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ANALYSIS OF SPERMATIDS MICRONUCLEI UNDER THE INFLUENCE OF ACRYLAMIDE

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ABSTRACT
The micronucleus test is an effective method for determination of the evolution of genotoxic or clastogenic agents of physical and chemical functions. The micronuclei are formed due to the condensation lagging off of acentric chromosomes, chromatid fragments or entire chromosomes and also due to non introduction of them in main daughter nuclei during metaphase or anaphase of cell division. Formation of micronuclei reflects chromosome damage and thus provide a marker for carcinogenesis analysis. Acrylamide is an important industrial chemical used in waste water treatment, adhesives and grout, cosmetics and also in laboratories. It is genotoxic, in *in vitro* and *in vivo*, in both somatic and germ cells. Acrylamide induces chromosomal aberrations, micronuclei formation, sister chromatid exchanges, SS-DNA breaks, polyploidy, aneuploidy and other mitotic disturbances, dominant lethal and specific locus mutations. Therefore a study was made on micronuclei assay to evaluate the degree of genotoxicity of acrylamide and its toxicologic effects on rat's reproductive system after intraperitoneal injection of different doses of acrylamide. The Acrylamide treatment to rats caused damage not only to peripheral blood cells and reticulocytes but also to spermatids. The formation of micronuclei was evidence in our present study though acrylamide may not be used directly. The indirect consumption of acrylamide definitely causes damage to almost all type of cells in the rats.

INTRODUCTION

The micronucleus test (MNT) is an effective method for the evaluation of genotoxicity or clastogenicity of various physical and chemical agents. Since the micronuclei (MN) are formed during the cell division due to lagging of acentric chromosomes, chromatid fragments or entire chromosomes (Uma Devi and Sharma, 1991; Jagetia,1994). Acentric chromosome fragments or whole chromosomes that are not included in the main daughter nuclei during metaphase or anaphase of cell division (Lindberg *et al.,* 2007) can produce micronuclei. The most frequently used genotoxicity test in mammals is the micronucleus test, which provides a simple and rapid indirect measure of structural and numerical chromosomal aberrations (Heddle *et al.,* 1991) and is scientifically accepted by supranational authorities such as Organization for Economic Co-Operation and Development [OECD], International conference on harmonization [ICH] and European Union [EU]. Although MNT has several advantages over other genotoxicity tests, it can be performed only in dividing cells. In constrast to most *in vitro* genotoxicity assays, the MNT in rats provides a higher systematic complexity, eliminate promutagens and mutagens.

Testing chemicals has ability to induce numerical or structural damage to chromosomes and are easily accomplished by micronucelus assay. A micronucleus is literally a small nucleus. The cell organelle contains genetic material of fragmented DNA. During cell division the genetic material replicates and then divides easily between the two daughter cells that are produced. If this process is disrupted, the chromosomes are broken or damaged by chemicals or radiation, then the distribution of genetic material between the two daughter nuclei during cell division may be affected and formed new nuclei may be micronucelus which is clearly observed under microscope. The production of micronuclei is specific to a particular cancer observation. This observation specifies a specific tumor and its determination using micronuclei is more useful tool for the identification of markers in the detection of cancers related to various tissues and stages.

Acrylamide (AA), a chemical used in industries for various purposes, is produced on heating of carbohydrate rich foods at 175ºC and also available relevant amounts of AA in French fries, baked potatoes and coffee (Zyzak *et al.,* 2003; Andrzejewski *et al.,* 2004). Epidemiological studies have been carried out to examine the risk of cancer as a result of consumption of food with high concentration of AA (Mucci *et al.,* 2006).

Acrylamide is a genotoxic molecule in *in vitro* and *in vivo* for both somatic and germ cells. It induces chromosomal aberrations, micronuclei formation, sister chromatid exchanges, unscheduled DNA synthesis, S-S DNA breaks, polyploidy, aneuploid and causes some other mitotic disturbances and also lethal to various organisms (WHO, 2002; Dearfield *et al.,* 1995). In bacterial mutations AA is exhibited negative results without metabolic activity, whereas glycidamide (GA), the epoxide metabolite of AA, induces

positive response in Salmonella GA 100 and GA 1535 (Hashimoto and Tanii, 1985). In bacterial systems the product of acrylamide, glycidamide, functions as mutant (Hashimoto and Tanii, 1985).

Chronic administration of AA was identified as a multisite carcinogen in mice, but understanding mechanisms of action are not fully understood so far (Bull *et al.,* 1984a,b; Friedman *et al.,* 1995). The genotoxicity and carcinogenicity of AA is attributed to the three mechanisms a) Radical mediated polymerisation b) Michael reactivity *i.e.,* the addition of carban ions to α–β–unsaturated chemicals and c) oxidation to GA by cytochrome P450 enzymes (Dearfield *et al*., 1995).

Ghanayem *et al*., (2005b) have investigated the role of cytochrome P450 in the metabolism of AA using mice differing in CYP2EI expression (wild type CYP2EI +/- and CYP2EI-/-m knockout mice). Additionally Ghanayem *et al.* (2005a) also confirmed that the AA to GA transformation leads to the formation of GA-DNA adducts in liver, lung and testes. In a recent *in vivo* study, pre-treatment with 1-aminobenzotriazole, a non specific P450 inhibitor abrogated or reduced the dominant lethal effect of AA in mice suggesting that GA is the cause of germ cell mutation in mouse spermatids (Alder *et al.,* 2000; Sumner *et al.,* 1999).

Therefore to analyse the spermatid analysis in testis of rats experiments were conducted using acrylamide as treatment and the results are reported as mentioned below.

MATERIALS AND METHODS

The male wistar rats weighing about 150-200g and 3 months old were purchased from Sri Venkateswara Enterprises, Bangalore.

These rats were acclimatized for seven days after arrival from the supplier. Control and treatment groups consisted of six animals each. Temperature was maintained at 71 ± 3ºF with relative humidity of 30-70% on 12:12hr (5am-5pm) light: dark cycle. Animals were housed individually in polycarbonate cages and provided food (Purina certified Rodent Chow 5002 and tap water ad libitum). These animals were treated with AA of conc. of 16, 32, 48, 64, 80 and 94 mg, respectively, for each group. Sample of blood was collected after 24 hr, 48 hr, 72hr of treatment for micronuclei assay. Approximately 120μL of blood was collected from the tail vein of each animal. Tube contains 350 μL of heparin. 10 μL of blood was collected spread on slides immediately after sampling (Wolf and Luepke, 1997).

Testes and epididymis of all test and control rats were isolated for further experimentation at the end of 24 hr of each dose of AA treatment after sacrificing the animals using decapitation. The normal and induced tissues were stored for further analysis.

Micronuclues Assay of Spermatids

Seminiferous tubules were removed from testes through tunica albuginea the collected cell suspension of it was filtered through a 70 mesh nylon filter and cell suspension was collected into 15mL centrifuge tube and centrifuged at 500rpm for 5mins. The pellet was collected and washed twice in testis isolation medium.

Three spaced drops were placed on precleaned microscope slide in horizontal position. To sediment the cells three drops of Helly's fixative was placed in order to touch the suspension. After 10 mins the slides were transferred to fresh fixative for 1hr and washed with 70% Ethanol. Later the slides were immersed in 1% acid solution for 10 mins and stained in Schiffs solution for 10 mins. Then the slides were rinsed with tap water for 5 mins and later with distilled water. After two washes with xylene, the cell suspension was embedded in Depex mounting medium. The stained slides were observed under Olympus BX60 microscope under bright field illumination of 1000x for spermatids with/without micronuclei. The spermatids micronuclei were calculated for control and treated rat samples of 3000 spermatids for each group.

RESULTS

About 3000 erythrocytes and speramatids were scored separately for the presence of micronuclei for each dose of acrylamide and time interval. The highest frequency of micronucleated erythrocytes was observed in the positive control group treated with 8mg of cyclophosphamide. In negative control or vehicle control group rats [*i.e.,* 6 rats treated with saline alone] the erythrocytes did not show any formation of micronuclei (Data presented elsewhere). The testis of treated rats with AA showed the highest frequency (13.5%) of micronucleated spermatids (MN-S) in cyclophosphamide per 100g of body weight, the positive control, treated group spermatids.

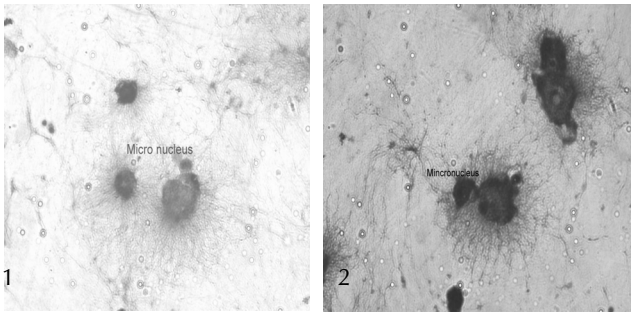

Figure 1 and 2: Acrylamide induced micronucleus in rat spermatids X 1000

The micronuclei formation in testicular spermatids was time dependent and dose dependent (Table 1; Figs. 1 and 2). The formation of altered spermatids was low in 24 hr, medium in 48hr and high in 72hr of exposure to acrylamide (Fig. 3). In time dependent system of 16mg to 96mg the formation of micronuclei was found to be in increasing order. The fold increase of micronuclei was observed within the group of 8mg and also among the increased groups of all types of doses. The fold increase of formation was found in all time and dose dependent treatment of acrylamide and the increase of micronuclei was in linear ascending order (Fig. 4).

DISCUSSION

Micronuclei test (MNT) has been a most suitable genotoxicity test along with peripheral blood micronucleus sperm count test and sperm morphology tests. These observations would ensure a 100 per cent accurate genotoxicity testing (Muller

Table 1: Effect of Acrylamide on micronucleus formation in rat spermatid cells

Treatment	Dose mg/ 100g of the body weight	Sampling time (hr)	No.of spermatids containing MN	% of MNS Mean ± SE	Fold increase in micronuclei formation Group (hr) With in Conc.	24	48	72
Negative control	Saline	24	0	0				
		48	0	0				
		72	0	0	0	0	0	0
Positive control	8mg	24	70	2.33 ± 0.40	_			
		48	254	8.46 ± 1.35	3.64			
		72	405	13.5 ± 0.404	5.8			
Acrylamide	16 mg	24	44	1.46 ± 0.176	_	_	_	_
		48	64	2.13 ± 0.185	1.5			
		72	86	2.86 ± 0.348	1.96			
Acrylamide	32 mg	24	62	2.06 ± 0.176	_	_	_	_
		48	81	2.70 ± 0.435	1.31	1.41	-	-
		72	113	3.76 ± 0.145	1.83	_	1.26	_
Acrylamide	48 mg	24	75	2.50 ± 0.458	_	_	_	1.31
		48	118	3.93 ± 0.352	1.57	1.71	_	_
		72	164	5.46 ± 0.317	2.18	_	1.84	_
Acrylamide	64 mg	24	97	3.23 ± 0.338	_	_	_	1.9
		48	154	5.13 ± 0.463	1.58	2.21	_	_
		72	195	6.50 ± 0.624	2.01	_	2.4	_
Acrylamide	80 mg	24	138	4.60 ± 0.416	_	_	_	2.27
		48	188	6.26 ± 0.233	1.46	3.15	_	_
		72	257	8.56 ± 0.721	1.86	_	2.93	_
Acrylamide	96 mg	24	170	5.66 ± 0.409	_	_	_	2.99
		48	225	7.50 ± 0.378	1.32	3.88	_	_
		72	328	10.93 ± 0.466	1.93	_	3.52	_
							_	3.82

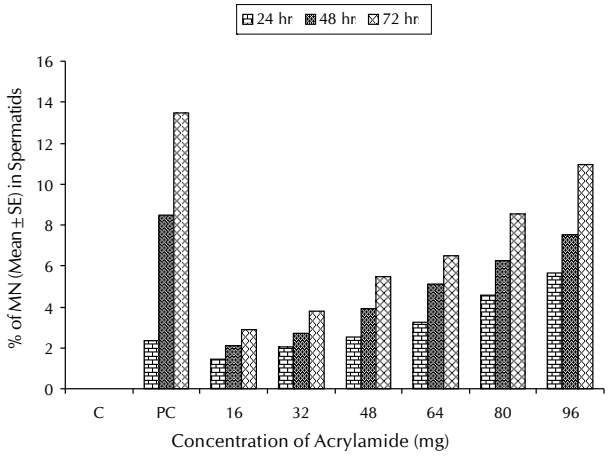

Figure 3: Effect of Acrylamide on micronucleus formation in rat spermatid cells

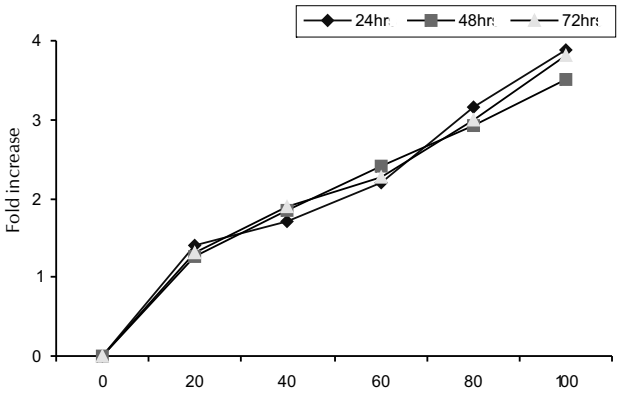

Figure 4: Concentration of Acrylamide vs fold increase in micronuclei formation

and Streffer, 1994) for Chromosomal aberrations (CA) that contribute to cancer development in humans and experimental animals upon exposure to various pollutants of environment. Recently, peripheral blood MNT has been reported as a useful technique to study the effect of Enviromental mutagens and promutagens in the chick embryo (Begum, 2009) on exposure to AA during its development.

In view of several advantages of the MNT, we have used this assay to measure the genetic damage in spermatids of rats exposed to different doses of Acrylamide. A test for micronuclei in spermatids collected from Sprague-Dawley rats yielded negative results at the concentrations up to 0.05 mg/mL (Lahdetie *et al.,* 1994). On the acrylamide treatment rats, the frequency of micronuclei formation in spermatids was steadily increased in rats with the increase in concentration and exposure time of the dose. A dose related increase in the incidence of MN is the criterion for a positive effect. So, based on this finding, AA can be considered to be inducer of micronucleus, which implies cytogenic damage to spermatids (Xiao and Tates, 1994).

Acrylamide upon oxidation is converted to glycinamide which inturn interact with DNA and forms as DNA adduct. This adduct formation blocks the binding of chromosome to the tubulin protein during cell division and then retards the chromosomes to form a micronuclei. The present result also supports the formation of MN in spermatids by acrylamide (Table 1). A statistically significant increase in the number of micronuclei was noted at 96mg/100g body weight of rat. Most striking

feature of the reproductive toxicity of AA was reduced sperm reserves in cauda epididymidis isolated from rats treated with Acrylamide (Data will be published elsewhere). Even the lowest dose of AA (16mg) reduces the number of sperms in left cauda epididymidis to half level. The sperm reserves further decreased in an acrylamide dose dependent manner. In the present study AA is found to induce the formation of abnormal sperms and decreases the sperm count in the rats (Wyrobek *et al.,* 1983). AA exposure in hamsters caused testicular atrophy with damage to spermatids and mature spermatozoa (Sakamoto *et al.,* 1988). Reduced sperm motility and impaired fertility have also been reported in treated mice and rats.

Most of the earlier reports performed to evaluate AA induced chromosomal alterations in mammalian system *in vivo* were employed *i.p*. injection of AA at concentration in the range of 25-200 mg/kg. All these results have suggested an increase in chromosomal aberrations in spermatocytes of mice (Alder, 1990), but the frequency of aneuploid sperm detected by fluorescence in situ hybridization (FISH) was not increased by single *i.p*. injections of 60 or 120 mg/kg. AA in male mice (Schmid *et al.,* 1999) consistent with AA induction of chromosomal aberrations in sperm, the frequency of zygotes with chromosomal aberrations in specific to MN was significant. These zygotes of MN may have altered growth and may cause lethality to embryo. In conclusion the micronuclei formation was observed more in 96mg/100g body weight rat spermatids. These spermatids may cause damage to embryo upon fertilization with ovum.

REFERENCES

Alder, I. D. 1990. Clastogenic effects of Acrylamide in different germ –cell stages of male mice, In: Biology of Mammalian Germ Cell Mutagenesis, Banbury Report Vol.34.Allen,J; Bridges, B; Lyon, M; Moses, Meds. Cold Spring Harbour laboratory Press: Cold Spring Harbor, NY. pp. 115-131.

Alder, I. D., Baugmgartner, A., Gonda, H. and Friedman, M. 2000. 1-Aminobenzotriazole inhibits Acrylamide –induced dominant lethal effects in spermatids of male mice. *Mutagenesis*. **15:**133- 136.

Andrzejewski, D., Roach, J. A., Gay, M. L. and Musser, S. M. 2004. Analysis of coffee for the presence of Acrylamide by LC-MS/MS. *J. Agric. Food Chem*. **52:** 1996-2002.

Begum, R. 2009. Micronuclei analysis: Stress of Acrylamide and Cadmium chloride on chick embryo. *J. Adv. In Life Science.*

Bull, R. J. Robinson, M., Laurie, R. D., Stoner, G. D., Greisiger, E., Meier, J. R. and Stober J. 1984a. Carcinogenic effects of Acrylamide in Sencar and A/J mice. *Cancer Res.* **44:** 107-111.

Bull, R. J., Robinson, M. and Stober, J. A. 1984b. Carcinogenic activity of Acrylamide in the skin and lung of Swiss-ICR mice. *Cancer. Lett.* **24:** 209-212.

Dearfield, K., Douglas, L., Geroge, R., Ehling, U.H., Moore, M. M. and Sega, G. A. 1995. Acrylamide a review of its genotoxicity and an assessment of heritable genetic risk. *Mutat. Res.* **330:** 71-99.

Friedman, L. H., Dulak, M. A. and Stedham, 1995. A life time oncogenicity study in rats with Acrylamide. *Fundam. Appl. Toxicol.* **27:** 95-105.

Ghanayem, B. I., Witt, K. L., El-Hadri, L., Hoffler, U., Kissling, G. E., Shelby, M. D. and Bishop, J. B. 2005a. Comparison of germ cell mutagenicity in male CYP2E1-null and wild – type mice treated with Acrylamide: evidence supporting a glycidamide-mediated effect. *Biol. Repord.* **72:** 157-163.

Ghanayem, B. I., Witt, K. L., Kissling, G. E., Tice, R. R. and Recio, L., 2005b. Absence of Acrylamide-induced genotoxicity in CYP2E1-null mice: evidence consistent with a glycidamide mediated effect. *Mutat. Res.* **578:** 284-297.

Hashimoto, K. and Tanii, H. 1985. Mutagenicity of acrylamide and its analogues in *Salmonella typhimurium*. *Mutat. Res.* **158:**129–13

Heddle, J. A., C immino, M.C., Hayashi, M., Romagana, F., Shelby, N.D., Tucker, J. D., Vanparys, P. H. and Mac Greger, J.T. 1991. Micronuceli as an index of ctyogentic damage: past, present and future-Environ. *Mol. Mutagen.* **18:** 277 -291.

Jagetia , 1994. Chronic toxicity and oncogenicity study on Acrylamide incorporated in the drinking water of fischer 344 rats. *Toxicol. Appl. Pharmacol.* **85:** 154-168.

Lahdetie, J., Suutari, A. and Sjoblom, T. 1994. The spermatid micronucleus test with the dissection technique detects the germ cell mutagenicity of Acrylamide in rat meiotic cells. *Res.* **309:** 255-262.

Lindberg, H. K., Wang, X. and Jarventaus, H. 2007. Origin of nuclear buds and micronuclei in normal and folate-deprived human lymphocytes. *Mutat. Res. Fund. Mol. M.* **617:** 33-45.

Mucci, L. A., Adami, H. O. and Wolk, A. 2006. Prospective study of dietary Acrylamide and risk of colorectal cancer among women. *Int. J. Cancer:* **118:**169-173.

Muller, W. U. and Streffer, C. 1994. Micronuleus assay, In; Obe G, Editor, advances in mutagenesis Research-5. New Yark;Springer

Sakamoto, J., Kurosaka, Y. and Hashimoto, K. 1988. Histoligical changes of Acrylamide-induced testicular lesions in mice. Exp Mol. *Pathology*. **48:**324-334.

Schmid, T. E., Wang, X. and Alder, I. D. 1999. Detection of aneuploidy by multicolor FISH in mouse sperm after in vivo treatment with Acrylamide, colchine, Diazepam, or thiabenzole. *Mutagenesis*. **14:** 173-179.

Sumner, S. C. J., Fennel, T. R., Moore, T. A., Chanas, B., Gonzalez, F. and Ghanayem, B. I. 1999. Role of cytochrome P4502E1 in the metabolism of Acrylamide and Acrylonitrile in mice. *Chem. Res. Toxicol:* **12:** 1110-1116.

Uma Devi, P. and Sharma, A. S. K. 1991. Mouse bone-marrow response to low doses of whole-body gamma irradiation: induction of micronuclei. *Int. J. Radiat Biol.* **57:** 97-101.

WHO, 2002. WHO to hold urgent expert consultation on acrylamide in food after findings of Swedish National Food Administration. *Cent. Eur. J. Public Health.* **10:** 162–173.

Wyrobek, A. J., Gordon, L. A., Burkhurt, J. G., Frances, M. W., Kappa, R. W. and Jr, LetzG. 1983. An evaluation of mouse sperm morphology test and other sperm tests in non-human mammals. *Mutat. Res.* **115:** 1-172.

Xiao, Y. and Tates, A. D. 1994. Increased frequencies of micronuclei in early spermatids of rats following exposure of young primary spermatocytes to acrylamide. *Mutat. Res.* **309:** 245–254.

Zyzak, D. V., Sanders, R. A., Stojanovie, M., Tallmadge, D. H., Eberhart, B. L., Ewald, D. K., Gruber, D. C., Morsch, T. R., Strothers, M. A., Rizzi, G. P. and Villagran, M. D. 2003. Acrylamide formation mechanism in heated foods. *J. Agric. Food Chem*. **51:** 4782-4787.