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Embryo-feto-toxicity of Anticancer Drug, Heptaplatin in Laboratory Mice

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Abstract: The present study was conducted to test the possible teratogenic and toxic effects of anti-cancer drug heptaplatin (SKI 2053) on developing embryos and fetuses in gestating SWR/J mice. Dose levels of 5.0, 10.0 and 12.5 mg heptaplatin/kg b.wt. were intraperitoneally administered to pregnant mice on days 6-8, 9-11 and 12-14 of gestation. On day 17 of gestation, all fetuses were removed and examined for toxic phenomena (embryo-fetal toxicity) by taking observation on live fetuses and embryonic resorption. Fetuses were also examined for external, internal and skeletal malformations. None of the dams treated with heptaplatin at any of the dose levels used in the present study died during the experimental period. Higher doses of heptaplatin caused greater embryonic resorption and reduced number of live fetuses. However, no loss of body weight was noticed in fetuses at any of the dose levels administered. At highest dose of heptaplatin (12.5 mg kg^{-1}), tail deformity was observed in the form of short and curve tails whereas no other anatomical or skeletal malformations were noticed in any of the fetuses. In addition to mild embryo-fetotoxicity, the study indicates mild teratogenic effects of hetaplatin as reflected in fetal abnormalities at low frequency. These results have significant implications for protracted use of this drug.

Key words: Heptaplatin, anti-cancer, teratogenic, embryonic resorption embryo-fetotoxicity

INTRODUCTION

Heptaplatin is a new drug developed with third generation platinum compounds and is claimed to have fewer and milder side effects as compared to drugs currently being used for treatment of cancers (Galanski *et al*., 2005). Early studies have shown that this drug has a remarkable suppressive activity against many drug-resistant tumors in humans including stomach carcinoma and resistant cancerous cells (Xu *et al*., 2005; Ahn *et al*., 2002). Initial clinical trials also indicate that it may be more effective with lower toxicities and fewer side effects compared to cisplatin (Kim *et al*., 1995a). It has been suggested that heptaplatin enhances the, receptivity of tumor cells to radiotherapy at doses less than those used with cisplatin (Ryu *et al*., 2004). However, another study suggested that the double therapy administered to patients of advanced stomach carcinoma with heptaplatin and fluorouracil-5 is less effective and may be more toxic at higher doses (Min *et al*., 2004; Ahn *et al*., 2003).

Heptaplatin is used singly as well as in combination with other drugs in chemotherapy of several cancers, especially those resistant to chemotherapies such as advanced or recurrent stomach cancer following chemotherapy and leukemia (Jung *et al*., 2004; Hong *et al*., 1995; Kim *et al*., 1995b). This drug is also used for the treatment of some solid tumors such as those of breast, lung, colorectal region, head, neck and cervix (Zang *et al*., 1999; Kim *et al*., 1999a; Min *et al*., 2004; Kang *et al*., 2005).

Although, efficacy of heptaplatin to treat tumors equals or exceeds that of other chemotherapies, whose administration is generally associated with side effects like, renal toxicity, nausea, vomiting, alopecia, azotemia, proteinurea, myelosupression, thrombocytopenia, mucositis and neurotoxicity, its use has only rarely shown incidence of vomiting, renal toxicity, neurotoxicity and proteinurea (Jung *et al*., 2004; Ryu *et al*., 2004; Ahn *et al*., 2002; Kim *et al*., 1992, 1994; Lee *et al*., 1992). It has been found that radioactively marked (^{14}C-SK 2053 R) heptaplatin is distributed over the whole tissues of the body except the central nervous system (CNS) and is especially concentrated in the digestive tract, urine and the secretory organs (Cho *et al*., 1995, 1996).

Very few studies have been conducted on experimental animals to examine the toxicological and

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teratogenic effects of heptaplatin (Jong *et al*., 1999). Investigation on the toxic effects of this drug is still lacking (Jung *et al*., 2004; Kim *et al*., 1999b). Therefore, the present study was undertaken to investigate the teratogenic, toxic and growth suppressing effects of heptaplatin on embryos and fetuses of SWR/J mice when administered to pregnant females at different stages of gestation.

MATERIALS AND METHODS

The present study (research project) was conducted from June, 2009 to May 2010 at the Department of Zoology, College of Science, King Saud University, Riyadh.

Inbred normal SWR/J male and female mice, 8-10 weeks old and weighing 26.2 -28.4 g were used in the present study. Animals were kept and bred under controlled room 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*.

In each box, 4-5 nulliparous females were caged together with a single male. The day the vaginal plug was detected was considered as day 0 (D0) of gestation and the pregnant females were moved to separate cages. A total of 150 pregnant females were divided into 10 groups of 15 members each. Three doses of heptaplatin in sterile normal saline (SK Pharmaceutical, Seoul, Korea), namely, 5.0, 10.0, 12.5 mg kg^{-1} b.wt. (BW) were intraperitoneally administered to females of three separate groups given at three stages, namely, 6-8, 9-11 and 12-14 days of gestation. One group of pregnant females served as control and received 0.4 mL of the vehicle alone (sterile normal saline).

On day 17th of gestation, pregnant females from all the groups were sacrificed by cervical dislocation, abdominal wall was opened and both the uterine horns were promptly exposed to their full extent. The number of resorbed and intact fetuses was recorded. The uterine horns were then opened to determine the number of live and dead fetuses. Spontaneous movement, reddish color, size and/or movement induced with a forceps on the neck or the head of the fetus were used as criteria distinguish between live and dead fetuses. The relative positions of fetuses and resorption of dead ones were also recorded. Live fetuses were carefully examined under a stereoscopic microscope for gross malformations and were accordingly classified as normal or abnormal. Normal and abnormal live fetuses were moved to paper towels, dried up and weighed. Live fetuses in each dose level were cleaned and stained according to the method of McLeod (1980) for the study of skeletal abnormalities. Fetuses in the control group were also similarly prepared for skeletal and anatomical examination.

The experiment was conducted in three replicates and the data was analyzed using a 2×2 contingency table for the actual number of resorptions observed. Significance of the difference between means of heptaplatin-treated and control group was calculated according to Student's t-test (Sokal and Rohlf, 1981).

RESULTS

None of the dams treated with heptaplatin at any dose died during the experimental period; nor did they show any overt signs of maternal toxicity.

When the dams were treated with heptaplatin at 10 and 12.5 mg kg^{-1} b.wt. during 6-8 days of pregnancy, mean number of live fetuses on day 17 was significantly lower ($p<0.01$) and correspondingly, percentage of dead embryos and fetuses had gone up as compared to the controls (Table 1). There was no significant change in fetal body weight under different treatments as compared to the controls. Treatment during the same period at lower dose (5 mg kg^{-1} b.wt.) did not affect any of the parameters studied. Two pups borne to the dames of this group (6-8 days' gestation) treated with 12.5 mg kg^{-1} b.w. showed teratogenic effects in the form of curved and short tails (Fig. 1).

In the case of treatment with heptaplatin during 9-11 days of pregnancy, only the dose of 12.5 mg kg^{-1} b.wt. showed significant negative effect ($p<0.01$) on number of live fetuses and related increase in number of dead embryos and fetuses on day 17 of gestation (Table 2). Only a single malformed fetus with congenital defects was recorded at this dose level. No effect was noticed on the body weight of the fetuses at this stage also.

Fig. 1: Pups obtained from the dams treated with 12.5 mg kg^{-1} b.wt. on days 6-8 of gestation showing curved and short tails

Table 1: Effect of various doses of heptaplatin administered to pregnant SWR/J mice on 6-8 days of gestation on the fetuses

Dose (mg kg^{-1} b.wt.)	No. of dams used	No. of implantation sites	No. of implantation sites/dam (Mean±SE)	No. of live fetuses/dam (Mean±SE)	Total No. of resorptions (%)	Live fetal body weight in g dam^{-1} (Mean±SE)	Abnormalities observed (%)
Control	15	171	0.41±11.40	0.44±11.07	5 (2.9)	0.02±0.84	None
5	15	171	0.46±11.40	0.47±10.67	11 (6.4)	0.03±0.79	None
10	15	160	0.55±10.67	1.19±4.74**	89 (55.6)	0.05±0.79	None
12.5	15	170	0.51±11.33	1.24±3.87**	112 (65.9)	0.04±0.76	2 (2.85)

**Significantly different from the control group at p<0.01

Table 2: Effect of various doses of heptaplatin administered to pregnant SWR/J mice on 9-11 days of gestation on the fetuses

Dose (mg kg^{-1} b.wt.)	No. of dams used	No. of implantation sites	No. of implantation sites/dam (Mean±SE)	No. of live fetuses/dam (Mean±SE)	Total No. of resorptions (%)	Live fetal body weight in g dam^{-1} (Mean±SE)	Abnormalities observed (%)
Control	15	171	0.41±11.40	0.44±11.07	5 (2.9)	0.02±0.84	None
5	15	170	0.56±11.33	0.56±10.93	6 (3.5)	0.05±0.82	None
10	15	157	0.59±10.46	0.83±9.33	17 (10.8)	0.03±0.79	None
12.5	15	156	0.32±10.32	1.17±4.40**	90 (57.7)**	0.04±0.77	1 (1.52)

**Significantly different from the control group at p<0.01

Table 3: Effect of various doses of heptaplatin administered to pregnant SWR/J mice on 12-14 days of gestation on the fetuses

Dose (mg kg^{-1} b.wt.)	No. of dams used	No. of implantation sites	No. of implantation sites/dam (Mean±SE)	No. of live fetuses/dam (Mean±SE)	Total No. of resorptions (%)	Live fetal body weight in g dam^{-1} (Mean±SE)	Abnormalities observed (%)
Control	15	171	0.41±11.40	0.44±11.07	5 (2.9)	0.02±0.84	None
5	15	168	0.43±11.20	0.78±9.13	31 (18.4)**	0.03±0.86	None
10	15	154	0.57±10.27	1.03±7.27*	45 (29.3)**	0.04±0.79	None
12.5	15	161	0.57±10.73	1.15±7.06*	47 (29.2)**	0.03±0.76	1 (0.88)

*Significantly different from the control group at p<0.05, **Significantly different from the control group at p<0.01

When heptaplatin was administered during 12-14 days of pregnancy, fetal mortality was significantly higher than the controls at the dose levels of 10 and 12.5 mg kg^{-1} b.wt. Embryonic resorption frequency was higher under all the tree dose levels, but fetal body weight and all other considered parameters remained un-affected by the drug at this stage (Table 3).

Except for tail deformity, no other anatomical or skeletal malformations were noticed in any of the fetuses obtained from females treated with heptaplatin at any stage of gestation used in the present study.

DISCUSSION

Absence of mortality in dams treated with heptaplatin indicates that the drug is not lethal to mice at the administered regimen. This observation is in contrast to trials with cisplatin which has shown considerably lethal effects on experimental mice when administered at doses of 0.3-3 mg kg^{-1} b.wt. during days 6-8 or 11-14 of gestation (Keller and Aggarwal, 1983).

In the present study, heptaplatin has shown mild lethal effect on embryos as indicated by lower number of fetuses at higher doses of 10 and 12.5 mg kg^{-1} b.wt. with no adverse effect at 5 mg kg^{-1} b.wt. Similar observations were reported for heptaplatin by Chung *et al.* (1998). It has been shown earlier that heptaplatin is less damaging to embryos in comparison with cisplatin and carboplatin (Cho *et al.*, 1996). Kai *et al.* (1989) have also shown that administering carboplatin to pregnant rats may induce death of the embryos and delay their development if administered late during pregnancy. Cho *et al.* (1996) have suggested that despite its lipophilic characteristic, heptaplatin scarcely penetrates the blood-placenta barrier and it cannot penetrate the blood-brain barrier, which may be the reason for its lower toxicity to embryos. Kim *et al.* (1994) have also shown that distribution of radio labeled heptaplatin (^{14}C-SKI-2053R) administered at 20 mg kg^{-1} b.wt. was higher in uterus, placenta and mammary glands of pregnant rats, but the radioactivity was significantly lower in amniotic fluid and was negligible in embryonic tissue.

It is not clear whether the higher frequency of embryonic resorption recorded during our study, especially at advanced stage of gestation, was a direct or indirect effect of heptaplatin. Chung *et al.* (1998) suggested that the resorption may be the result of insufficient hormone concentration or deficient placenta function or may be direct effect of heptaplatin on the embryos. Bajt and Aggarwal (1985) pointed out that the observed decrease in each of Luteinizing Hormone (LH) prolactin and progesterone after treating the female rats with cisplatin on day-6 may be responsible for embryonic resorption observed on days 7 and 9 of gestation; because continuation of gestation depends primarily on this hormone system.

In addition to mild embryo-fetotoxicity, our study also indicates mild teratogenic effects of heptaplatin as reflected in fetal abnormalities at low frequency, which is similar to the findings of Chung *et al.* (1998) and Kim *et al.* (1999b). These long term consequential aspects of the drug administration under protracted regimes need to be thoroughly investigated.

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REFERENCES

Ahn, J.H., Y.K. Kang, T.W. Kim, H. Bahng and H.M. Chang *et al*., 2002. Nephrotoxicity of heptaplatin: A randomized comparison with cisplatin in advanced gastric cancer. Cancer Chemother. Pharmacol., 50: 104-110.

Ahn, J.H., Y.K. Kang and T.W. Kim, 2003. Heptaplatin is more nephrotoxic than cisplatin. Kidney, 12: 141-141.

Bajt, M.L. and S.K. Aggarwal, 1985. An analysis of factors responsible for resorption of embryos in cisplatin-treated rats. Toxicol. Applied Pharmacol., 80: 97-107.

Cho, Y.B., K.H. Kim and D.K. Kim, 1995. Pharmacokinetics, tissue distribution and excretion of cis-malonato [(4R, 5R)-4, 5-bis (aminomethyl)-2 isopropyl 1-1, 3-dioxolane] Platinum (II) in dogs. Drug Metab. Dispos., 23: 1280-1285.

Cho, Y.B., D.K. Kim, K.H. Kim and G. Miyamoto, 1996. Pharmacokinetics of cis-malonato [(4R, 5R) -4, 5- bis (aminomethyl)-2-isopropyl-1, 3-dioxolane] platinum (II) in rats. Azneimittelforschung, 46: 629-634.

Chung, M., J. Kim and J. Rob, 1998. Embryotoxic effects of SKI 2053R a new potential anticancer agent in rats. Reprod. Toxicol., 12: 375-381.

Galanski, M., M.A. Jakupec and B.K. Keppler, 2005. Update of the preclinical situation of anticancer platinum complexes: Novel design strategies and innovative analytical approaches. Curr. Med. Chem., 12: 2075-2094.

Hong, W.S., H.T. Kim, K.H. Kim and D.K. Kim, 1995. *In vitro* antitumor activity of a new platinum complex, cis-malonoto [(4R, 5R)-4, 5-bis (aminomethyl)-2-isopropyl-1,3-dioxolane] platinum (II) (SKI 2053R), against human lung and stomach cancer cell lines. Anticancer Res., 15: 51-54.

Jong, C.K., H.K. Kap, I.P. Gong, C.K. Hyuong and K.C. Moon, 1999. Teratogenicity study of SKI 2053R, a new platinum anticancer in rabbits. Applied Pharmacol., 7: 292-299.

Jung, K.H., D.H. Lee, H.K. Kim, J.Y. Han and I.J. Jang *et al*., 2004. Phase I clinical study of heptaplatin (H) and paclitaxel (P) in previously treated patients with advanced solid tumor. J. Clin. Oncol., 22: 2122-2122.

Kai, S., H. Kohmura, K. Ishikawa, Y. Makihara, S. Ohta, S. Kawano and N. Takahashi, 1989. Teratogenic effects of carboplatin an oncostatic drug administered during the early organogenetic period in rats. J. Toxicol., 14: 115-130.

Kang, J.H., H.J. Kuh, J.H. Lee, J.Y. Shin, K.S. Lee, J.A. Jung and D.Y. Chang, 2005. Phase I/II clinical and pharmacokinetic trial of heptaplatin and 5-FU combination treatment in advanced head and neck cancer. J. Clin. Oncol., 23: 5550-5550.

Keller, K.A. and S.K. Aggarwal, 1983. Emryotoxicity of cisplatin in rats and mice. Toxicol. Applied Pharmacol., 69: 245-256.

Kim, H.O., K.S. Kang, D.J. Shin, J.J. Cho, B.H. Kim, K.W. Seo, K.H. Nam and Y.S. Lee, 1992. Subacute toxicity of cis- malonato [4R , 5R)-4 , 5-bis (aminomethyl) -2- isopropyl-1, 3-dioxolane] platinum (II) (SKI 2053R) in rats. Korean J. Toxicol., 8: 217-233.

Kim, D.K., J.S. Ahn, G. Ryu, K.H. Kim and C.W. Park *et al*., 1994. General pharmacology of cis-malonato [(4R, 5R)-4, 5-bis-aminomethyl)-2-isopropyl-1, 3-dioxolane] platinum (II). Arzneimittelforschung, 44: 1080-1088.

Kim, D.K, H.T. Kim, Y.B. Cho, J.H. Tai, J.S. Ahn, T.S. Kim, K.H. Kim and W.S. Hong, 1995a. Antitumor activity of cis-malonato [(4R, 5R)- 4, 5-bis (aminomethyl)-2-isopropyl-1, 3-dioxolane] platinum (II), a new platinum analogue, as an anticancer agent. Cancer Chemother. Pharmacol., 35: 441-445.

Kim, D.K., H.T. Kim, J.H. Tai, Y.B. Cho and T.S. Kim *et al*., 1995b. Pharmacokinetics and antitumor activity of a new platinum compound, cis-malonato [(4R, 5R)-4 ,5-bis (aminomethyl)-2-isopropyl-1, 3-dioxolane] platinum (II), as determined by *in vivo* pharmacodynamics. Cancer Chemother. Pharmacol., 37: 1-6.

Kim, J.S., K.H. Kim, J. Park, H.C. Kim and M.K. Chung, 1999a. Teratogenicity study of SK 2053R a new platinum anticancer agent in rabbits. J. Applied Pharmacol., 7: 292-299.

Kim, N.K., S.A. Im, D.W. Kim, M.H. Lee and C.W. Jung *et al*., 1999b. Phase II clinical trial of SKI 2053R, a new platinum analog, in the treatment of patients with advanced gastric adenocarcinoma. Cancer, 86: 1109-1115.

Lee, Y.S., K.S. Kang, D.J. Shin, J.J. Cho, H.O. Kim, B.H. Kim and Y.K. Lim, 1992. Subacute toxicity of cis-malonato [4R, 5R) 4,5-bis (aminomethyl)-2-isopropyl-1, 3-dioxolane] platinum (II) (SKI-2053R) in beagle dogs. Korean J. Toxicol., 8: 235-253.

McLeod, M.J., 1980. Differential staining of cartilage and bone in whole mouse fetuses by Alcian Blue and Alizarin Red S. Teratology, 32: 38-39.

Min, Y.J., S.J. Bang, J.W. Shin, H. Kim-do and J.H. Park *et al*., 2004. Combination chemotherapy with 5-fluorouracil and heptaplatin as first- line treatment in patients with advanced gastric cancer. J. Korean Med. Sci., 19: 369-373.

Ryu, M.R., S.Y. Paik and S.M. Chung, 2004. Combined effect of heptaplatin and ionizing radiation on human squamous carcinoma cell lines. Mol. Cells, 19: 143-148.

Sokal, R.R. and F.J. Rohlf, 1981. Biometry the Principles and Practice of Statistics in Biological Research. 2nd Edn., W.H. Freeman, New York.

Xu, H.S.M., C.S. Choi, Y.D. Min, K.C. Kim, K.J. Kim and C.H. Choi, 2005. Concentration-dependent collateral sensitivity of cisplatin-resistant gastric cancer cell sublines. Biochem. Biophys. Res. Commun., 328: 618-622.

Zang, D.Y., K.H. Lee, J.S. Lee, J.H. Lee and W.K. Kim *et al*., 1999. Phase II trial of novel platinum analog, SKI-2053R, in patients with previously untreated extensive-stage small-cell lung cancer. Am. J. Clin. Oncol., 22: 495-498.