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Cytogenetic Effects of Heptaplatin on SWR/J Mouse Bone Marrow Cells

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Abstract.- In the present study, the cytogenetic effects of the platinum based anti-tumour drug heptaplatin were analysed by measuring chromosomal aberrations and the mitotic index, in bone marrow cells of SWR/J mice. A single 5, 10 or 12.5 mg/kg body weight (b.wt) intraperitoneal dose of heptaplatin was administered to the experimental animal groups while the control group were injected *i.p.* with normal saline (0.4 ml). Animals from all the groups were sacrificed at sampling times of 24, 48, and 72 h and their bone marrow cells were scrutinized for cytogenetic damage. It was observed that with the three disparate doses of heptaplatin significantly high levels of chromosomal aberration were seen in animals in the initial 24 h, compared to the normal group but these gradually reduced after 48 and 72 h. A similar trend was observed in aberrations with G, without G and in the mitotic index for all three dosages levels. The present study demonstrates that a single *i.p.* administration of 5, 10 or 12.5 mg/kg b.wt. of heptaplatin significantly reduces the mitotic indices and highly significantly induces chromosomal aberrations (both numerically and structurally) in proliferative bone marrow cells of SWR/J mice.

Keywords: Heptaplatin, cytogenetic effects, carcinogenesis, chromosome aberrations.

INTRODUCTION

Heptaplatin (HTP), cis-malonato[(4R,5R)-4,5-bis(aminomethyl)-2-isopropyl-1,3-dioxolane] platinum (II) (SKI-2053R, Sunpla®), is a novel platinum cognate (Kim *et al.,* 1994) known for its better antineoplastic traits (Chung *et al.,* 1998) compared to other platinum based drugs since it possess greater penetration through multilayers of tumour cells which is an important characteristic in the treatment of solid tumours. HTP is a third generation platinum drug derived from cisplatin and synthesized by Sunkyong Industries Co., Ltd. (Republic of Korea) (Chung *et al.,* 1998; Zhang *et al.,* 2014). HTP is being used in treatment plans for gastric carcinomas (Kim *et al.,* 1999; Liu *et al.,* 2014), and a combination of heptaplatin, UFT-E and leucovorin chemotherapy has a better efficacy and controllable toxicity in advanced gastric cancer. Heptaplatin exerts effects similar to cisplatin when combined with 5-FU in advanced gastric cancer patients with tolerable toxicities thereby replacing the hitherto most widely used clinical regime of combination chemotherapy with 5-fluorouracil (5-FU) and cisplatin which is associated with more toxicity issues. HTP is less associated with metallothioneins and is therefore more effective against cells exhibiting cisplatin-resistance related to a high level of metallothioneins. In combination with low-dose PTX (paclitaxel) HTP also shows promising potential against head and neck squamous cell cancers (HNSCC) (Lee *et al.,* 2006), and has been shown to exhibit an inhibitory effect in oxaliplatin and irinotecan colon cancer cell lines (Wei *et al.,* 2014). However, genotoxic studies using a cytogenetic approach in animal models have not yet been reported for HTP. The objective of the present study, therefore was to analyse the cytogenetic ramifications of HTP using *in vivo* SWR/J mouse bone marrow cells.

MATERIALS AND METHODS

Ethics statement

The study plan was approved by the Institutional Review Board of King Saudi University and the experiments on animals were performed in accordance with the guidelines issued by the Animal Care and Use Committee of the University.

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Animals

The study was conducted with seventy two (thirty six males and thirty six females), 8-10 weeks inbred SWR/J mice, weighing 26.3-28.4 g. The animals were procured from the Experimental Animal Care Center of King Saud University and were maintained on a 10/14 h light/dark cycle in a regulated environment at a controlled temperature of 22±1°C and a relative humidity of 45±5%. The animals were fed on standard pellet food for mice and water *ad libitum.*

Dosing and experiment schedule

The seventy two animals were divided into four groups each containing nine male and nine female mice. Group I mice were injected (*i.p.*) with 0.4 ml of the vehicle normal saline and served as the control group, while groups II, III and IV were administered (*i.p.*), with 5.0, 10.0 and 12.5 mg/kg b.wt. of heptaplatin (SK pharmaceutical, Seoul, Korea) prepared in normal saline, respectively. Six animals (three male and three female) from each group were sacrificed by cervical dislocation 24, 48 and 72 h after heptaplatin administration. The evaluation of heptaplatin induced cytogenetic ramifications was accomplished by scrutiny of chromosomal aberrations in bone marrow cells derived from the experimental mice. Chromosome slide preparations were performed in accordance with Preston *et al.* (1987) and Al-Hawary and Al-Saleh (1989). Briefly, SWR/J mice were injected *i.p.* with 0.4 mL of 0.05% colchicine, MIS after 90 min the animals were sacrificed by cervical dislocation and bone marrow cells were collected from femur bones by flushing 0.075 M, pre-warmed (37°C) it with KCl, and incubated at 37°C for 25 min. The material was centrifuged and the cell pellet was fixed in aceto-methanol (acetic-acid:methanol, 1:3, v/v). Centrifugation and fixation (in the cold) were repeated three times, and the final suspension was dropped onto clean glass slides, flame dried, and stained after a week in 10% buffered Giemsa, pH 6.8. To identify possible chromosomal aberrations, a total of 50 metaphase chromosomes from each mouse were analysed using the 100x oil immersion objective of a Zeiss microscope. The chromosome aberrations scanned were: chromatid gaps (G), chromatid breaks (B), fragments (F), ring chromosomes (R), deletion (D), centromeric attenuation (CA), centric fusion (CF), pulverized chromosomes (PC) and end to end association (EE). The chromatid gaps were characterized and scored according to Matsuoka *et al*. (1979). In order to evaluate heptaplatin mediated ramifications on the mitotic index, 6000 bone marrow cells were scrutinized in control and treated groups. The mitotic index (MI) for each treatment was determined by the formula:

MI = No. of dividing cells x 100/No. of cells examined

The data acquired for male and female mice of a group were pooled together and statistically analysed employing a SAS computer program and a student's t-test (Sokal and Rohlf, 1981). The 100 x oil immersion objective with 10 x eyepiece under bright illumination was used for taking photomicrographs of selected metaphases from the prepared chromosomal slides of bone marrow cells.

RESULTS

The effects of heptaplatin on the MI of bone morrow cells of SWR/J mice are shown in Table I. In comparison with the control, treated mice showed a significant reduction in the overall MI in bone marrow cells. The reduction in MI existed across all three heptaplatin doses and treatment durations. After 24 h, the reduction in MI exhibited dependence on the dose of heptaplatin; *i.e.*, higher dose induced greater reduction in MI (Table I, MI 24 h). Likewise after 24 h the difference in the MIs of treated mice were significantly ($P<0.05$) different from the MI of the control animals. A single 5mg/kg b.wt. *i.p.* dose of heptaplatin significantly ($P<0.05$) decreased the MI percentage in bone marrow cells of 8-10 weeks old SWR/J mice 24 h after the injection, but this effect was not observed after 48 or 72 h (Table I). Moreover, this treatment also induced highly significantly ($P<0.01$) chromosomal aberrations in the bone marrow cells 24 h after heptaplatin administration, but no significant changes were observed 48 or 72 h after heptaplatin administration (Table II).

A single high dose of heptaplatin (10 and 12.5 mg/kg b.wt. *i.p.*) meanwhile, significantly ($P<0.05$) decreased the percentage of the MI in bone

Table I.- Heptaplatin induced ramifications on the mitotic index of bone morrow cells of SWR/J mice after 24, 48 and 72 h of administration.

Groups	Heptaplatin dose (mg/kg)	No. of animals per treatment	No. of cells examined	No. of dividing cells (H)			Mitotic index (%) (H)		
				24	48	72	24	48	72
I	0	6	6000	254	254	254	4.23	4.23	4.23
II	5	6	6000	212	237	249	3.53*	3.95	4.15
III	10	6	6000	205	210	245	3.43*	3.50*	4.08
IV	12.5	6	6000	203	207	238	3.38*	3.45*	3.97

*Differences are statistically significant from the control group at $p<0.05$.

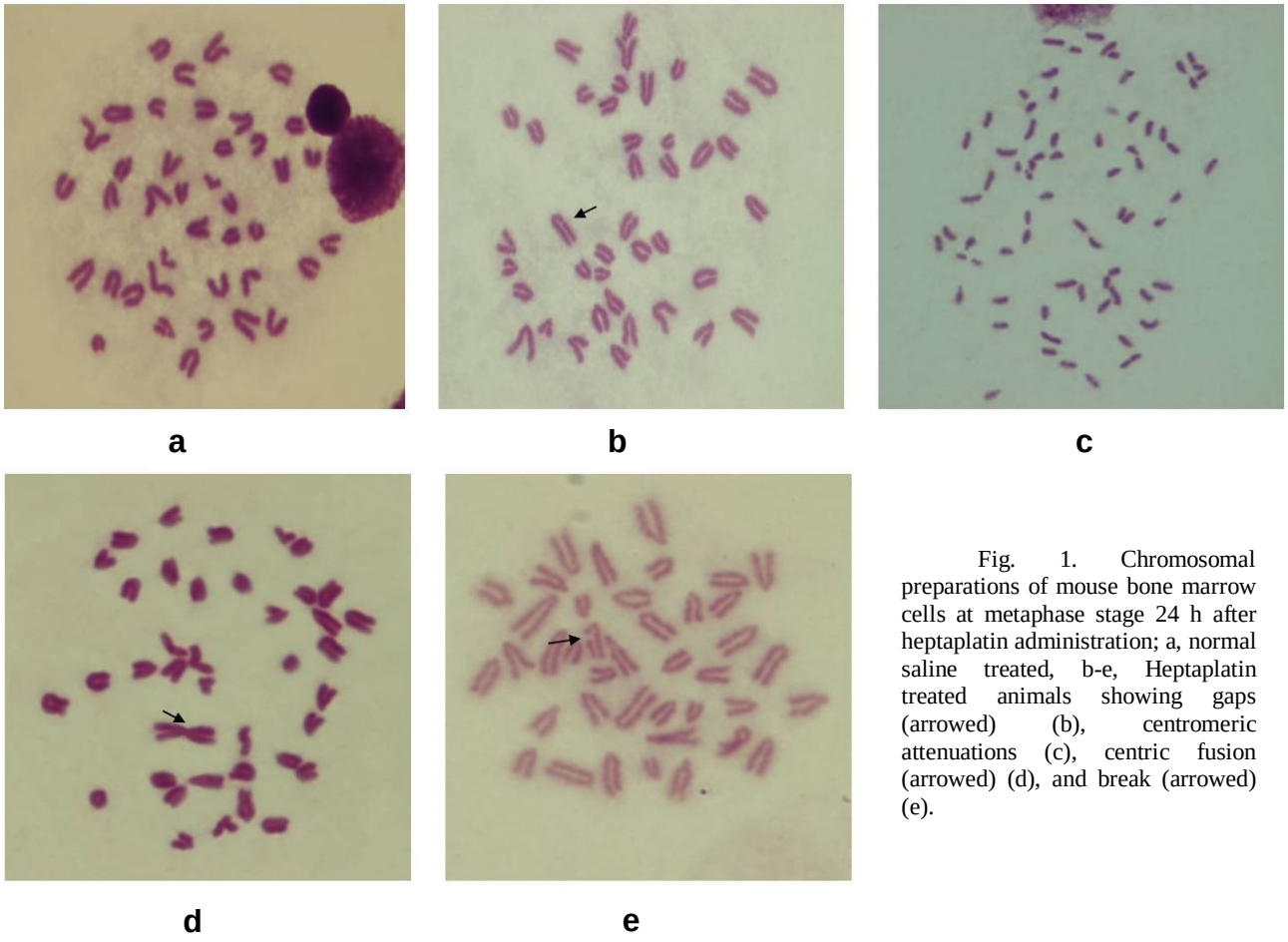

Fig. 1. Chromosomal preparations of mouse bone marrow cells at metaphase stage 24 h after heptaplatin administration; a, normal saline treated, b-e, Heptaplatin treated animals showing gaps (arrowed) (b), centromeric attenuations (c), centric fusion (arrowed) (d), and break (arrowed) (e).

marrow cells of 8-10 weeks old SWR/J mice after 24 and 48 h of injection, but this effect was not observed after 72 h (Tables II). Moreover, this treatment also highly significantly ($P<0.01$) induced chromosomal aberrations in the bone marrow cells of mice 24 and 48 h after heptaplatin administration but it did not induce significant changes 72 h after the administration of heptaplatin. Table II and Figure 1 show that the percentages of total numeric chromosomal aberrations, with and without gaps, observed in bone marrow cells were highly significantly increased ($p < 0.01$) in heptaplatin administered groups compared with the control group. With higher doses (10 and 12.5 mg/kg b.wt. *i.p.*) the highly significant percentages of chromosomal aberrations were observed untill 48 h

Table II.- Effects of various doses of heptaplatin on the chromosomal aberrations in bone morrow of SWR/J mice after 24, 48 and 72 h of administration.

Dose (mg/Kg)	No. of animals used	No. of cells examined	No. and types of numerical chromosomal aberrations		Total		No. and types of structural chromosomal aberrations								Total numerical chromosomal aberrations			
			Hypoploidy (2N-)	Hypoploidy (2N+)			G	B	F	D	CA	CF	PC	EE	With G		Without G	
			No	No	No	%									No	%	No	%
24 h administration																		
Control	6	300	0	2	2	0.7	2	16	2	3	4	6	0	0	33	11	31	10.3
5	6	300	1		2	0.7	5	30	7	8	5	3	0	1	59	19.7**	54	18**
10	6	300	2	1	3	1	7	44	8	4	5	1	1	2	72	24**	65	21.7**
12.5	6	300	2	3	5	1.7	8	48	11	8	4	2	1	1	83	27.7**	75	25**
48 h administration																		
Control	6	300	0	2	2	0.7	2	16	2	3	4	6	0	0	33	11	31	10.3
5	6	300	0	1	1	0.3	2	19	3	3	3	4	0	3	37	12.3	35	11.7
10	6	300	2	2	4	1.3	4	26	9	6	7	3	0	2	57	19**	53	17.7**
12.5	6	300	2	1	3	1	7	31	10	2	6	5	0	1	62	20.7**	55	18.3**
72 h administration																		
Control	6	300	0	2	2	0.7	2	16	2	3	4	6	0	0	33	11	31	10.3
5	6	300	1	0	1	0.3	0	13	7	4	3	1	0	1	29	9.7	29	9.7
10	6	300	2	1	3	1	0	16	5	6	2	3	0	2	34	11.3	34	11.3
12.5	6	300	3	1	4	1.3	0	18	5	4	6	3	0	1	37	12.3	37	12.3

*Differences are statistically significant from the control group at P<0.05

**Differences are statistically significant from the control group at p<0.01.

G, Gap; B, Break; F, Fragment; D, Deletion; CA, Centromeric Attenuation; CF, Centric Fusion; PC, Pulverized Chromosomes; EE, End to End association.

whereas at a lower dose (5mg/kg b.wt. *i.p.*) highly significant percentages of chromosomal aberration were limited to 24 h after the heptaplatin administration. The present results clearly demonstrate that a single *i.p.* administration of 5, 10 or 12.5 mg/kg body weight of heptaplatin significantly reduces the MIs and induces highly significantly chromosomal aberrations (both numerically and structurally) in proliferative cells of the bone marrow of SWR/J mice.

DISCUSSION

Cancer is a complex disease of uncontrolled cellular proliferations. It has a significant impact on the global health structure (Are *et al.*, 2013; Jemal *et al*., 2011; Siegel *et al.,* 2014). The development of advanced diagnostic strategies have served to increase the number of new cases of cancer being reported. According to Globocan (2012), there were 14.1 million new cases of cancer in the year 2012 and 8.2 million deaths associated with the disease reported globally (Ferlay *et al*., 2014). Moreover, countries with low and middle income status are burdened with and increasing incidence of cancer (Ferlay *et al.*, 2014).

Platinum-based chemotherapeutic drugs have been key players in systemic anticancer chemotherapies for some time. Such drugs include heptaplatin along with others such as cisplatin (DDP), carboplatin, oxaliplatin, nedaplatin and lobaplatin. Although platinum based chemotherapeutic drugs are important components of the major therapeutic regimes for cancer treatment they are also responsible for causing significant complications in the overall health of patients. One of these complications involves toxicity to non-cancerous cells leading to organ impairments. In addition, prolonged administration of chemotherapy results in development of resistance in cancer cells against drugs, restricting their usefulness.

Compared to cisplatin and other platinum based drugs, heptaplatin is able to penetrate deeper through multilayers of tumour cells (Lee *et al*., 2006b). Higher doses of heptaplatin have been linked to greater embryonic resorption, reduced number of live foetuses, and increased instances of tail deformity in mice (Al-Anazi *et al.*, 2010). Dose levels are however, not correlated with body weight loss, anatomical (other than tail deformities) or skeletal malformations in mouse foetuses (Al-Anazi *et al*., 2010). Although it is known therefore, that heptaplatin possesses mild embryo-foetotoxicity and mild teratogenic effects (Al-Anazi *et al.*, 2010), the present study has been conducted to evaluate the cytogenetic effects of this drug in experimental animals. It has shown that all dose levels of heptaplatin significantly increased the percentages of chromosomal aberrations in bone marrow cells of the heptaplatin-treated groups used in the present study. To the best of our knowledge, no other reports have been documented concerning such an effect for this drug. Further studies are therefore needed to confirm our results and to investigate the mechanism(s) responsible for the induction of such chromosomal aberrations.

CONCLUSION

It was observed that the effect of the heptaplatin dose was high during the initial 24 h but gradually reduced, so mice sacrificed after 48 exhibited fewer aberrations while those sacrificed after 72 h showed significantly fewer aberrations than the mice sacrificed after 24 h. A similar trend was observed in both types of aberrations (*i.e.* with gaps and without gaps) at all three dosage levels. The present study demonstrates that a single *i.p.* administration of 5, 10 or 12.5 mg/kg body weight of heptaplatin significantly reduces the mitotic indices and highly significantly induces chromosomal aberrations (both numerically and structurally) in proliferative cells of the bone marrow of SWR/J mice. The study suggests that a lower dose of heptaplatin reduces both the frequency of chromosomal aberrations and minimizes the reduction in the MI in SWR/J mice after prolonged exposure.

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