Clastogenic effects of carboplatin on SWR/J mouse bone marrow cells

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ABSTRACT

The clastogenic effect of the anticancer drug carboplatin was investigated in SWR/J mouse bone marrow cells. Males and females were used for each treatment time.The animals aging from 10- 12 weeks and weighting from 29.2 - 32.7 g were injected intraperitonealy with 10 mg/kg of carboplatin solution. A control group (3 males and 3 females) received only isotonic sterile saline (0.4 ml/animal). The animals were sacrificed 6, 12, 24, 48 and 72 h after the injection. The chromosome preparations were obtained from bone marrow cells. Chromatid and chromosome aberrations were investigated in 50 metaphases per animal.

No significant differences in the percentage of mitotic indices and in the frequency of chromosome aberrations were observed between the treated male and female mice at any time intervals used, therefore, data from the two sexes were pooled and analyzed statistically. A significant ($P<0.01$) decrease in the percentage of mitotic indices in bone marrow cells of treated mice was observed at 6, 12 and 24 h following the injection. Moreover, such treatment also significantly ($P<0.01$) increased the frequencies of chromosome aberrations in bone marrow cells of carboplatin-treated mice at all time intervals used following the injection, but it did not induce any significant changes in the diploid number of chromosomes ($2N^{-}/2N^{+}$) at any of the intervals used in this study. The chromosome aberrations induced by this drug included both chromatid and chromosome abnormalities, however, the most frequent types were chromatid gaps and breaks, the former being more frequent.

Key Words: *Carboplatin, chromosome aberrations, bone marrow cells, mice, clastogenic effects.*

INTRODUCTION

Platinum-derived drugs are playing an increasing important role in the treatment of a variety of neoplasms (Olivi *et al.*, 1993). The use of cisplatin, however, is limited by significant dose related toxicity, notably, nephrotoxicity, emesis, ototoxicity and peripheral neuropathy (VanHoff *et al.*, 1979; Olivi *et al.*, 1993). To improve the therapeutic index of platinum compounds, new analogs have been developed (Evans *et al.*, 1983), and carboplatin is one of these platinum derivatives that has been introduced into clinical practice.

Carboplatin has less non-hematologic toxicity, a similar antineoplastic activity and a better therapeutic index (Ettinger *et al.*, 1993). It

is less emetogenic, nephrotoxic and ototoxic than cisplatin (Foster *et al.*, 1985). Carboplatin has been used mainly for first or second line therapy of advanced ovarian carcinoma of epithelial origin and small cell carcinoma of the lung (Ettinger *et al.*, 1993; Sandman *et al.*, 1999; Thomas and Rosenberg, 2002; Romanini *et al.*, 2003). It has also activity similar to cisplatin in head and neck cancer and in genitourinary cancer patients (Smith *et al.*, 1985; Basauri *et al.*, 1986; Feng *et al.*, 1996; Chung *et al.*, 1998). The activity of carboplatin has been also observed in pediatric brain tumors (Canetta *et al.*, 1987; Gaynon *et al.*, 1990).

Despite its effectiveness in the suppression of cancer cells, the administration of carboplatin is associated with a variety of side effects which include myelosuppression, alopecia, rash, and other mild effects (Gaynon *et al.*, 1987; Muggia, 1989). Moreover, carboplatin has also embryotoxic and teratogenic effects (Kai *et al.*, 1988 a & b & c; Chung *et al.*, 1998). However, only few studies have been carried out to investigate its mutagenic and clastogenic effects (Quintana *et al.*, 1994; Gonzalez Cid *et al.*, 1995; Jirsova and Mandys, 1996; Mylonaki-Charalambours *et al.*, 1998).

The use of antitumor drugs for the treatment of cancer have always posed a risk for the patients to be subjected to the long-term side effects of the drugs applied. Since carboplatin is a drug widely utilized for clinical treatment of a variety of human malignancies (Ettinger *et al.*, 1993; Sandman *et al.*, 1999), the aim of the present study was to investigate the clastogenic effect of carboplatin on SWR/J mouse bone marrow cells.

MATERIALS AND METHODS

Inbred SWR/J male and female mice, 10-12 weeks old and weighing 29.2-31.7 g were used throughout the study. Animals were kept and bred in an environmentally controlled room at a temperature of 22 ±1°C, a relative humidity of 45±5 % and a light-dark cycle of 10/14 h. Rodent chow (commercially available in Saudi Arabia) and water were offered *ad libitum*. A total of 18 males and 18 females were used and divided into 6 groups, each group contained 3 males and 3 females. Animals of groups II-VI were treated with a single intraperitoneal (ip) injection of 10 mg/kg body weight of carboplatin (Faulding Pharmaceuticals Plc., UK) dissolved in sterile normal saline. Animals of group I were injected (ip) with the vehicle only (0.4 ml saline) and served as control. The animals were killed by cervical dislocation 6, 12, 24, 48 or 72 hr following the injection and the clastogenic effect of the drug on those animals using *in vivo* bone marrow cells, was evaluated.

The methods of Preston *et al.* (1987) and of Al-Hawary and Al-Saleh (1989) were used for chromosome preparations. A minimum of 10 slides were prepared and 50 well spread and distinctly identifiable metaphase from each mouse were selected. Each selected metaphase was examined using the 100X oil immersion objective of a Zeiss microscope for detecting possible chromosome aberrations. Prior to scoring the drug effect on the chromosomes, the slides were covered and coded. The chromosome aberrations scanned were: chromatid gaps (G), isochromatid gaps (IG), chromatid breaks (B), isochromatid breaks (IB), fragments (F), ring chromosomes (R), deletion (D), pulverized chromosomes (PC) and aneuploidy ($2N^-/2N^+$). The gap was scored as a complete discontinuity narrower than the width of a chromatid according to the criterion of Matsuoka *et al.* (1979). Photomicrographs of selected metaphases were taken under bright illumination, using 100X oil immersion objective and 10X eyepiece.

The data obtained were statistically analyzed using a SAS computer program and a student-t test (Sokal and Rohlf, 1981).

RESULTS

In the present work, no significant differences in the percentage of mitotic indices or in the frequencies of chromosome aberrations were observed between carboplatin-treated male and female mice at any time intervals used. Accordingly, the data obtained from the two sexes were pooled together and statistically analyzed.

A single intraperitoneal injection of 10 mg/l carboplatin/kg body weight highly significantly ($P<0.01$) decreased the percentage of mitotic index in bone marrow cells of 10-12 weeks old SWR/J mice at 6, 12 and 24 h following the injection, however, such effect was not observed at 48 or 72 h (Table 1). Moreover, such treatment also highly significantly ($P<0.01$) induced chromosome aberrations in bone marrow cells of carboplatin-treated mice at 6, 12, 24, 48 and 72 h following the injection, but it did not induce significant changes in the diploid number of chromosomes ($2N^-/2N^+$) at any of the intervals used in this study (Table 2). The chromosome aberrations induced by carboplatin included both chromatid and chromosome abnormalities, however, the most frequent types were chromatid gaps and breaks, the former being more frequent (Table 2).

Table (1): Effect of the dose level 10 mg/kg of carboplatin on the mitotic index in the bone marrow cells of SWR/J mice.

Time after treatment (hr)	*Number of Animals used*	*Number of cells screened*	*Number of dividing cells screened*	*Mitotic index (%)*
Control	*6*	*6000*	*263*	*4.38*
6	*6*	*6000*	*202*	*3.37***
12	*6*	*6000*	*210*	*3.50***
24	*6*	*6000*	*209*	*3.48***
48	*6*	*6000*	*266*	*4.43*
72	*6*	*6000*	*271*	*4.52*

**** Differences are highly significant from the control group at P<0.01.**

Table (2): Effect of dose level 10 mg/kg of carboplatin on chromosome anomalies in bone marrow cells of SWR/J mice.

Time interval (h)	No.of animals used	No.of cells screened	No .of aberrant cells scored	2N'/2N* (%)	No.of aberrant metaphases								% of total aberration	% of total aberration without gabs
					G	IG	B	IB	F	R	D	PC		
Control	6	300	8	3 (1.00)	3	-	2	-	1	-	2	-	2.67	1.67
6	6	300	30	9 (3.00)	13	2	4	-	1	-	-	-	10.00**	5.67*
12	6	300	63	11 (3.67)	38	6	8	-	3	2	1	-	21.00**	10.00**
24	6	300	99	12 (4.00)	77	7	9	-	2	1	9	4	33.00**	13.67**
48	6	300	25	10 (3.33)	9	-	1	-	1	-	3	-	8.33**	5.33*
72	6	300	38	12 (4.00)	17	2	1	1	2	-	5	-	12.67**	6.67**

*Differences are statistically significant compared to control group at P<0.05.
** Differences are statistically significant compared to control group at P<0.01.
G = Chromatid Gaps B = Chromatid Breaks F = Fragment D = Deletion
IG = Isochromatid Gaps IB = Isochromatid Breaks R= Ring chromosomes PC= Pulverized chromosomes

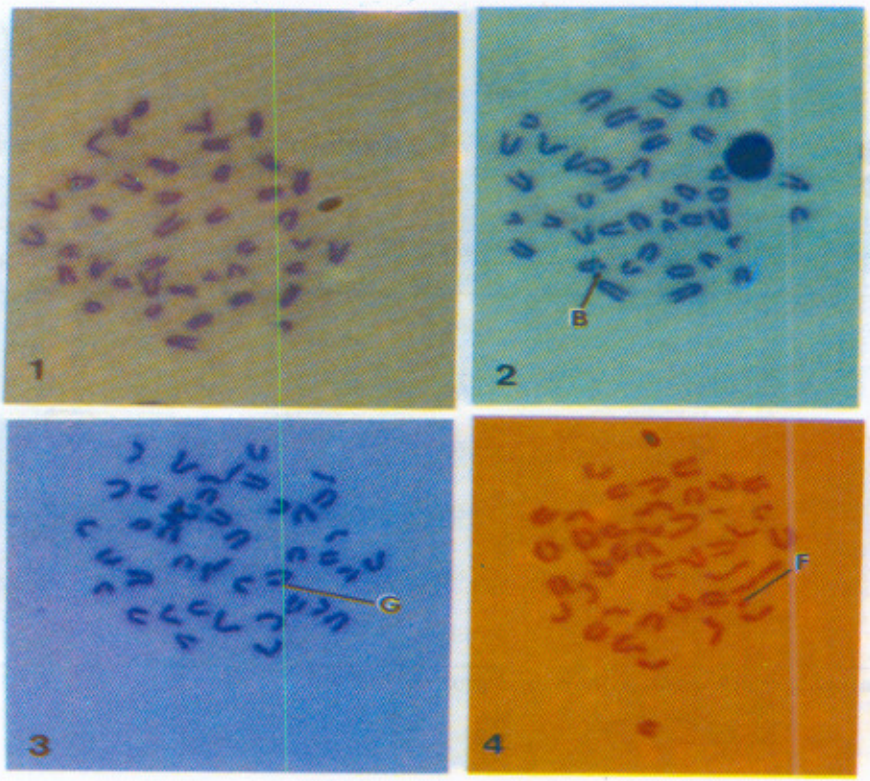

Fig. (1): Photomicrograph of mouse bone marrow cells at metaphase stage, (1) normal metaphase stage, (2), (3) and (4) abnormal metaphase from a carboplatin treated adult mouse after 24hr showing chromatid break, chromatid gap and fragment, respectively.

DISCUSSION

The present results clearly demonstrate that a single intraperitoneal administration of 10 mg carboplatin/kg body weight significantly decreased the percentage of mitotic indices and significantly increased the incidence of chromosome aberrations in proliferating bone marrow cells of SWR/J mice. Both chromatid and chromosome aberrations were observed. However, the most frequent types were chromatid gaps and breaks, the former being more frequent. Accordingly, high incidence of chromatid gaps and breaks may indicate that the clastogenic damage induced by carboplatin occurs during the S phase or after DNA synthesis (G_2) (Traganos *et al.*, 1980; Edelweiss *et al.*, 1995).

In the present study, carboplatin decreased the percentage of mitotic index and induced chromosome aberrations as early as 6 h, but the highest frequency of aberrations was produced 24 hr following treatment when compared with the control group. However, there was a decrease after 6, 12 and 24 hr in the percentage of mitotic indices and there is a increase in the aberrant metaphases after 6, 12, 24, 48 and 72 hr compared with control. The lowering effects of carboplatin on the mitotic indices and on chromosome aberrations in bone marrow cells with time might reflect the short *in vivo* half-life of this drug and the instability of its metabolite (s) (Dorr, 1988), or it might be due to its elimination from the body of treated animals with time (Tates and Natarajan, 1976; Abou-Tarboush and El-Ashmaoui, 2001). Moreover, this gradual reduction in mitotic index and in aberration frequency with time, could be explained by the fact that mammalian cells remove all or part of DNA damaged by carboplatin through excision repair, recombination events, cell death or all of these events (Plooy *et al.*, 1985). In this context, Brendel and Ruhland (1984) stated that about 50 % of both interchromatid cross- linkages and DNA-protein cross-linkages are removed within 30 hr. Furthermore, Matter (1976) indicated that a critical concentration of a reactive chemical compound or its metabolite (s) in the target tissue cells is extremely important for the production of mutagenic or clastogenic events. Moreover, Sram *et al.* (1981) reported that it is not necessary for the mitotic index to coincide with the aberration frequency in bone marrow cells of treated animals, and this could explain the return of the mitotic indices (but not the aberration frequencies) to their normal values after the 24 h treatment.

Similar results were reported in Ehrlich ascites tumor cells (Quintana *et al.*, 1994), cultured human lymphocytes (Gonzalez Cid *et al.*, 1995; Mylornaki-Charalambours *et al.*, 1998), rats (Jirsova and Mandys, 1996) and in mice (Mylonaki-Charalambour, 1998).

The present study deserves to be extended to patients to evaluate the probable clastogenic action of carboplatin in people submmitted to chemotherapy and to characterize the repair mechanisms of cells in a clinical situation. More studies should be conducted on the mutagenicity of platinum compounds that are effective as antitumor agents, in search of an agent less harmfull than carboplatin and cisplatin to normal cells for future modifications of chemotherapy (Edelweiss *et al.*, 1995).

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الملخص العربي

التأثيرات المحثة لتكسر الكروموسومات لعقار الكاربوبلاتين على خلايا نخاع عظام فئران السلالة SWR/J

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تم في هذا البحث دراسة التأثيرات المحثة لتكسر الكروموسومات لعقار الكاربوبلاتين المضاد للسرطان وذلك في خلايا نخاع عظام السلالة النقية SWR/J من الفئران المختبرية . استخدمت في هذه الدراسة ست مجموعات اشتمل كل منها على ثلاثة ذكور وثلاث إناث بلغت أعمارها ما بين ١٠–١٢ أسبوعاً وأوزانها ما بين ٢٩,٢–٣٢,٧ جم . تم حقن أفراد المجموعات من ٢–٦ بجرعة واحدة قدرها ١٠ مجم/كجم من وزن الجسم عند الحقن من العقار عن طريق التجويف البطني ، أما المجموعة الضابطة (المجموعة ١) فقد حقنت أفرادها بـ ٠,٤ مل من المحلول الملحي الفسيولوجي فقط . ولقد تم قتل الحيوانات عن طريق فصل العنق عن بقية الجسم بعد ٦ ، ١٢ و ٢٤ و ٤٨ و ٧٢ ساعة من المعاملة وتم الحصول على تحضيرات الكروموسومات من خلايا نخاع العظام . كما تم دراسة التغيرات الكروموسومية في ٥٠ خلية في المرحلة الاستوائية جيدة الفرد وواضحة لكل حيوان .

أوضحت نتائج هذه الدراسة عدم وجود فروق ذات دلالة معنوية في نسب المؤشرات الميتوزية أو في متوسطات عدد العيوب الكروموسومية بين الذكور والإناث ، ولذا فقد تم ضم نتائج الذكور والإناث معاً عند تحليلها إحصائياً . ولقد أظهرت نتائج هذه الدراسة أن هناك انخفاضاً ذو دلالة معنوية ($p<0.01$) في نسب المؤشرات الميتوزية في خلايا نخاع العظام بعد ٦ ، ١٢ ، و ٢٤ ساعة من المعاملة فقط . كما أظهرت نتائج هذه الدراسة ، أيضاً ، زيادة ذات دلالة معنوية ($p<0.01$) في متوسطات عدد العيوب الكروموسومية الكلية في خلايا نخاع العظام عند كل الفترات الزمنية التي فحصت فيها ، ولقد كان معظم هذه العيوب من النوع التركيبي وعلى هيئة تغيرات كروماتيدية . إلا أن المعاملة بالجرعة ١٠ مجم/كجم من عقار الكاربوبلاتين لم تستحث أية تغيرات معنوية في العدد الكروموسومي الثنائي في خلايا نخاع العظام عند أي من الفترات الزمنية التي فحصت فيها . واشتملت الشذوذات التي استحدثت بهذا العقار الطبي على شواذ كروماتيديه وكروموسوميه ، وان اكثرها تكرارا هي الفراغات والكسرات الكروماتيديه.