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ACRYLAMIDE INDUCED GENOTOXIC EFFECT IN GOLDEN HAMSTER (*Mesocricetus auratus)* GENOME

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ABSTRACT

This work aims at dislosing the capability of acrylamide in causing genotoxic effect in golden hamster genome. To achieve such a purpose, five different doses that ranged from 1 to 20 mg/ kg b. wt. were chosen and tested employing three genotoxic bioassays. These assays are : 1-Estimation of cell proliferation, 2-Analysis of chromosomal abnormalities; and 3-In vivo induction of sister chromatid exchanges.

The results obtained showed that all tested doses were found to be toxic and cellular toxicity was observed. Analysis o chromosomal aberrations revealed that acrylamide is a positive clastogenic agent. Sister chromatid exchange assay indicated that acrylamide is a powerful inducer of primary genetic damage.

INTRODUCTION

Acrylamide (AA) is genotoxic and has been classified as a probable human carcinogen. Human exposure to AA may be high by the consumption of starch-based food that has been treated at high temperature, e.g. potato chips and crisps. For risk assessment, extrapolation to the expected low doses to humans will be more reliable when data from low experimental doses can be used. There have been several studies upon the effects of a series of low doses in the sensitive flow cytometer-based micronucleus assay acrylamide in mice, paying special attention to deviations from the expected linear dose-response function (Zetterberg, 2003). Two experiments were performed with CBA mice, injected i.p. with different doses of AA. In one experiment the effects of 22 doses (two mice per dose) ranging from 0 to 100 mg/ kg b.w. were studied. In the second experiment seven doses (five mice per dose) ranging from 0 to 30 mg/ kg b.w. were used. In both experiments, a clear increase of the frequency of micronucleated erythrocytes was seen, already at the lowest doses used. The dose-response function was found to be linear with a tendency to have a steeper rise at the lowest doses. The low DNA content of the micronuclei indicated an absence of whole chromosomes, i.e. no aneugenic effect of AA. (Zetterberg, 2003).

The reactive industrial chemicals acrylamide (AA) and N-methylolacrylamide (MAA) are neurotoxic and carcinogenic in animals, MAA showing a lower potency that AA. The causative agent in AA-induced carcinogenesis is assumed to be the epoxy metabolite, *glycidamide* (*GA*), which in contrast to AA gives rise to stable adducts to DNA. The causative agent in MAA induced carcinogenesis is so far not studied. The two AAs were studied in *mice* and *rats* using analysis of hemoglobin (Hb) adducts as a measure of in vivo doses and the in vivo micronucleus (MN) assay as an end-point for chromosome damage. Male CBA mice were treated by intraperitoneal (i.p.) injection of three different doses and male sprague-

Dawley rats with one dose of each AA. Identical adducts were monitored from the two AAs [N- (2-carbamoylethyl) valine] and the respective epoxide metabolites [N-(2-carbamoy 1-2 hydroxyethyl) valine]. Per unit of administered amount, AA gives rise to hgiher (three to six times) Hb adduct levels than MAA in mice and rats. Mice exhibit, compared with rats, higher in vivo doses of the epoxy metabolites, indicating that AAs were more efficiently metabolized in the mice. In mouse the two AAs induced dose-dependent increases in both Hb adduct level and MN frequency in peripheral erythrocytes. Per unit of administered dose MAA showed only half the potency for inducing micronuclei compared with AA, although the MN frequency per unit of in vivo dose of measured epoxy metabolite was three times higher for MAA than for AA. No increase in MN frequency was observed in rat bone marrow erythrocytes, after treatment with either AA. This is compatible with a lower sensitivity of the rat than of the mouse to the carcinogenic action of these compounds (Paulsson *et al., 2002 & 2003*).

The present investigation aims at disclosing the capability of acrylamide (AA) in causing micro as well as macro genetic damage in golden hamster genome. So *Mesocricetus auratus,* 2n = 44 was employed in this study and five different doses of acrylamide were chosen and tested for the possible genotoxic effect. These doses are 1, 5, 10, 15, 20 mg/ kg b.wt. Two genotoxic bioassays were used in this study. They are :

1- Analysis of chromosomal abnormalities in bone-marrow cells.
2- *In vivo* induction of sister chromatid exchanges.

MATERIALS AND METHODS

Analysis of chromosome behavior :

Each animal had orally received daily the proper dose for 7 days. The animals were killed by decapitation 24 hr after the last dose. For each treatment, four animals were used. Animals of the control group (4 for each treatmen) received equivalent amounts of deionized water. Three hours prior to killing, the animals were injected with 0.6 mg/ kg of colchincine.

After killing, the adhering soft tissue and epiphyses of both tibiae were removed. The marraw was aspirated from the bone, transferred to phosphate buffered saline, centrifuged at 1000 rpm for 5 minutes and the pellet resuspended in 0.075 M KCl. Centrifugation was repeated and the pellet was resuspended in fixative (methanol : acetic acid, 3 : 1). The fixative was changed after 2 hours and the cell suspension was left overnight at 4 C.

Slide prepartion and staining

Cells in fixative were dropped on very clean glass slides and air-dried. Spreads were stained with 10% Giemsa at PH 6.8 for 5 min.

Screening of slides

Slides were coded and scored for chromosomal aberrations e.g., gaps and deletion, fragment, break, stickiness and polyploidy. A mitotic index based on at least 1000 counted cells was recorded. For chromosomal

abnormalities, at least 200 metaphase cells per dose were recorded. Comparison with control was also statistically tested when needed.

Sister chromatid exchange technique

Experimental design

Typically 2-3 month old hamester, 2n = 44 were used. Four animals per dose were used and analysis of at least 100 cells per animals was carried out. Five selected doses were administrated. A dose response curve was established and extended over at least a 10 fold dose range and contained five informative doses plus that of the control group.

Bomodeoxyuridine tablet preparation

Bromodeoxyuridine tablets were prepared as described Allen *et al.,* 1978; Allen, 1982; and Seehy *et al.,* 1983 as follow : Bromodeoxyuridine tablets were prepared by using pellet press (Parr instrument co. Moline, III., USA), equipped with a 0.178 in diameter punch and die. Approximately, 200 mg of pure Bromodeoxyuridine powder were weight, placed in the die, and pressed. In order to maintain consistent compaction hardness (and thus the dissolution rate) among tablets, utilization of the same personal and die adjustment when pressing the powder was conducted. BrdU tablets were protected from light and stored in a freezer until usage.

BrdU treatment

The animals were lightly anesthetized by placing it in a closed container with ether until immobile (about 2 min). After removal from the container, each animal was restrained on its back. A small vial of anesthesia was placed near its nose for use in prolonging the inactive state. The lower lateral region was swabbed with alcohol in order to mat the fur down. Clean scissor or a scalpel was used to make a small (approx. 1 cm) subcutaneous incision. In order to spread open a deeper subcutaneous pocket, forceps were used, and the tablet was inserted. The wound was then closed with 2-3 outclip sutures taking full care not to break the tablet and the animal was returned to its cage. The animals received the proper doses of Acrylamide 8 hr after BrdU treatment. Each animal was injected intravenously with 20 □g colcemid (0.1 ml/ animal, in tail vain) at hr 19- (following BrdU treatment). Control marraw cells harvested 2 hr later revealed a high fraction of metaphases of optimal sister chromatid differentiation after staining.

Marrow cells harvest and slide preparation

The animal was killed by cervical dislocation. Both femurs were immediately removed, and cleaned of extraneous tissues. Bone tips were cut away so that a small syringe needle (i.e. 26 gauge) can be inserted and femoral contents were flushed with phosphate buffered saline (8 g NaCl, 0.2 g KCl, 2.17 g Na_2HPO_4 + H_2O_3 0.2 g KH_2PO_4, are dissolved in 1 L and pH is adjusted to 7.0) into a small common tube (total cell solution volume of about 8 mL). Cell suspension was centrifuged at 1000 rpm for 5 min. the supernatant was discarded and cell pellet disrupted by flicking the base of the tube. A hypotonic solution of potassium cloride (0.075 M) was added to give a light cloudy solution (about 8 mL), and let stand for 12 min. the cell suspension was centrifuged, and the supernatant was discarded, cell pellets

were fixed in a fixative solution (3 parts methanol : 1 part glacial acetic acid) for 10 min. then centrifuged and the supernatant was discarded. Fixation was repeated for 10 min, followed by centrifugation and the supernatant was discarded. Final fixation was perfomed in 4-5 mL fresh fixative. The slides were prepared as follow: 3 drops of freshly fixed cells were added to clean dry side, dropping the cells from about 1-2 ft distances. Cell density was checked through the microscope and more drops were added if needed. The slides were then stored protected from light.

Slide staining .Staning was performed by the method of Goto *et al.* (1978). The slides were stained with 50 □g / mL of Hoechest 33258 dye in distilled water, pH 7.0 for 10 min (protected from light). The slides were rinsed in water, and covered by a layer of Mc Ilvaines buffer [add 18 mL of solution A (1.92% citric acid) to 82 mL of solution B (2.% disodium phosphate) and adjust the pH of 7.0 or 7.5 with further mixing], mounted by cover slip and subjected to light with intesity <= 400 nm, at a distance of about 2 inches for 20 min. during this time, slides were placed on a wormer tray at 50 C. the slides were then rinsed in distilled water and immersed in 4% Giemsa dye, rinsed in water and allowed to dry for subsequent light microscope analysis.

Screening of sildes and analysis

Scanning slides for mitotic spreads was conveniently accomplished with a 25 x magnification objective, and analysis was with a 100 x objective. For control of bias, all prepared slides were coded prior to scoring. There are two ways for counting sister chromatid exchange frequencies i.e., (1) from the microscope images of second division cells, (2) the cells may be photographed and SCE frequencies counted from the microscope images. An interstitial exchanged segment was counted to be 2 SCEs.

Usually, wide ranges of SCE values were encountered specially in treated cells, and then the analysis of variance using F-test was applied. To evaluate the differences in mean of SCE frequencies between treated and control groups, Duncan's multiple range test was used (Snedecor, 1958).

RESULTS

Cell proliferation :

The effect of acrylamide on cell proliferation was investigated by estimating mitotic activity (or Mitotic index; MI). Table (1) shows that mitotix index in the control group was found to be 24.22%. It decreased to be 18.04; 12.12; 8.32; 4.18; and 2.22% for the tested doses 1, 5, 10, 15 and 20 mg/ kg b.wt., respectively. This result, however, reflects the cellular toxicity caused by the tested chemical compound (Figure, 1).

Table (1) Mitotic activity in bone-marrow cells after treatment with Acrylamide (AA).

Dose; mg/ kg b.wt.	MI ± S.E.
Control	24.22 ± 2.40
1	18.04 ± 2.10
5	12.12 ± 1.80
10	8.32 ± 1.20
15	4.18 ± 0.40
20	2.22 ± 0.34

*** Estimated as mitotic index.**

Fig1

Chromosomal abnormalities :

Cytological observations obtained after treatment of golden hamster with the tested compound acrylamide are tabulated in Table (2).

Table (2) * Chromosomal abnormalities in bone-marrow o golden hamester after treatment with acrylamide (AA).

**Dose; mg/kg b.wt	Fragment	Stickiness	Gap	Robertsonian centric Fusion	Ring Chromosome	Polyploidy	Total aberrant metaphases
Control	-	2	-	-	-	-	2
1	4	4.5	2	1	-	1	12.5
5	6.5	8.5	6.5	1	2.5	1	26
10	8.5	10	6.5	4	6	4	39
15	12	12.5	8.5	6	8.5	4	45.5
20	16	14	10	6	10.5	10.5	53

*** : Percent of Metaphases .**
**** : 200 Metaphase were examined for each dose.**

Different types o aberrations were observed. These aberrations are : fragments, gaps, stickiness; Robertsonian Centric Fusion (RCF), Ring chromosome and polyploidy. In the negative control only 2% of metaphases showed stickiness, and other types of aberrations were not obtained – After treatment fragment ranged from 4-16%; stickiness from 4.5 – 14%; gaps from 2-10% RCF from 1-6%; ring chromosome from zero to 10.5%; and polyploidt ranged from 1-6.5%. Total aberrant metaphase ranged from 2% for the control group to 53% after treatment with 20 mg/ kg b.wt., respectively (Figure, 2). These result, however indicate that acrylamide, at the level of this

study, is a positive clastogen . Induction of polyploidy indicates that acrylamide was proven to be capable of interferring with spindle fibers.

***In vivo* induction of sister chromatid exchanges :**

Averages of sister chromatid exchange frequencies induced after acrylamide treatment are given in Table (3) and illustrated in figure (3). Sister chromatid exchanges in the negative control were found to be 4.2 per cell. It increased to be 7.7 ; 9.8; 16.6; 21.3; and 28.2 for the tested doses 1, 5, 10, 15; and 20 mg/ kg b. wt., respectively.

Statistical analysis revealed that all tested doses were found to be different significantly from the negative control.

Duncan's multiple range test (Table, 4) for mean differences showed that each dose was proven to be different significantly from the other doses, except the mean difference between 1 and 5 mg/ kg b. wt. This result, however, gave an evidence for a positive linear-response relationship between the tested doses and frequency of SCEs. Figures (1-6) illustrat the effect of acrylamide upon the chromosomes of golden hamester

Table (3) Averages of sister chromatid exchanges in bone marrow after treatment with Acrylamide (AA)

Dose; mg/ kg b.wt.	X ± S.E.	Range
Control	4.2 ± 0.82	1 – 6
1	* 7.7 ± 0.9	2 – 10
5	* 9.8 ± 1.1	3 – 14
10	* 16.6 ± 1.3	3 – 21
15	* 21.3 ±2.1	5 – 26
20	** 28.2 ± 3.2	5 – 34

*** : Significant at 0.05 level of probability.**

Table (4) Duncan's multiple range test for mean differences of SCEs after treatment with Acrylamide.

Dose; mg/ kg b.wt.	X	X-Xc	X-X1	X-X5	X-X10	X-X15
20	28.2	* 24.0	* 20.5	* 18.4	* 11.6	* 6.9
15	21.3	* 17.1	* 13.6	* 11.5	* 4.7	
10	16.6	* 14.4	* 8.9	* 6.8		
5	9.8	* 5.6	2.1			
1	7.7	* 3.5				
C	4.2					

*** : Significant at 0.05 level of probaility.**

2,3

4,5

fig6

DISCUSSION

Becalski *et al.* (2003) analyzed 30 samples of food for acrylamide. Concentrations of acrylamide varied from 14 ng/g (bread) to 3700 ng/ g (potato chips). Acrylamide was formed during model reactions involving heating of mixtures of amino acids and glucose in ratios similar to those found in potatoes . In model reactions between amino acids and glucose, asparagine was found to be the main precursor of acrylamide. Rosen & Hellenas (2002) found a wide range of acrylamide in cooked foods (30 to 10.000 mg/ kg).

However, acrylamide could not be detected in unheated control or boiled foods (Tareke *et al*, 2002). These authors found a temperature dependence of acrylamide formation. Ahn *et al*, (2002) confirmed that acrylamide is absent from the raw or boiled foods but present at significant levels in fried, grilled, baked and toasted foods.

The highest result was 12000 microg (kg) acrylamide in overcooked oil-fried chips.

Cytological examination revealed that acrylamide was found to be effective in inducing significant decreases in cell proliferating rate, giving an evidence on its cellular toxicity. Chromosomal abnormalities indicated that acrylamide is a strong clastogenic agent.

According to Seehy *et al.* (1983) the following criteria in order to call an incrase of SCEs significant or relevant were used : 1- In a rank test the value of p should b < 0.05 ; 2 – the difference between the mean SCE values of the matching negative control and the experiment in question must be 1.5 or more 3- A dose – response relationship should prevail.

The results obtained, however, showed that acrylamide was proven to be positive in inducing primary genetic damage in golden hamster genome . This conclusion reflects the possible mutagenic activity o acrylamide.

The mutagenic effect of foods and formation of acrylamide in diet by high temperature were discussed and reported by several workers (Berg *et al.,* 1990; Bull, 1992 & 1995; Champman & Maclean, 1993; Chen *et al.,* 1990; FAO, WHO; 1980 & 1992; Felton *et al.,* 1984; Guy, 1993; and Zetterberg, 2003).

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تأثير السمية الوراثية المستحدثة بواسطة الأكريلاميد على التركيب الوراثى لحيوان الهامستر

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أجرى هذا البحث لمعرفة السمية الوراثية لمركب الأكريل أسيد الذى يتكون على درجات الحرارة العالية بين الأحماض الأمينية والسكر كما فى تحمير الأطعمة الغنية بالنشا. ثم اختيار حيوان الهامستر الذهبى لحساسية الفائقه في الكشف عن السمية الوراثية وتم اختيار خمس جرعات هى ١ ، ٥، ١٠، ١٥، ٢٠ ملليجم / كجم وزن حيوان.

ثم توظيف ثلاث اختبارات هي :

1- دراسة تكاثر الخلية .
2- تحليل الشذوذ الكروموسومى فى نخاع العظام.
3- استحداث تبادل الكروماتيدات الشقيقة فى الكائن الحى.

أظهرت النتائج المتحصل عليها ما يلي :

1- مركب الأكريل أسيد سام خلوياً على مستوي الجرعات المستخدمة.
2- مركب الأكريل أسيد ذو قدرة تكسيرية موجة لإنتاجية شذوذ كروموسومي عالى علاوة على قدرته على التفاعل مع خيوط الغزل وإنتاجه لتضاعف كروموسومي.
3- أظهر الأكريل أسيد قدره على أحداث ضرر أولى للمادة الوراثية لقدرته على إنتاج زيادات جوهرية من تبادل الكروماتيدات الشقيقة والتي أظهرت علاقة خطية مع الجرعات المستخدمة.