Carcinogenicity of Cadmium Chloride Via Intraperitoneal Injection in albino Rats

*AL-Kaisie,B.I. **AL-Mussawei,M.T. *Maccawei,T.M.

Date of acceptance 6/9/2006

Summary

In the present research, study was done on the carcinogenic effects of $CdCl_2$ which Injected I/P in albino male rats.

The study includes determine LD50 of $CdCl_2$ 40 in albino male rats 6 weeks of ages which randomly divided into 4 equal groups and one of them left as control group, other 3 group injected I/P with $CdCl_2$ at dose 30 mg/kg B.Wt. ,23 mg /kg B.Wt. and 20 mg/kg B.Wt. for 24 hrs, 48 and 96 hrs. The safe dose 10 mg/kg B.Wt. was used for I/P Injected to group chosen for which include 15 albino male rats and equal group used as control group, then the following parameters were studied :

1. Cytogenetic investigation : include Micronuclei MN and chromosomal aberration which shown to be significantly Increase and mostly as breaks in chromosomes.
2. Clinical signs: Vomation , bloody foamy cough , dyspnea and diarrhea in treated group were observed.
3. Histopathological changes: lungs of treated rats showed grossly masses embedded and /or raised upon lung.The masses diagnosed lately asadenocarcinoma which characterized by tubular or papullar form with acini formation which composed of columnar or cuboidal cells and vascularized connective tissue stalkes.Emphysema and coagulation were also observed.

Introduction

Cadmium is a naturally occurring metal that is used in various chemical forms in metallurgical and other industrial processes and in production of pigments .Environmental exposure can occur via the diet and drinking water (1). Cadmium is absorbed efficiently by the lungs (30 to 60 %) than by gastrointestinal tract , the latter being as a turable process(2).

Cadmium is transported in the blood and widely distributed in the body but accumulates primarily in the liver and kidneys(3).Inhalation exposure to Cadmium and Cadmium compounds may result in effects including headache, chest pain, muscular weakness, Pulmonary edema and death (4).

There is limited evidence from epidemiologic studies for Cadmium – related respiratory tract cancer and Cadmium is placed in weight of – evidence group BI- Probable human carcinogen (1).

Inhalation exposure to Cadmium dust , Fumes , aerosols and some Cadmium Compounds causes irritation of the respiratory tract , emphysema and death for acute exposure to high cadmium concentration (4).

Materials and Methods

1. Median lethal dose LD50 (5) :

40 white male rats six – weeks olds randomly divided equally into four groups ,1st group injected Intraperitoneal (I/P) with 30.0 mg/kg B.Wt. Cadmium Chloride CdCl2, 2nd

* Department of Pathology/ Vet. College/ Baghdad University
** Market research and consumer protection center/Baghdad university

group with 23.0 mg/kg B.Wt. I/P CdCl2 ,3rd group with 20.0 mg/kg B.Wt. CdCl2 I/P at 24 hrs,48 hrs and 9 hrs respectively,whil the last group left as control.

2- Animal groups : Two randomly equal groups each contain 15 albino male rats at six weeks old were used were used for study , The 1st group injected I /P with 10 mg/kg B.Wt. CdCl2 and 2nd group were left as control group .

3- Clinical signs : Observed in animal groups dialy for 90 days.

4- Medium Preparation : (6)

- PMI 1640 medium 10 g.
- Newborn bovine Serum.
- Penicillin 1000 U.
- Streptomycin 100 µg.
- Brduurd 1 %
- Sodium Pyrovate 1 %
- Sodium Bicarbonate 1 %

5- Micronuclei preparation : According to reference (5), 0.5 blood samples were taken from rats tail veins and added to 4.0 ml minimum essential medium(MEM)enriched with 10 % heat inactivated fetal calf serum and phytohem -agglutinin (PHA, Serva).In Sterile plastic culture tubes.After 44 hrs . of incubation at 37° ,Cytochglasin – B (Cyto –B,Sigma),In Final concentration of 3 µg / ml of the culture medium was added .

The stock solution of Cyto-B was prepared by dissolving 1.0 mg of lyophilized material in 1.1 ml dimethyl sulfoxide (DM50) and kept at – 70° , 20 µl of the stock solution was added to each culture tube .The cultures were harvested after incubation for 72 h.

A mild hypotonic solution of 0.1 M KCL was used for 3 min., and a further 10 min of centrifugation at 200 xg after removal of supernatant the pellet was fixed with freshly prepared methanol/glacial acetic acid (3:1) and centrifuged as described before.

This procedures was repeated 4 times ,all supernatant were removed and the pellet resuspended in few drops of freshly prepared fixative ,then spread on clean slides and stained with Giemsa.

6- Chromosomal preparation :

According to reference (6) , heparinized tubes at 0.5 ml of blood incubate at 37° for 72 hrs. and then add 0.1 ml colchicine before 3 hrs. of cell culture period end , then centrifuged (1800Xg) for 10 min.

Slides were stained with 1:20 Giemsa solution and studied by using an oil immersion objective (100X). The steps 5 and 6 done for 30,60 and 90 days of groups also.

7- Histopathological Preparation :

Evaluations of Histopathological lesions in lung tissue were carried out by taken specimen (1 X 1 X 1 cm3) from lung at 90 days of treated group at the (end of experiment) fixed with Bouin's solution for minimum four days , dehydration to 70% isopropyl alcohol and embedding in paraffin, the blocks were sectioned at 7.5 microns and affixed to glass slide and stain with hematoxylin and eosin stain (7) .

Results and Discussion

1- Median lethal dose (LD 50) :

The LD50 of I/P injection of CdCl2 in albino rats was 30.0, 23.0 and 20.0 mg / kg B.Wt. at 24 , 48 and 96 hrs. The maximum dose which did not produce mortality in 96 hrs. was 20 mg / kg B.Wt., respectivelly using standard graphical procedures (Fig:I). According to reference (4) the oral value for animals range from 225-890 mg / kg B.Wt., for elemental CdCl2 ,72 mg / kg for cadmium oxide and 590-1125 mg / kg B.Wt., for Cadmium stearat .

2- Micro nuclei assay : The data tabulated in table (1).

Table (1) Micronuclei frequency in albino rats at 20 mg/kg B.Wt. of $CdCl_2$.

Days	Groups	Micronuclei per 500 CB cells
30	Treated control	24.4 (920) 4.1 (5420)
60	Treated control	20.1(1121) 2.1(5000)
90	Treated control	15.8(1465) 5.3 (4234)
Mean±S.E.	Treated control	20.1 ± 0.06 3.9 ± 0.8

3- Chromosomal aberration assay :

The data tabulated in table(2)

Table (2) Chromosomal aberration induced by 20 mg/kg B.Wt $CdCl_2$ in albino rats:

Days	Groups	Cells with Chromosomal aberrations		Number of Breaks		
		No.	percent	Total	per cell	Per chromosome
30	Treated control	48 /	24 P<0.01 7	76 /	0.38 P<0.01 0.07	0.0189 P<0.01 0.0035
60	Treated control	32 7	32 P<0.01 7	63 7	0.63 P<0.01 0.07	0.0199 P<0.01 0.0035
90	Treated control	28 7	28 P<0.01 7	56 7	0.56 P<0.01 0.07	0.0289 P<0.01 0.0035

The percentage of cells chromosomal aberration rate per cells and breaks rate per chromosome (figure 2) were showon presented in CdCl2 treated group with significant increase in rate of MN in lymphocytes (figure : 3) in total thus obviously refer to that CdCl2 are able to induce chromosomal aberrations and MN.,and that agree with reference (8) which indicated that several Inorganic cadmium compound have capability to cause aberration in genetic material particularly chromosom .

4- Clinical Signs :

Treated rats showed illness , vomiting diarrhea , dyspnea , cough with foamy bloody sputum that agree with reference (9) which indicate that exposure to 1 mg / m3 CdCl2 for 8 hours is immediately dangerous to human, and to other reference which identified that 0.5 mg Cd /m3 as the threshold for respiratory effect resulting from an 8 hours exposure(10).

5- Histopathological lesion:

Lung tissue of CdCl2 treated rats showed emphysema , bronchiolitis , alveolitis with hyperemia and congestion also clotted arteries present in lung (Figure : 4).

The most important lesion presented in the lung was lung carcinoma which characterized grossly by smaller masses embedded with parenchyma or raised above the surface of lungs .Microscopically , the carcinoma composed of columnar and cuboidal cells that form tubules and acini or papillary growth (Fig: 5 and 6) into alveoli with well vascularized connective tissue stalkes .

The neoplastic cells also form in broad sheets and cords and that agree with reference (9) which indicate that CdCl2 have potential carcinogenic effects , when chronic exposure to CdCl2 at concetration (10, 12.5 , 25 or 50 µg / m3) produced related increase in the frequency of primary lung carcinoma (10) .

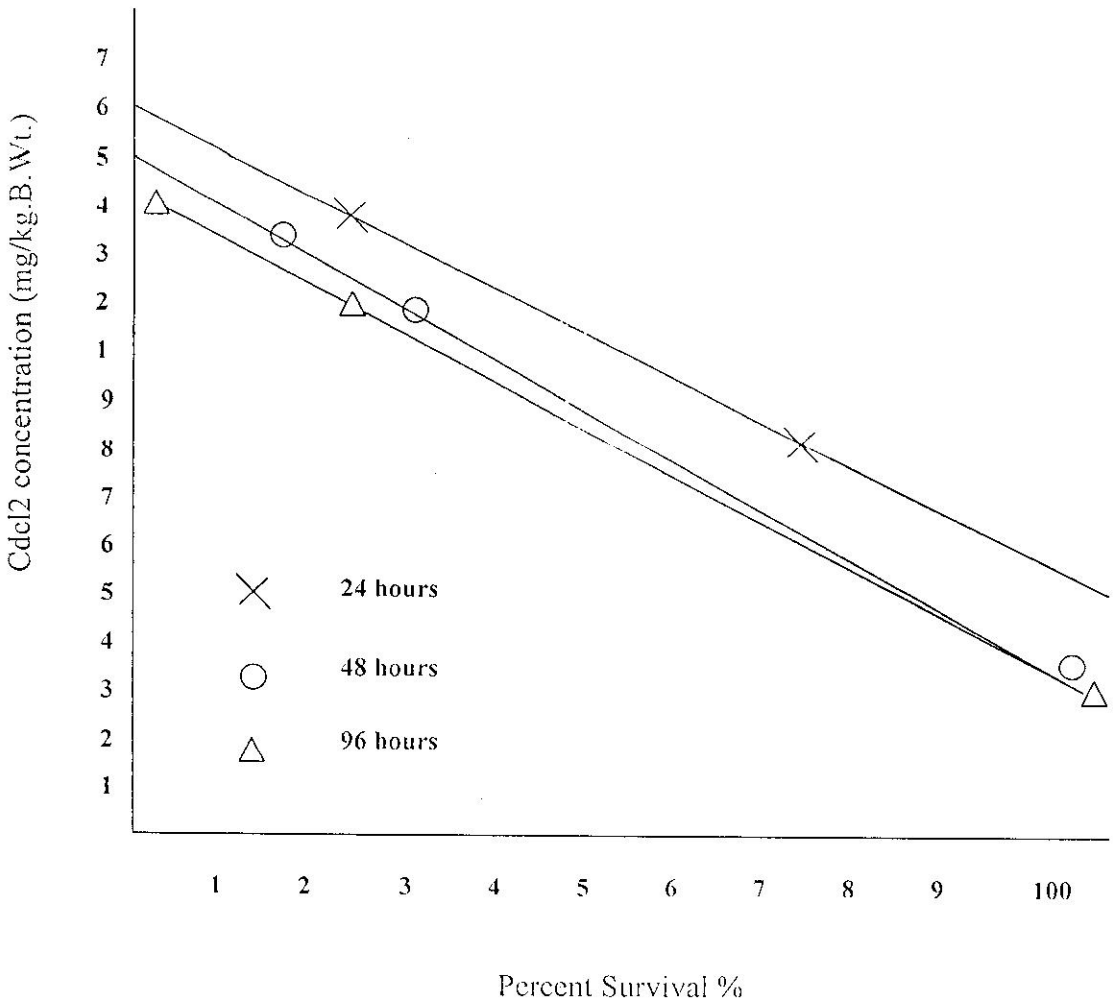

Fig I : CdCl2 LD50 for albino rats at 24 , 48 and 96 hrs. Intersection of dashed lines with survival lines (Solid) indicates LD50 concentration .

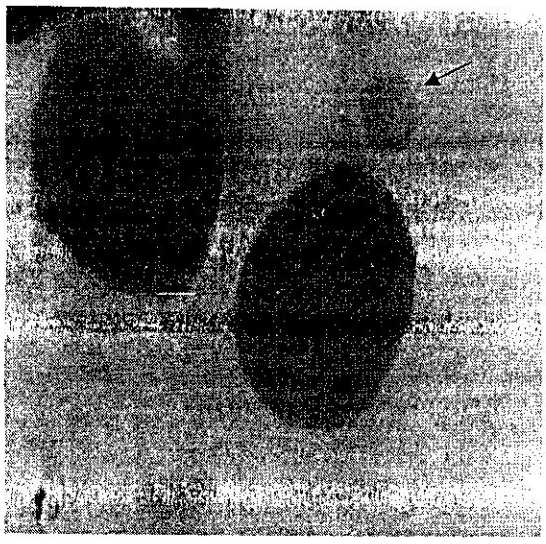

Fig 2 : Micronuclei in cytoplasm of divided lymphocyte in 30 days at 20 mg/kg B.Wt. CdCl2 treated rats .by Giemsa stain (100 X).

Fig 3 : Chromosomal aberrations with breaks in 90 days at 20 mg/kg B.Wt. CdCl2 treated rats ,by Giemsa stain (100 X).

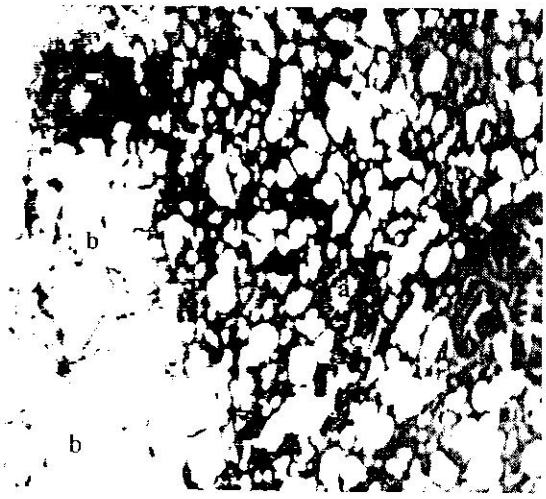

Fig : 4 Section in the lung at 20 mg/kg B.Wt CdCl2 treated rats at 90 days alveolitis (a), emphysema (b),coagulation area (c) and granuloma (d) by H & E stain(20 X)

Fig 5: Lung carcinoma : Acinar formation (a) with slough of carcinoma epithelium into the lumen;Acini lined by Columnar epithelium (b) , by H & E stain(20 X)

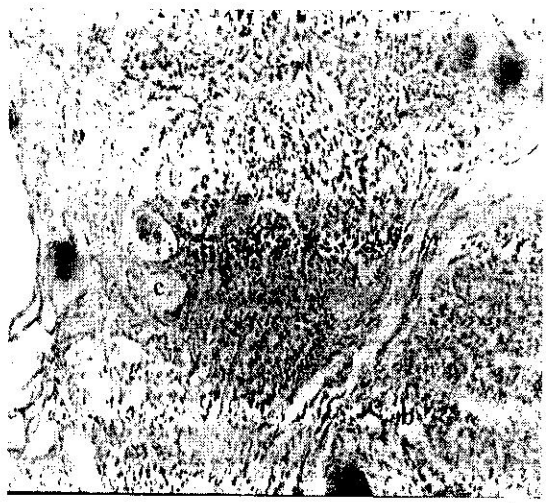

Fig 6 : Lung with focci of inflammatory scattered throughout lung (a) , irregular acinar formation (b) ,cuboidal cell forming acini (c) , by H & E stain (20 X)

References

1- ATSDR (1989) . Agency for toxic substances disease registry . Toxico- logical profile for cadmium . ATSDR / U . S . Public Health service ,ATSDR / TP -88 / 08 .

2- Norberg , G.F . ; Kyellstrom,T. and Nordberg,M.(1985) . Kinetics and Metabolism in:Friberg,L.;C.G.Elinder,T.Kyellstro m and G .F .Nordberg Eds.Cadmium and Health :Atoxicological and epidemiological appraisal. Vo.I.Exposure,dose and metabolism .CRS press,Boca Raton, FL . , PP .103-178.(Cited in ATSDR,1989).

3- Goyer,R.(1991). Toxic effect of metals in : Amdur,M.O.;J.D.Doull And C.D.Klaassen , Eds .Casarett and Doull's .Toxicology 4th ed . Pergamon Press,Newyork.PP.623-680.

4- USAF,(1990) . Cadmium in : Installation Restoration Program Toxicology Guide vol.5 Harry G.Armstrong Aerospace Medical Research laboratory ,Wright patterson AFB,OH.

5- Abbas ,N.Balasem and Abdul Sahib K.Ali(1991) . Establishment of dose response relationships between doses of Cs-137 X – rays and frequencies of micronuclei in human peripheral blood lymphocytes . Mutation Research .25:133-138.

6- Humason,G.(1967).Animal tissue techniques 56 g.2nd ed. SanFranciscow , W.H. Freeman.

7-Takenaka , S. ; H .Oldiges;H.Konig,D.Hochranainer and berdorster(1983).Carcinogenicity of Cadmium Chloride in Waster rats .]. NattCancer Inst.70:367-373.

8- http://Digital Fire.Com 1,2003.

9-Friberg,L.M.;Piscato,G.F.Norberg and T.Kyellstrom(1974) . Cadmium . In environment. 2nd Ed.CRC Press,Boca Raton,FL.

10- WHO(World health organization) (1989) . Recommended health – based limits in occupational exposure to heavy metals. Geneva (cited ATSDE ,1989)Retrieve Toxicity profile condensed version.

التأثير المسرطن لكلوريد الكادميوم المحقون في خلب الجرذان البيض

بشرى ابراهيم القيسي* منى تركي الموسوي** طالب عبد الامير مكاوي*

* فرع الامراض/ كلية الطب البيطري/ جامعة بغداد
** مركز بحوث السوق والمستهلك/ جامعة بغداد

الخلاصة

خلال الدراسة الحالية سجل التأثير المسرطن لكلوريد الكادميوم المعطى عن طريق الخلب في ذكور الجرذان البيض .

شملت الدراسة تحديد LD50 لكلوريد الكادميوم حيث قسمت 40 من ذكور الجرذان البيض بعمر ستة اسابيع الى اربعة مجاميع متساوية حقنت ثلاثة منها :30.0 و 23.0 و 20.0 ملغرام/ كغم من وزن الجسم كلوريد الكادميوم على التوالي وتركت الأخيرة كمجموعة سيطرة، ثم حددت الجرعة القاتلة للنصف في المدد 24 و 48 و 96 ساعة ، ثم تحديد جرعة آمنة والتي مقدارها 10 ملغرام/ كغم من وزن الجسم وذلك باستخدام مجموعتين من ذكور الجرذان البيضاء ، وبواقع 15 ذكر في كل مجموعة حيث حقنت احداها بالجرعة الآمنة في الخلب وتركت الاخرىكمجموعة سيطرة، وسجلت خلالها المعايير الآتية وللمدد 30 و60 و 90 يوماً من التجربة:

1- دراسة وراثية خلوية شملت تحديد النوى الصغيرة في خلايا اللمفوسايت وتحديد الإنحرافات الكروموسومية ، حيث سجلت المجاميع المعاملة إرتفاعاً معنوياً في اعداد النوى الصغيرة ومعدل الأنحرافات وخاصة الكسور منها .

2- دراسة العلامات السريرية : وتمثلت التقيوء ، البهر ، الإسهال مع السعال ذو الرغوة الدموية.

3- دراسة الامراضية النسجية : درست التأثيرات المسرطنة لكلوريد الكادميوم على نسيج الرئة حيث لوحظ عيانياً وجود كتل بارزة على سطح الرئة أو منطمرة في نسيج الرئة ومجهرياً سجل النفاخ والتجلط مع السرطانة الرئوية التي أخذت الشكل الغداني أو الحليمي مع تكوين الحويصلات المبطنة بالخلايا العمودية أو المكعبة وزيادة وعائية النسيج الضام للسرطانة الرئوية مقارنة بمجموعة السيطرة التي لم تظهر أي من العلامات.