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MODULATION OF RADIATION-INDUCED ORGANS TOXICITY BY CREMOPHOR-EL IN EXPERIMENTAL ANIMALS

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Pharmacological and cytogenetic evaluations of the protective effects of polyethoxylated castor oil cremophor-EL (cremophor) against hepato, renal and bone marrow toxicity induced by gamma irradiation in normal rats were carried out. A single dose of irradiation (6 Gy) caused hepatic and renal damage manifested biochemically as an elevation in levels of serum alanine and aspartate aminotransferase as well as an increase in blood urea. Cremophor administration at a dose level of 50 μl kg^{-1} intravenously 1 day before exposure to irradiation (6 Gy) protected the liver and kidney as indicated by the recovery of levels of hepatic aminotransferase, urea and lipid profiles to normal values. Gamma irradiation of male rats caused a decrease in reduced glutathione and an increase in the oxidized form in rat-liver homogenate. A highly significant increase in the incidence of micronucleated normochromatic erythrocytes and micronucleated polychromatic erythrocytes was observed after irradiation exposure. The induced genotoxicity in the bone marrow cells was corrected by pretreatment with cremophor. The findings of this study suggest that cremophor pretreatment can potentially be used clinically to prevent irradiation-induced hepato, renal and bone marrow toxicity without interference with its cytotoxic activity.

KEY WORDS: irradiation, cremophor, organ toxicity, micronucleus.

INTRODUCTION

Serious problems are generally encountered after acute exposure to high doses of radiation [1]. It has been reported that radiation could induce hyperlipidaemia with elevation of triglycerides concentration in the blood [1, 2]. It was also reported that ionizing radiation affected liver and kidney functions [3, 4]. Acute and delayed hypoplasia or aplasia of the bone marrow and other lymphohaematopoietic organs has been observed following radiation treatment, and is further accentuated when radiation is combined with chemotherapy [5, 6]. Prolonged genetic instability manifested by delayed reproductive death, an increased rate of point mutations, chromosomal rearrangements [7] and micronuclei [8, 9] have been reported after exposure to ionizing radiation.

Cremophor-EL (cremophor), a polyethoxylated castor oil widely used pharmaceutically for solubilization of water insoluble drugs and vitamins, is capable of reversing the multidrug resistance (MDR) of mammalian tumor cell lines *in vitro* [10, 11]. Further studies also demonstrated that the survival of mice transplanted with a MDR tumor was significantly increased by co-administration of cremophor [12]. Recently, Bertoncello *et al*. [13] have shown that cremophor has a haematopoietic radioprotective effect.

The present study was undertaken to investigate the possible protective effect of cremophor against radiation-induced hepato, renal and bone marrow toxicity.

MATERIALS AND METHODS

Animals

Male adult Wister albino rats weighing 120–170 g and female Swiss albino mice weighing 20–22 g, were obtained from the animal house of the National Cancer Institute (NCI), Cairo University. The animals were allowed free access to a standard diet. A line of Ehrlich ascites carcinoma (EAC) was supplied by courtesy of Dr. C. Benckhujsen, Amsterdam, The Netherlands.

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Irradiation

Whole-body gamma-irradiation was performed at the National Centre for Radiation Research and Technology, Cairo, Egypt, using an AECL Gamma Cell-40. Animals were irradiated at an acute single-dose level of 6 Gy delivered at a dose rate of 0.012 Gy s^{-1}.

Drug

A stock solution of cremophor (Sigma Chemical Co. USA) was prepared in a 1 : 20 (v/v) ratio in BSS [a Herpesbuffered balanced salt solution containing 2% NBBS (Newborn bovineserum)], sterilized by filteration and stored at 4 °C under protection from light until required for injection.

Experimental design

A total of 72 rats were divided into 12 groups of six animals. Six groups were injected intravenously (i.v.) with cremophor (50 μl kg^{-1}) [13] or 0.05 ml of BSS containing 2% NBBS respectively, 1 day before a single radiation dose level of 6 Gy. Six additional groups were used as controls and received i.v. injection of either cremophor or BSS containing 2% NBBS.

One, 3 and 7 days after exposure to gamma radiation, four different groups of animals were anaesthetized by ether, then blood samples were taken by heart pincture and the serums separated. The levels of serum cholesterol, triglycerides and total lipids were determined following the methods of Allain *et al*. [14], Fossati and Prencip [15], and Knight *et al*. [16], respectively. Serum urea and creatinine levels were determined according to the methods of Hallet and Cook [17] and Bonsnes and Taussky [18], respectively. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were estimated using the method of Reitman and Frankel [19]. Liver 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 levels were determined within the liver homogenate using the method of Hissin and Hilf [20]. The biochemical parameter data were statistically analyzed and Student's *t*-test was used to evaluate the significance of differences observed.

Bone marrow was separated and the micronuclei were prepared according to the method described by Jagetia and Ganapathi [21]. The slides were stained with May Grunwald (Sigma Chemical Co., USA) and mounted in DPX, coded and examined under a light-transmission microscope (Licka) using a 100× plannapochromat oil immersion abjective. The data regarding the micronucleated polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) were collected. At least 500 PCEs or NCEs were recorded and the presence of micronuclei was found in the same field for each animal. The control group data were pooled together and the mean was calculated and used for comparison with other experimental groups. The incidences of micronuclei were analyzed and the significance of the observed difference was assessed using a Chi-square test (RXC) contingency.

For evaluation of antitumor activity, EAC cells were inoculated i.p. into 40 female Swiss albino mice (2.5×10^6 cells per mouse). Twenty-four h later, the mice were randomly divided into four groups of 10 animals each, and treated as previously stated. The survival of tumor-bearing animals was recorded daily and the antitumor activity was evaluated by recording the mean survival time (MST) of the animals, over a period of 32 days.

RESULTS

Effect of cremophor pretreatment and irradiation on: Lipids

Table (I) shows the effect of whole-body gamma irradiation (6 Gy) and/or cremophor (50 μl kg^{-1}) on the lipid profile of male rats at different time intervals after radiation exposure. Gamma irradiation (6 Gy) induced a significant increase in the cholesterol level on the first and third day post-irradiation, which differed by 19 and 78% from the control values, respectively. In contrast, a significant decrease in the cholesterol level on the seventh day post-irradiation was markedly observed. Administration of cremophor before irradiation restored the normal level on the first and third day post-irradiation.

Irradiation caused an increase in serum triglycerides after 3 and 7 days post-irradiation. The increases were 46 and 70% compared to the control group, respectively (Table I). Pretreatment with cremophor restored serum triglycerides to a normal level. Total lipid levels increased after 1 and 3 days and decreased after 7 days post-irradiation. Normal values were restored when the animals were pretreated with cremophor.

ALT and AST

Gamma irradiation (6 Gy) induced an increase in the activity levels of both serum ALT (49 and 739%) and AST (27 and 522%) on the third and seventh post-irradiation days, respectively. Treatment with cremophor before exposure to radiation, significantly reduced the activity of both ALT (33 and 77%) and AST (14 and 75%) (Table II), which still remained significantly higher than the control or cremophor-treated groups.

Kidney function

Exposure of male rats to gamma radiation (6 Gy) induced a significant increase in serum urea (34, 85 and 120%) after 1, 3 and 7 days post-irradiation, respectively (Table III). Cremophor pretreatment restored the normal value of serum urea at all time intervals. Neither gamma irradiation nor cremophor had any effect on serum creatinine at all time intervals after exposure.

Reduced and oxidized glutathione

Irradiation of male rats caused a decrease in reduced glutathione by 36, 40 and 30% in liver homogenate, while oxidized glutathione increased by 66 and 68%

Table I
Effect of cremophor pretreatment on irradiation-induced lipids alterations in male rats. Cremophor (50 μl kg $^{-1}$ i.v.) was administrated 1 day prior to irradiation (6 Gy). Blood samples were obtained 1, 3 and 7 days post-irradiation. All data represent mean value ± SEM ($n = 6$). Cr : cremophor group; I : irradiated group; Cr + I : cremophor + irradiated group

		1 day			*3 days*			*7 days*		
	Control	*Cr*	*I*	*Cr + I*	*Cr*	*I*	*Cr + I*	*Cr*	*I*	*Cr + I*
Cholesterol (mg dl^{-1})	74.32 ± 1.52	66.60^{a} ± 2.14	88.69^{a} ± 4.69	79.93 ± 3.62	72.82 ± 3.89	132.88^{a} ± 8.76	86.59^{a,b} ± 2.18	82.32^{a} ± 2.18	52.65^{a} ± 4.87	57.85^{a} ± 3.31
Triglycerides (mg dl^{-1})	65.29 ± 8.30	61.22 ± 10.01	81.00 ± 2.89	51.39^{b} ± 5.85	66.26 ± 3.14	95.80 ± 3.39	51.00^{b} ± 2.36	88.91 ± 14.08	111.45^{a} ± 12.63	64.33^{b} ± 6.38
Total lipids (g l^{-1})	2.80 ± 0.17	2.46 ± 0.14	3.71^{a} ± 0.24	3.38 ± 0.19	2.62 ± 0.18	3.68^{a} ± 0.27	2.98^{b} ± 0.06	2.73 ± 0.09	1.19^{a} ± 0.27	2.72^{b} ± 0.06

^{a}Different from control at $P < 0.05$. ^{b}Different from the irradiated group at $P < 0.05$.

Table II
Effect of cremophor pretreatment and/or irradiation on serum ALT and AST of male rats. Cremophor (50 μl kg $^{-1}$ i.v.) was administrated 1 day prior to irradiation (6 Gy). Blood samples were obtained 1, 3 and 7 days post-irradiation. All data represent mean value ± SEM ($n = 6$). Cr : cremophor group; I : irradiated group; Cr + I : cremophor + irradiated group

		1 day			*3 days*			*7 days*		
	Control	*Cr*	*I*	*Cr + I*	*Cr*	*I*	*Cr + I*	*Cr*	*I*	*Cr + I*
ALT (unit ml^{-1})	60.39 ± 2.33	76.21^{a} ± 1.64	60.60 ± 2.14	68.03 ± 4.58	63.73 ± 3.36	90.26^{a} ± 4.83	60.82^{b} ± 6.14	77.93^{a} ± 3.24	507.21^{a} ± 44.14	119.52^{a,b} ± 8.59
AST (unit ml^{-1})	95.83 ± 3.49	86.98 ± 6.23	110. ± 9.61	84.18^{b} ± 3.49	99.45 ± 4.37	121.86^{a} ± 5.25	104.60^{b} ± 3.14	106.57 ± 7.43	596.85^{a} ± 37.75	146.36^{a,b} ± 11.89

^{a}Different from control at $P < 0.05$. ^{b}Different from the irradiated group at $P < 0.05$.

and decreased by 80% 1, 3 and 7 days post-irradiation, respectively (Table IV). Treatment of male rats with cremophor (50 μl kg^{-1} i.v.) before irradiation protected them against the effect of radiation (6 Gy) on the GSH and GSSG levels in the liver homogenate, where their values became within normal limits (Table IV).

Incidence of micronuclei and cell proliferation

The incidences of micronuclei (MN) in PCEs and NCEs of albino male rats bone marrow cells were determined in different groups of animals; the data are presented in Figs (1, 2 and 3).

Irradiation induced a very highly significant increase in the incidence of MNPCEs compared to the control group (Fig. 1). The overall increase was about 3 orders of magnitude above the control value. A similar increase in PCEs with one, two and three MNs were also noted (Fig. 2). There is a notable increase in the frequency of MNPCEs within the duration of the post-irradiation interval; these differences were statistically significant.

The frequency of MNPCEs induced in the bone marrow of albino rats treated with cremophor showed no significant changes compared to the control group (Fig. 2). However, only the data of the PCEs with MN has been observed.

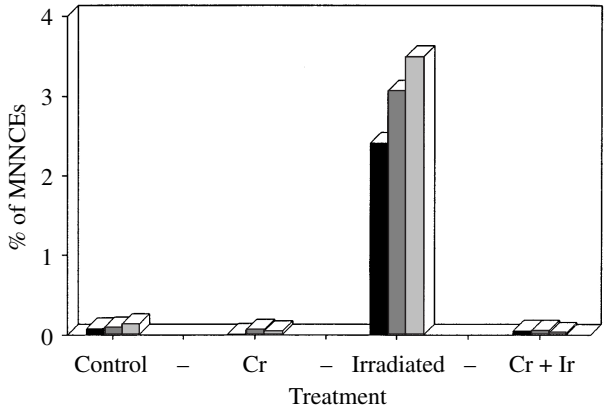

Fig. 1. Effect of cremophor (50 μl kg^{-1}) and/or irradiation (6 Gy) on the incidence of MNNCEs in bone marrow of male albino rats, at 1, 3 and 7 days intervals. ■ day 1, ▩ day 3, ▨ day7.

Cremophor treatment 24 h before irradiation restored the normal levels of total MNPCEs, MNNCEs and MNPCEs with one, two and three MN (Fig. 1, 2), respectively, at all time intervals when compared to the irradiated group.

Table III
Effect of cremophor pretreatment and/or irradiation on serum urea and creatinine of male rats. Cremophor (50 μl kg^{-1} i.v.) was administrated 1 day prior to irradiation (6 Gy). Blood samples were obtained 1, 3 and 7 days post-irradiation. All data represent mean value ± SEM ($n = 6$). Cr : cremophor group; I : irradiated group; Cr + I : cremophor + irradiated group

		1 day			*3 days*			*7 days*		
	Control	*Cr*	*I*	*Cr + I*	*Cr*	*I*	*Cr + I*	*Cr*	*I*	*Cr + I*
Urea (g l^{-1})	0.35 ± 0.02	0.39 ± 0.01	0.47 ± 0.02[a]	0.38 ± 0.02[b]	0.43 ± 0.03	0.65 ± 0.02[a]	0.34 ± 0.02[b]	0.46 ± 0.03[a]	0.77 ± 0.08[a]	0.33 ± 0.03[b]
Creatinine (mg dl^{-1})	0.94 ± 0.07	1.01 ± 0.11	0.96 ± 0.08	0.90 ± 0.07	1.01 ± 0.06	0.94 ± 0.11	0.83 ± 0.03	0.83 ± 0.03	0.97 ± 0.04	0.90 ± 0.11

[a] Different from control at $P < 0.05$. [b] Different from the irradiated group at $P < 0.05$.

Table IV
Effect of cremophor pretreatment and/or irradiation on liver homogenate content of GSH and GSSG in male rats. Cremophor (50 μl kg^{-1} i.v.) was administrated 1 day prior to irradiation (6 Gy). Liver homogenate was obtained 1, 3 and 7 days post-irradiation. All data represent mean value ± SEM ($n = 6$). Cr : cremophor group; I : irradiated group; Cr + I : cremophor + irradiated group

		1 day			*3 days*			*7 days*		
	Control	*Cr*	*I*	*Cr + I*	*Cr*	*I*	*Cr + I*	*Cr*	*I*	*Cr + I*
GSH (μg g^{-1} tissue)	1348.56 ± 150.00	1635.01 ± 98.97	871.14[a] ± 82.98	1050.17 ± 78.58	1455.57 ± 140.58	811.46[a] ± 93.86	1050.17 ± 109.69	1348.56 ± 128.06	938.77[a] ± 34.46	1002.43 ± 100.03
GSSG (μg g^{-1} tissue)	234.12 ± 26.71	227.40 ± 23.76	388.56[a] ± 28.54	203.92 ± 29.97	227.40 ± 11.36	395.27[a] ± 26.81	244.19[b] ± 24.42	217.33 ± 27.90	47.23[a] ± 8.65	212.30[b] ± 12.98

[a] Different from control at $P < 0.05$. [b] Different from the irradiated group at $P < 0.05$.

Table V
Effect of cremophor pretreatment and irradiation on mean survival time of mice-bearing EAC-cells

Treatment	*Mean survival time (days)*[a]
Control	16.30 ± 1.62[b]
Cremophor	18.80 ± 0.98
Irradiation	18.80 ± 1.72
Cremophor + irradiation	20.20 ± 3.60

[a] Mean survival time = avarage survival days of mice-bearing EAC-cells.
[b] Data represent mean value ± SEM (N-10)

Table (V) and Fig. (4) show the effect of cremophor pretreatment and irradiation on the MST of EAC-bearing mice. Untreated tumor-bearing animals showed a MST of 16.30 days, whereas cremophor and irradiated groups showed a non-significant change in the MST.

DISCUSSION

Cremophor, a polyethoxylated lipid widely used pharmaceutically for solubilization of water insoluble drugs and vitamins, is capable of reversing the MDR of mammalian tumor cell lines *in vitro* [12]. Few reports have been published on the effect of cremophor as a chemo- or radioprotector against the side effects induced by either radiation [13] or chemotherapy [22]. This study is presented to investigate the possible protective effects of cremophor against irradiation-induced biochemical and cytogenetic changes in experimental animals.

The results of the present study showed that whole-body gamma irradiation (6 Gy) induced hyperlipidaemia particularly on the first and third days post-treatment. These data confirm previous reports that the lethal dose of ionizing radiation induces hyperlipidemia [1, 23], with inhibition of lipoprotein lipase in experimental rabbits [24]. Cremophor administration before irradiation restored normal lipid-level profiles. Although the mechanism of protection is not clear, Sertich *et al*. [25] showed that a reduction in the membrane cholesterol content results in an increase in membrane permeability and fluidity. This is in agreement with our results where cremophor pretreatment decreased the triglycerides and cholesterol levels (Table I), causing a significant fluidization of the membrane of leukemic cells [12].

Gamma irradiation caused a marked increase in the activity of aminotransferase (ALT and AST) in the rats serum. These data agree with that reported by Roushdy *et al*. [4] who found that irradiation caused a significant increase in ALT and AST activities after 5 and 10 days. The increase in the serum aminotransferase activities could be due to liver damage induced by free radicals

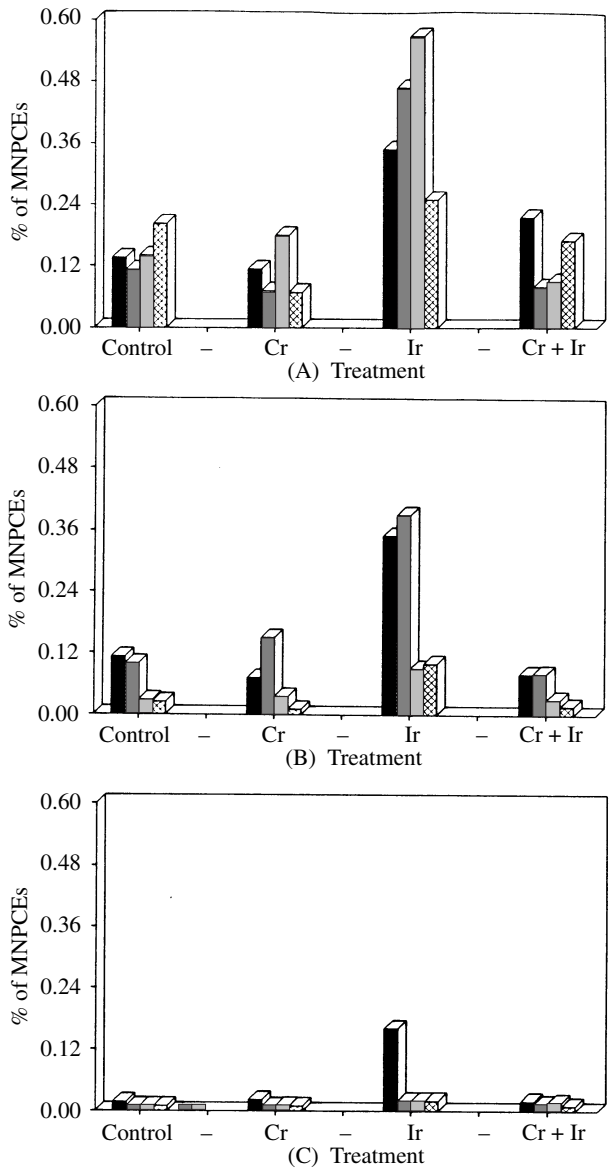

Fig. 2. Effect of cremophor (50 μl kg^{-1}) and/or irradiation (6 Gy) on the incidence of MNPCEs in bone marrow of male albino rats, at day 1 (A), day 3 (B) and day 7 (C) intervals. ■ MNP total, ▩ MNPw1MN, ▨ MNPw2MN, ▩ MNPw3MN.

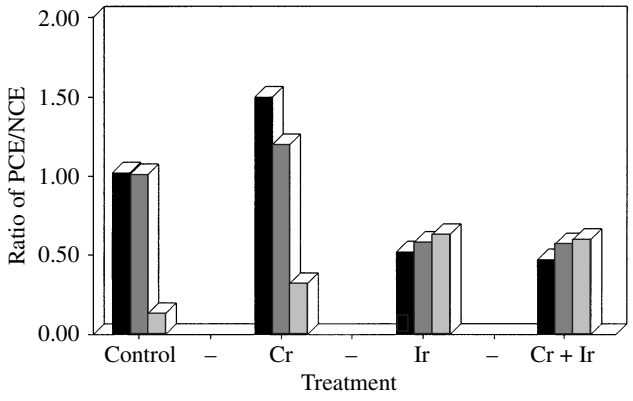

Fig. 3. Effect of cremophor (50 μl kg^{-1}) and/or irradiation (6 Gy) on the ratio of PCEs, NCEs in bone marrow of male albino rats, at 1, 3 and 7 days intervals. ■ day 1, ▩ day 3, ▨ day7.

generated after radiation exposure [26]. Our studies showed that irradiation of male rats increased serum urea without significant changes in serum creatinine (Table III). It is known that radiation causes an increase in glutamate dehydrogenase enzyme levels, which might increase carbamoyl phosphate synthetase activity leading to an increase in urea concentration [27].

Cremophor pretreatment caused a significant recovery of the liver and kidney damage induced by irradiation. This was observed by significant restoration of aminotransferases activity and urea concentration. The mechanism by which cremophor mediated its effects is unclear. However, it is possible that cremophor can inhibit free radical liberation induced by radiation or can scavenge these free radicals, so preventing the damage induced on liver and kidney cells (Tables II and III). This was indirectly suspected in our study, since cremophor pretreatment increased the reduced glutathione (Table IV), which is known to be a free radical scavenging agent [28–30].

The results obtained in this study demonstrate that irradiation caused a very significant increase in the incidence of MNPCEs and MNNCEs (Figs 1, 2). On the other hand, cremophor pretreatment induced a significant decrease in the incidence of MNPCEs and MNNCEs compared with the irradiated group. This suggests a protective role of cremophor. Bradly *et al.* [31] reported that cremophor activates accessory cells, and/or modulates accessory factors regulating haematopoitic progenitor cells directly or indirectly through the operation of cytokine cascades. It reduces the level of serum inhibitory activity which suppresses the proliferation of haematopoietic progenitor cells. Moreover, cremophor showed no evidence of localized cytotoxicity or marrow destruction as reported by flow cytometric studies by Woodcock *et al.* [12], who demonstrated that a very high concentration of cremophor (10% v/v) did not lyse mammalian cell membranes. The decrease in the number of MNPCEs and MNNCEs in the cremophor-treated irradiated groups (Figs 1, 2) could be attributed to the recovery of the mitotic activity of bone marrow cells which probably increased the rate of proliferation of surviving cells in the bone marrow [32]. The decreased ratio of PCEs and NCEs on the seventh day post-irradiation, was due to a decline in the number of PCEs relative to NCEs in the bone marrow after irradiation. Protection of bone marrow tissue by cremophor against radiation cytotoxicity is expressed as an increase of the PCEs/NCEs ratio (Fig. 3). These results are supported by the findings of Webster *et al.* [33], which indicate that cremophor can limit radiation associated myelosuppression and could accelerate haematopoietic recovery following treatment with radiomimetic agents. Bertoncello *et al.* [31] claimed that light and electron microscopic examination of femurs from cremophor-treated mice showed no evidence of localized cytotoxicity or marrow destruction. Similarly, Woodcock *et al.* [12] reported no destruction of mammalian cell membranes exposed to cremophor treatment. Webster *et al.* [33] noted no obvious toxic effects

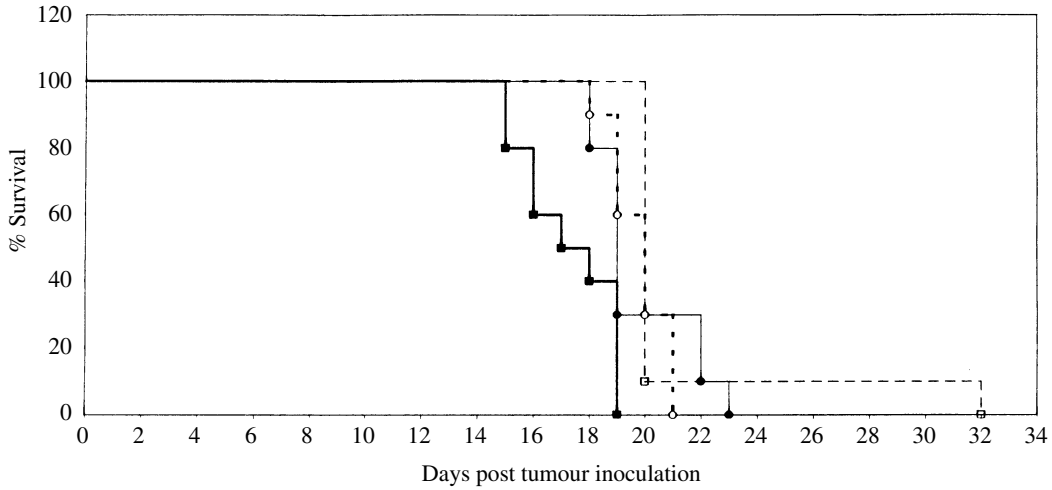

Fig. 4. Effect of cremophor (50 μl kg^{-1}) pretreatment and irradiation (6 Gy) on mean survival time of mice bearing EAC-cells. −■− control; −−○−− cremophor; −●− irradiation; −□− cremophor + irradiation.

attributable to cremophor infusion in patients pretreated with cytotoxic drugs. Cremophor was tried clinically as a substitution or adjunct to cytokines for accelerating haematopoietic recovery following cytotoxic treatment. Further studies are needed to confirm the protective role of cremophor at a certain dose level.

The question now arises, does cremophor interfere with the antitumor activity of radiation? Our results showed that cremophor did not affect the mean survival time (MST) of mice bearing EAC cells treated by radiation (Table V), whether a similar dose or a more effective dose of cremophor is needed, requires more investigation.

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