethylated castor oil that has been used as a vehicle for oral and intravenous administration of water-insoluble compounds in humans **[45]**. It is characterized by being missible with serum due to its protein-binding characteristics **[42]**. It has been reported that cremophor-EL had a radiprotective effect in hematopoietic system **[5]** and in organ toxicity induced by radiation **[30]** in experimental animals.

The present study was undertaken to investigate whether cremophor has potential ability to

ameliorate the toxic effects induced by cisplatin in different organs and bone marrow cells, as well as the effects of cremophor on the antitumor activity of cisplatin in Ehrlich Ascites Carcinoma (EAC) bearing mice.

PATIENTS AND METHODS

Animals:

Male adult Wister albino rats weighing 120-170 g and females Swiss albino mice weighing 20-22 g were obtained from the animal facility of the National Cancer Institute (NCI), Cairo University. Animals were fed on a standard diet and allowed free access to water. A line of Ehrlich ascites carcinoma (EAC) was kindly supplied Dr. C.Benckhujsen, Amesrerdan, The Netherlands.

Materials:

Stock solution of cremophor (Sigma, St. Louis, USA) was prepared as a 1:20 (V/V) in BSS (Herpes buffered balanced salt solution containing 2% NBBS (New born bovine serum), sterilized by filtration, stored at 4˚C and protected from light until used. Lyophilized cisplatin (Cisplatyl 50, Laboratoire Roger Bellon, France) was dissolved in normal saline.

Experiment design:

A total of 24 male adult Wistar albino rats were divided into 4 groups each of 6 animals. Two groups were injected intravenously i.v. with cremophor (50 µl/kg) [5] and 0.05 ml BSS containing 2% NBBS respectively, 5 min prior to a single i.v. injection of cisplatin (7.5 mg/kg) [1] via the tail vein. Two additional groups served as controls and received i.v. injection of either cremophor or BSS containing 2% NBBS.

Seven days after the last injection, animals were anesthetized with ether and blood samples were withdrawn by heart puncture and serum was separated. Serum urea nitrogen and creatinine were determined according to the methods of Hallet and Cook [16] and Bonsnes and Taussky [6], respectively. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated using the method of Reitman and Frankel [31].

Livers were removed and washed with ice cold saline and blotted with a piece of filter paper, weighed and homogenized in ice cold distilled water. Reduced and oxidized glutathione were determined in liver homogenate using the method of Hissin and Hilf [17].

Bone marrow smears were prepared and allowed to dry overnight. Differential staining to distinguish polychromatic erythrocytes (PCE) and nonchromatic erythrocytes (NCE) with May Grunwald Giemsa stains (Sigma, St Louis, USA) was performed according to the method described by Jagetia and Ganapathil [18]. A total of 500 PCEs were examined and the percentage of micronucleated PCEs (MN-PCEs) were estimated for each individual rat using oil immersion lens (100 x). The number of NCEs was also counted in the fields of recorded PCEs and scored for MN. The mitotic index (MI) was also estimated for bone marrow cells under different experimental conditions.

For evaluation of antitumour activity, EAC cells were inoculated intraperitoneally (i.p.) into 40 female Swiss albino mice (2.5 x 10^6 cells/mouse). Twenty-four hours later, mice were randomly divided into 4 groups, 10 animals each and treated as previously mentioned. Survival of tumour-bearing animals was recorded daily and antitumor activity was evaluated by recording the mean survival time (MST) of animals over a period of 45 days.

Statistical analysis:

Data analysis was carried out using one way ANOVA followed by Tukey-Kramer as a post-ANOVA test. The 0.05 level of probability was used as the criterion for significance.

RESULTS

Table (1) shows the effect of cremophor (50 µl/kg) given i.v. 5 min before cisplatin (7.5 mg/kg) on the renal function of normal rats. Seven days after treatment, cisplatin caused a significant increase (528 and 1307%) in the levels of serum urea nitrogen and creatinine respectively compared to control. Prior treatment with cremophor before cisplatin significantly reduced the level of urea and creatinine in serum by 41 and 76%, respectively compared to cisplatin alone.

Serum ALT and AST levels increased by about 180% after cisplatin treatment compared to control. However, serum ALT and AST decreased by 21% when rats were treated with cremophor before cisplatin compared to cisplatin alone (Table 1).

Table (2) shows the effect of cisplatin and cremophor pretreatment on liver content of reduced and oxidized glutathione in normal male rats. None of the treatment modalities did significantly after the levels of these non-protein sulfhydryl compounds.

Table (3) shows the mitotic indices for the different groups of animals. Cisplatin caused a significant decrease in the mitotic indices (MI) of bone marrow cells of male albino rats compared to either control or cremophor. However, pretreatment with cremophor resulted in a significant recovery of the MI by about 45%, suggesting a possible protective effect of cremophor against mitotic inhibition and/or mitotic delay.

The MNPCEs, MNNCEs data and PCEs/NCEs ratio are presented in table (4). Administration of cisplatin caused a significant increase in the spontaneous frequency of MNPCEs, MNNCEs and the PCEs/NCEs ratio, while pretreatment with cremophor resulted in a significant decrease in MNPCEs, MNPCEs as well as the PCEs/NCEs ratio suggesting a probable modulatory role against the induced cyto-and genotoxicity induced by cisplatin alone. However, the data of cremophor treatment alone show that cremophor has a non-significant effect on the incidence of MN of PCEs and NCEs.

Fig. (1) shows the effect of cremophor pretreatment and/or cisplatin on the mean survival time (MST) of EAC-bearing mice. Untreated tumor-bearing animals showed MST of 16.30 days, whereas administration of cisplatin (7.5 mg/kg) increased the MST to 29 days. Cremophor pretreatment (50 µl/kg) increased significantly the MST of tumor bearing mice treated with cisplatin to 32.40 days.

Table (1): Effect of cremophor pretreatment and/or cisplatin on serum urea, creatinine, ALT and AST in male albino rats.

Parameters	Treatment			
	Control	Cremophor	Cisplatin	Cremophor + cisplatin
Urea (g/l)	$0.35^{a}\pm0.050$	$0.46^{a}\pm0.070$	$2.20^{a}\pm0.070$	$1.29^{a}\pm0.070$
Creatinine (mg/dl)	$0.94^{a}\pm0.196$	$0.83^{a}\pm0.070$	$13.23^{a}\pm1.590$	$3.21^{a}\pm1.860$
ALT (unit/ml)	$60.39^{a}\pm5.70$	$77.93^{b}\pm7.93$	$108.13^{a,b}\pm24.34$	$85.26^{a}\pm7.15$
AST (unit/ml)	$95.83^{a}\pm2.45$	$106.57^{b}\pm20.94$	$172.47^{a,b,c}\pm23.58$	$85.26^{c}\pm9.67$

Cremophor (50 µl/kg, i.v.) was given 30 min prior to cisplatin (7.5 mg/kg, i.v.)
Blood samples were obtained 7 days after cisplatin administration.
All data represented mean values ± SD (n=6).
In the same row, groups having the same superscript are significantly different from each other at $p < 0.05$ using one way ANOVA followed by Tukey-Kramer as a post-ANOVA test.

Table (2): Effect of cremophor pretreatment and/or cisplatin on liver content of GSH and GSSG in male albino rats.

Treatment	GSH (µg/g tissue)	GSSG (µg/g tissue)
Control	1348.56±367.35	234.12±65.41
Cremophor	1348.56±313.61	217.33±68.33
Cisplatin	918.86±59.63	212.29±31.76
Cremophor + cisplatin	1153.15±233.95	212.29±10.60

Cremophor (50 µl/kg, i.v.) was given 30 min prior to cisplatin (7.5 mg/kg, i.v.).
Blood samples were obtained 7 days after cisplatin administration.
All data represented mean values ± SD (n=6).

Table (3): Changes in the mitotic index of bone marrow cells of male albino rats following treatment with cremophor and/or cisplatin.

Treatment	Changes in the total number of MNPCEs
Control	$20.40^{a,b}\pm4.92$
Cremophor	$20.21^{c,d}\pm4.90$
Cisplatin	$6.45^{a,c}\pm2.33$
Cremophor + cisplatin	$11.70^{b,d}\pm3.72$

Cremophor (50 µl/kg, i.v.) was given 30 min prior to cisplatin (7.5 mg/kg, i.v.).
Blood samples were obtained 7 days after cisplatin administration.
All data represented mean values ± SD (n=6).
Groups having the same suprescript are significantly different from each other at $p < 0.05$. Using one way ANOVA followed by Tukey-Kramer as a post-ANOVA test.

Table (4): Percentages and ratio of MNPCEs and MNNCEs of bone marrow cells of male albino rats following tretment with cremophor and/or cisplatin.

Parameters	Treatment			
	Control	Cremophor	Cisplatin	Cremophor + cisplatin
No. of MNPCEs	500	500	500	500
Total MNPCEs (with 1,2 and 3 MN)	$0.146^{a}\pm0.025$	$0.143^{b}\pm0.034$	$0.331^{a,b,c}\pm0.06$	$0.165^{c}\pm0.02$
No. of MNNCEs	476	524	1526	595
Total NCEs (with 1 MN)	$0.082^{a}\pm0.006$	$0.082^{b}\pm0.003$	$0.21^{a,b,c}\pm0.04$	$0.075^{c}\pm0.011$
Ratio	$0.9^{a}\pm0.15$	$0.95^{b}\pm0.04$	$0.32^{a,b,c}\pm0.004$	$0.84^{c}\pm0.056$

Cremophor (50 µl/kg, i.v.) was given 30 min prior to cisplatin (7.5 mg/kg, i.v.).
Blood samples were obtained 7 days after cisplatin administration.
All data represented mean values ± SD (n=6).
In the same row, groups having the same superscript are significantly different from each. Other at $p < 0.05$ using one way ANOVA following by Tukey-Kramer as post ANOVA test.

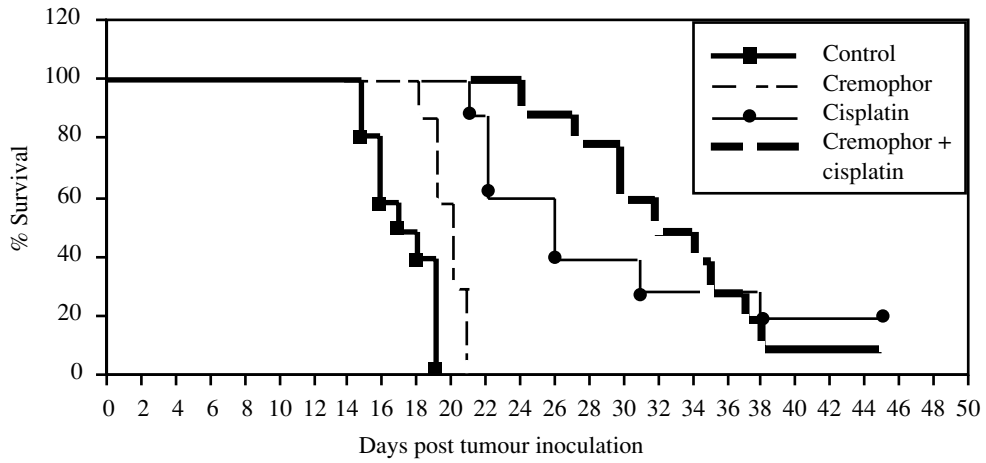

Fig. (1): Effect of cremophor (50 µl/kg, i.v.) and/or cisplatin (7.5 mg/kg, i.v.) on the mean survival time of EAC-bearing mice.

DISCUSSION

Cisplatin is an inorganic platinum compound with a broad spectrum antineoplastic activity against different types of human tumors [21]. Nevertheless, both clinical and experimental studies report a dose-limiting nephrotoxicity which restricts cisplatin's optimal usefulness in cancer chemotherapy [37]. Cremophor, is a polyethoxylated lipid widely used pharmaceutically to solublize water insoluble drugs and vitamins and it is capable of reversing the multidrug resistance of mammalian tumor cell lines in vitro [33,38].

Few reports have been published on the effect of cremophor as chemoprotector against the side effects induced by chemotherapy [25]. Results obtained from previous studies motivated as to initiate this study to investigate the possible protective effects of cremophor against cisplatin induced biochemical and cytogenetic changes in experimental animals.

In the present study, a single dose of cisplatin (7.5 mg/kg) induced nephrotoxicity as revealed from the increase in serum urea and creatinine 7 days after treatment of normal rats. These results are consistent with those reported by Al-Harbi et al. [1], Badary et al. [4], Wong et al. [44] and Sayed-Ahmad et al. [36], who demonstrated that cisplatin caused renal damage in rats with maximum lesions on day 7. It has been reported that administration of cisplatin to normal rats resulted in depletion of glutathione and subsequent potentiation of lipid peroxidation in kidney cortical slices [23] and there was a relation between the cisplatin-induced nephrotoxicity and depletion of glutathione content of kidney tissue [49]. The reduced glutathione was reported to protect cells from cytotoxic damage by many compounds [20] and it is generally known as a potent factor in the control of lipid peroxidation [47]. The role of GSH depletion with the consequent lipid peroxidation in cisplatin-induced nephrotoxicity is confirmed by

the data presented by Anderson [2] et al., who has demonstrated that administration of reduced glutathione protects against cisplatin-induced nephrotoxicity. On the contrary to the aforementioned findings, the liver contents of GSH and GSSG in this report were not affected following treatment with cisplatin.

The data so far obtained in this study demonstrated that cisplatin caused a significant increase in serum ALT and AST activities in male rats. The observed increase in serum aminotransferase activities could be a secondary event following cisplatin-induced liver damage with the consequent leakage from hepatocytes.

Cremophor pretreatment caused a recovery of both kidney and liver damage induced by cisplatin. This was observed by significant restoration of urea and creatinine concentration, as well as aminotransferases activities. It is possible that cremophor could inhibit free radicals generation induced by cisplatin, thus preventing the damage imparted on liver and kidney tissues. Cremophor was reported to be a scavenging agent for free radicals [35].

Cisplatin causes primarily intra-strand DNA-DNA cross links. It is highly mutagenic and carcinogenic in both in vitro and in vivo experimental animal models. The frequency of the mitotic indices of bone marrow cells (MI) showed a significant decrease. This depression in MI is considered as an indicator for assessment of its carcinogenic property [7,13,19,34]. In this study, a significant recovery was noted in cremophor pretreated cisplatin group. This recovery in the MI could be as an achievement for regenerating the survived bone marrow cells to proliferate by the effect of cremophor [30]. Also, the incidence of MNPCEs was significantly high when estimated after administration of cisplatin. The MNPCEs that appeared during the period of treatment (7 days) represented erythrocytes during the G2. G1/S phase of the cell cycle, respectively. This could be due to the formation of hydrogen peroxide and hydroxyl free radical within the cell which can cause DNA damage. These data are in good agreement with the reported findings [27,39] that demonstrated cisplatin to induce mutagenicity through the induction of larger scale damage may involve the induction of cross-links and subsequent processing to single-strand and double-strand breaks during repair. Consistent with this is the induction by cisplatin of significant levels of micronuclei (MN) and sister chromatid exchanges (SCEs), (HRRT) mutations and (GPA) glycopheron mutations in experimental animals. The protective effect of cremophor was assessed by measuring the frequency of the induced MN in PCEs and NCEs of bone marrow cells in vivo. Cremophor has been shown to have antioxidant potential in the repair processes of injured DNA caused by chemicals or by radiation [5,30,33]. In this study, cremophor did not show any alteration in the incidence of MN of PCEs or NCEs. However, pretreatment with cremophor prior to cisplatin showed a very significant decrease in the frequency of MNPCEs compared to cisplatin alone. Similar results were obtained where cremophor caused a significant decrease in the MN of PCEs and NCEs induced by gamma rays [5,30]. The ratio of PCEs to NCEs decreased significantly post cisplatin treatment which could be attributed to a decline in the number of PCEs compared to the NCEs in bone marrow cells. An increase of the ratio of PCEs to NCEs in the pretreated group of animals with cremophor prior to cisplatin treatment could be attributed to the protective role of cremophor against cisplatin-induced cytotoxicity.

Administration of cremophor with cisplatin increased the survival time of tumour bearing mice significantly compared with those treated with cisplatin alone (Fig. 1). Similar results have been reported by Woodcock et al. [46], who found that cremophor significantly increased the survival time of mice transplanted with an adriamycin-resistant tumor compared with those treated with adriamycin alone. Cremophor contains castor oil, which is a mixture of fatty acids [8], which may be more toxic to tumor than normal cells [9]. Moreover, Weber et al. [41] reported that cremophor interacts with P-glycoprotein expressed in modulating resistant cells with the consequent increase in cellular accumulation of anticancer drugs. The selectivity may be due to the apparent differences in the membrane compositions [43].

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