The Bioscan 5(4) : 567-569, 2010
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES

DOSE RESPONSE RELATIONSHIP FOR CISPLATIN INDUCED MICRONUCLEI IN BONE MARROW ERYTHROCYTES OF SWISS ALBINO MICE

K. RUDRAMA DEVI*, A. KOUSHIK[1] AND K. VENKAT REDDY[1]
Human Genetics Lab, Department of Zoology,
Osmania University, Hyderabad - 500 007
[1]Sree Datha Institute of Pharmacy Hyderabad - 500 007
E-mail: rudramadevi_k@yahoo.com

KEY WORDS
Cisplantin
Micronuclei
Erythrocytes
Mice

Received on :
12.07.2010
Accepted on :
21.10.2010

***Corresponding author**

ABSTRACT
Cis - Dichlorodiamminoplatinum - II (Cis - DDP) has been widely as anticancer chemotherapertic agent. The genotoxicity of CIs - DDP was investigated in *in vivo* mice system using micronucleus test in bone marrow erythrocytes of mice. Various doses of cisptatin *i.e.,* 2.5, 5 and 10 mg/kg were selected based on thearapertic concentration. The animals were scarified 6h after the last treatment and smears were stained with Giemsa May Grunwald stain. The prepared slides were screened for the presence of micronuclei in polychromatic erythrocytes of mice. A significant increase in the frequency of micronuclei in poly chromatic erythrocytes was noted when compared with control values.

INTRODUCTION

Cisplatin is one of the most widely used chemotherapeutic drugs. The antitumor properties of this drug were reported. It is effective against a wide variety of animal tumors and human cancers (Calabresi and Parks, 1985) like ovarian, head, neck, bladder, cervical, testicular teratoma, non-small-cell lung carcinoma and lung cancers, both as a single agent and in combination with other agents (Muggia, 1984; Mugdy *et al.,* 2003). CP is generally considered to exerts its cytotoxic effect by binding its highly reactive hydrated platinum complex to DNA (Erickson *et al.,*1981) resulting in mutation induction (Fichtinger *et al*., 1984). Along with its therapeutic activity of this drug it is known for its toxic effects like nephrotoxicity, neurotoxicity, ototoxicity, immunotoxicity, embryotoxicity, cytotoxicity, hepatotoxicity, mutagenecity, myelotoxicity, teratogenecity and severe nausea and vomitings (Lazar *et al*., 1978; Von Hoff *et al*., 1979; Sorsa *et al*., 1985; Anthony *et al*., 1988; Fillastre and Raguenez Viotte,1989; Barbara *et al*., 1996; Vijayalaxmi and D'Souza, 2004; Nersesyan and Muradyan, 2004; Thomas *et al*., 2004; Yingjun *et al*., 2008) in different test systems. It is also a potential human carcinogen as it develops secondary malignancies in patients who have been treated with CP.

In the present investigation the incidence of micronuclei in bone marrow erythrocytes of mice by cisplantin was carried out.

MATERIALS AND METHODS

Swiss albino mice weighing about 22-24 g aged 8-10 weeks old were utilized in the present study. The drug was supplied by Reddy Labs Hyderabad. For each dose group of five animals were used. The animals were fed with 2.5, 5 and 10 mg/kg adriamycin intraperitonally in two installments within 24 hr interval. The control group of mice received 0.5 physiological saline simultaneously. The animals were scarified 6 hr after the last administration, bone marrow preparations were made by an air drying technique and stained with May Grunwald and Giemsa stains according to the method described by smith (1975). For each animal 2000 polychromatic erythrocytes (RBC) and corresponding normochromatic RBC were scored for the presence of micronuclei the appearance of micronuclei in polychromatic erythrocytes was used as an indicator of genetic damage. The ratio of polychromatic to normochromatic RBC was utilized to estimate the effect on the proliferative activity of bone marrow. The data obtained from these studies were analyzed using t-test adopted form Gold stein (1965).

RESULTS

The results on the induction of micronuclei in bone marrow erythrocytes of mice were depicted in Table 1. In cisplatin treated animals there was an increase in the polychromatic cells with micronuclei (Table 1). The frequency in control

Table 1: Results on the frequencies of micronuclei in the bone marrow erythrocytes of mice treated with various doses of cisplatin

Treatment Cisplatin	Micronuclei polychromatic cells (P)	Micronuclei in normochromatic cells (N)	Micronuclei in total (P+N) cells	P/N ratio
Control	45/16000 (0.28)	15/16022 (0.09)	60/32022 (0.18)	0.99
2.5 mg/kg	99/16000 (0.62)*	34/16840 (0.20)	133/32840 (0.40)	0.95
5 mg/kg	130/16000 (0.81)*	45/17200 (0.26)	175/33200 (0.52)	0.93
10 mg/kg	198/16000 (1.24)*	70/20250 (0.35)	268/36250 (0.73)	0.79

*$p<0.05$

animal was 0.25% and the values were 0.60%, 0.85% and 1.20% after the treatment of 2.5, 5 and 10 mg/kg cisplatin respectively (Table 1). The percentage of normochromatic cells with micronuclei was 0.28% in control mice, while the frequencies were 0.62%, 0.81%, 1.24% at 2.5, 5 and 10 mg/kg cisplatin respectively (Table 1). The P/N ratio was 0.99 in control mice and it has decreased at all dose levels. The differences in the frequencies of micronuclei in polychromatic cells were significant between the control and cisplatin treated groups ($p<0.01$). Thus, the data showed dose response relationship in the frequency of micronuclei in polychromatic erythrocytes.

DISCUSSION

The *invivo* micronucleus test is one of best methods to screen the clastogenic effects of chemicals and drugs (Chaubey *et al.,* 1978) using this procedure the mutagenicity of various alkylating agents (Maier and Schmid, 1976; Rudrama Devi and Reddy, 1985, 1986, 1987) drugs (Rudrama Devi and Reddy, 1995) was also established.

The results clearly indicate that there was gradual increase in the frequency of various types of chromosomal aberrations with increase in dose and time intervals. Similar reports are also available on the toxic effects of platinum compounds. The chemotherapeutic agent cisplatin has been shown to be highly mutagenic, teratogenic, and carcinogenic (Morrison *et al*., 1981; Barbara 1996; Giri *et al*., 1998) in both *in vitro* and *in vivo* experimental models. Early activation of oncogenes was observed in human headneck tumors after treatment with the Cisplatin (Németh *et al*., 2002).

The cytotoxic and genotoxic effects of cisplatin was proved by using comet assay as an experimental protocol by chandrasekar *et al*. (2006); Misra and Choudhury (2006); Premkumar *et al*. (2006), Sabry *et al*. (2008) and micronucleus Nersesyan and Muradyan, (2004) in different experimental models.

The frequencies of chromosomal aberrations increased non-linearly after intraperitonial administration of cisplatin with an average of 2.7 aberrations per aberrant cell in bone marrow of mice (Giri *et al*.,1998) independent cytotoxic agent (Würschmidt *et al*., 2000) Apoptosis of normal cells of the small intestinal epithelium and the bone marrow was also observed by Tamaki *et al.,* (2003) after the administration of cisplatin in mice. The same was observed in the Hypopharyngeal Carcinoma Cell Lines by Byung *et al*. (2005).

The present results are comparable with that of Choudhury *et al.,* (2000) who reported the effect of Vinblastine (VBL); a vinaca alkaloid is a chemotherapeutic agent in male mice for cytotoxicity. The results showed decreased mitotic activity of cells and significant decrease in the percentage of micronucleus in somatic cells of mice. Decarbazine (DTIC) a chemotherapeutic agent has been successfully applied to treat various types of cancer such as Hodgkin's disease, malignant melanomas, soft tissue sarcomas and advanced neuroblastomas. In the bone marrow micronucleus assay DTIC induced micronuclei at range of 0-125 mg/kg. Present study proved the highly genotoxicity of CP in bone marrow cells of mice. Further observations are necessary to find out the mechanism by which cisplatin induce genetic damage.

REFERENCES

Anthony, Gringeri, Peter, C. Keng, and Richard, F. Horch. 1988. Diethyldithiocarbamate Inhibition of Murine Bone Marrow Toxicity Caused by cis-Diamminedichloroplatinum (II) or Diammine – (I,I-yclobutanedicarboxylato) platinum (II) I. *Cancer Research*. **48**: 5708-5712 .

Barbara, J. S., Sanderson, R. Ferguson Lynnette and A. Denny William. 1996. Mutagenic and carcinogenic properties of platinum-based anticancer-70, drugs. *Mutagenicity of Anticancer Drugs*. **355(1-2):** 59

Byung-Joo Lee[a], Kyong-Myong Chon[a], You-Sun Kim[b], Won G. An[c], Hwan-Jung Roh, Eui-Kyung Goh[a]. and Soo-Gen Wang[a]: 2005. Cisplatin, 5-Fluorouracil, and Radiation on Cell Cycle Regulation and Apoptosis in the Hypopharyngeal Carcinoma Cell Line *hemotherapy*. 103-110

Calabresi, P. and Parks, R. E. 1985. Antiproliferative agents and drugs used for Immunosuppression. In: *Goodman and Gilman's the Pharmacological basis of Ttherapeutics* **7th** Ed., pp. 1247-1307, Macmillan, new York .

Choudhury, R. C., Jagdale, M. B. and Misra, S. 2000. Effects of cisplatin in the male germ line cells of Swiss mice.*J. Chemother.* **12**: 352-359.

Chandrasekar, M. J. N., Bommu Praveen, M.I.J. Nanjan and Suresh, B. 2006. protective effect of Phyllanthus maderaspatensis in modulating cisplatin-induced nephrotoxicit and genotoxicity. *Pharmaceutical Biology*. **44**: 100-106

Chaubey, R. C., Kavi, B. R., Chauhan, P. S. and Sundaram, K. 1978. Micronucleus Test. Evaluation of chemicals and other environmental agents for in vivo chromosomal damage. In: *Proceedings of International Symposium on Environmental agents and their biological effects Int*. **3**:415-418.

Erickson, L. C., Zwelling, L. A., Ducore, J. M., Sharkey, N. A. and Kohn, K. W. 1981. Differential cytotoxicity and DNA cross linking in normal and transformed human fibroblasts treated with Cis-dichlorodiammine platinum (II). *Cancer Res.* **41**: 791-2784.

Fichtinger-Schempman, A. M., Van der Veer, J. L., Lohman, P. H. M. and Reddijk, J. 1984. A simple method for the inactivation of monofuctionally DNA – bound Cis-dichlorodiammine platinum (II). *J. Inorg. Biochem*. **21:** 103-112

Fillastre, J. P. and Raguenez-Viotte, G. 1989 Cisplatin nephrotoxicity *Toxicol Lett.* **46(1&3):** 163-75.

Giri, A., Khunriam, D. and Prasad, S. B. 1998. Cell mediated protection on cisplatin induced mutagenicity in mice. *Mutat Res.,*3. **421(2):** 139-48.

Lazar, P., Conran, C. and Damjanov, I. 1978. Embryotoxicity and teratogenicity of cisiamminedichloroplatinum (II). *Experientia***. 35**:

647-648.

Maier, P. and Schmid, W. 1976. Ten model mutagens evaluated by micronucleus test. *Mut. Res.* **40(4):** 325-327.

Morrison, W. D., Huff, V., Colyer, S. P., DuFrain, R. J. and Littlefiedl, L. G. 1981. Cytogenetic effects of cis platinum (II) diammineichloride in vivo. *Environ Mutagen.* **3(3):** 267-274.

Muggia, F. 1984. Introduction: cisplatin update. *Semin. Oncol.* **18:** 1-4.

Mugdy, S. Aly, Mohamad, B. Ashour, Soheir, M. El Nahas and Mona A. F. Abo Zeid, 2003. Genotoxicity and Cytotoxicity of the Anticancer Drugs Gemcitabine and Cisplatin, separately and in combination: *in vivo* studies. *J. Biological Sciences.* **3(11):** 961-972.

Misra, S. and Choudhury, R. C. 2006. Vitamin C modulation of cisplatin-induced cytogenotoxicity in bone marrow, spermatogonia and its transmission in the male germline of Swiss mice. *J. Chemother.* **18**(2): 182-7

Németh, A., Gyöngyi, Z., Nádasi, E., Ember, A., Olasz, L., Nyárády, Z., Skapinyeca, J. and Ember, I. 2002. Effect fo cisplatin treatment on early activation of oncogenes *in vivio. In Vivo.* **16(5):** 307-10

Nersesyan, A. and Muradyan, R. 2004. Sea-buckthorn juice protects mice against genotoxic action of cisplatin. *Exp. Oncol.* **26(2):** 153-5

Prem kumar, K., Thirunavukkarasu, C., Abraham, S. K., Santhiya, S. T. and Ramesh, A. 2006. Protective effect of saffron (*Crocus sativus L.)* aqueous extract against genetic damage induced by anti- tumor agents in mice. *Hum. Exp. Toxicol.* **25(2):** 79-84

Rudrama Devi, K. and Reddy, P. P. 1985. Bisazir induced cytogenetic damage in mice. *IRCS Med. Sci. and Bio.* **12**: 125-1246.

Rudrama Devi, K. and Reddy, P.P. 1986. Cytogetic effects of metepa in swiss albino mice. *Cell and chromosome Res.* **9(2):** 39-41.

Rudrama Devi, K. and Reddy, P.P. 1987. Evaluation of metepa induced cytogenetic damage in mice. *Agri. and Biological Res.* **4:** P 6-9.

Rudrama Devi and Reddy G. M. 1995. Mutagenicty of antihelmintic drugs 4 effect of tetramizole on somatic cells of swiss albino male mice. *Cell and Chromosome Research.* **18**: 91-94

Schmid, W. 1975. The micronucleus Test. *Mut. Res.* **31**: 9-12.

Sabry, M. Attia, Gouda, K. Helal, Mohamed, F. Abd-Ellah, Ahmed, M. Mansour and El-sayed M. El-sayed 2008. The effects of oral grape seed extract on cisplatin - induced cytogenotoxicity in mice. *Saudi Pharmaceutical J.* **16**: 2.

Sorsa, M., Hemminki, K. and Vainio, H. 1985. Occupationally exposure to anti cancwer drugs-Potential and real hazards. *Mutation Res.***154:**35-149.

Thomas Ludwig, Christoph Riethmu Ller, Michael gekle, Gerald Schwerdt, and Hans Oberlieithner. 2004. Nephrotoxicity of platinum complexes is related to basolateral organic cation transport *Kidney International.* **66C:** 196-202.

Tamaki, T., Naomoto, Y., Kimura, S., Kawashima, R., Shirakawa, Y., Shigermitsu, K., Yamatsuji, T., Haisa, M., Gunduz, M. and Tanaka, N. 2003. Apoptosis in normal tissues induced by anti-cancer drugs. *J. Int. Med. Res.* **31(1):** L: 6-16

Vijayalaxmi, K. K. and D'Souza, M. P. 2004. Studies on the Genotoxic Effects of Anticancer Drug Carboplatin in invivo Mouse. *Int. J. Hum Genet.* **4(4):** 249-255**.**

Von Hoff, D. D., Schilsky, R., Reichert, C. M., Reddick, R. L., Rozencweig, M., Young, R. C. and Muggia, F. M. 1979. Toxic effects of cisdichlordiammineplatinum-II in man. *Cancer treat. Rep.* **63:** 1527-1531.

Würschmidt, F., Bardenhjeuer, M. J., Müller, W. U. and Molls, M. 2000. Chromosomal aberrations induced in mice bonemarrow by treating with cisplatin and irradiation. *Strahlenther Onko.* 1**76(7):** 319-23.

Yingjun Liao, L. U., Xiuqiang, L. U., Chunwei, L. I., Gexin, Yapping Jin and Hao Tang: 2008 Selection of agents for prevention of cisplatin - induced hepatotoxicity. *Pharmacological Research.* **57(2):** 125-131.

NATIONAL ENVIRONMENTALISTS ASSOCIATION

AND ITS OFFICIAL ORGAN

The Bioscan

An International Quarterly Journal of Life Science

Started in 1988, the National Environmentalists Association has been reorganized in 2006 and now is an association functioning with full vigour and new impetus to meet its objectives with the co-operation of like minded environment conscious academicians from different parts of the nation.

MEMBERSHIP OF THE ASSOCIATION

Any graduate having interest in environmental conservation and protection of nature and natural resources can be the member of the association.

To be the member of the association the application form given below should be duly filled up and sent to the Secretary of the association along with a demand draft of Rs. 750/- (After the 25% concession) for annual membership and Rs. 7500/- (After the 25% concession) for life membership.

FELLOWSHIP OF THE ASSOCIATION

The Association is awarding FELLOWSHIP to deserving academicians / researchers /scientists who are LIFE MEMBERS of the Association after reviewing their bio-data by the Fellows and the Executive Members of the association. The Fellows are privileged to write **F.N.E.A.** after their names .The prestigious Fellowship also includes a citation in recognition of their contribution to society in general and the endeavour for the noble cause of environment in particular.

AWARDS OF THE ASSOCIATION

The Association in its Seminars and Conferences provides the following category of awards on annual basis.

1. **The young scientists award** : It is given to the researchers below the age of 35 years.
2. **The senior scientists award** : It is awarded to the academicians above the age of 35 years.
3. **The best paper award**: It is awarded to the contributor of the Journal **The Bioscan** during the year.
4. **The best paper presentation award** : It is awarded to the scholar whose presentation is the best other than the young scientist category.
5. **The best oration award** : It is awarded to the scholar who delivered invited speech.
6. **The recognition award** : It is awarded to those senior scholars who have contributed to the subject through their continued research .
7. **The environmental awareness award** : It is awarded to those who, apart from their research contribution, have done commendable extension work for environmental betterment.

The number of recipients of award in each category will vary depending upon the recommendation of the panel of judges and the executive committee. The association has the provision to institute awards in the name of persons for whom a with desired sum is donated in consultation with the executive body.

PUBLICATION OF THE ASSOCIATION

In order to provide a platform to a vast group of researchers to express their views and finding of research as well as to promote the attitude of quality research among the scholars of younger generation the association publishes an international quarterly journal – **THE BIOSCAN (ISSN:0973-7049).** For the benefit of the potential contributors **instructions to authors** is given separately in this journal. However, the details regarding the journal and also the association can be seen on our website *www.thebioscan.in.*

Cont. ..... P. 578