تقدير السمية الوراثية للمضاد الحيوي سيفوتاكسيم في ذكور الفئران البيض Evaluation of the genotoxicity of the antibiotic cefotaxim in white male mice

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الملخص

سيفوتاكسيم مضاد حيوي نصف صنعي تنتج فعاليته المبيدة للجراثيم الى قدرته على تثبيط تخليق الجدار الخلوي البكتيري. وهو يمتلك فعالية مضادة لطيف واسع من البكتريا الموجبة والسالبة لصبغة جرام. اجري البحث في مختبرات قسم علوم الحياة\ كلية العلوم- جامعة تكريت والمركز العراقي لبحوث السرطان والوراثة الطبية- العراق.

تم تقييم السمية الوراثية للمضاد الحياتي سيفوتاكسيم في خلايا الثدييات بوساطة ما يأتي:-

1- تقدير الشذوذ الكروموسومي في خلايا نقي العظم (خلايا جسمية).
2- تقدير الشذوذ الكروموسومي في الخلايا الابتدائية المولدة للنطف (خلايا جرمية)
3- تقدير التأثير في الشكل الظاهري للنطف الناضجة (خلايا جنسية).
4- تقدير التلف الوراثي في خلايا الكبد بواسطة تقدير الهالة.

تمت معاملة ذكور الفئران بجرعات مختلفة عن طريق الحقن داخل الخلب لمدة ثلاثة أيام وخمسة ايام وعشرة ايام. كانت الجرعات المستخدمة 150، 300، و 600 ملغم\كغم من وزن الجسم يوميا.

أظهرت النتائج أن العقار سيفوتاكسيم تسبب في زيادة معنوية إحصائيا مرتبطة بالجرعة في نسب الشذوذ الكروموسومي في خلايا نقي العظم لذكور الفئران البيض بعد المعاملة بالجرعتين 300 و 600 ملغم\ كغم وزن الجسم. وبينت نتائج الدراسة عدم وجود تأثير في معدلات انقسام خلايا نقي العظم مقارنة بمجموعة السيطرة. كما تسبب المركب في حدوث زيادة معنوية إحصائيا في الشذوذ الكروموسومي في الخلايا الابتدائية المولدة للنطف بعد المعاملة بالجرعة 600 ملغم\ كغم وزن جسم. كما وأظهرت نتائج الدراسة وجود زيادة معنوية في قيم OTM لخلايا الكبد ترافقت مع الجرعة 600 وكانت الزيادة مرتبطة ايجابيا مع مدة المعاملة، كما ظهرت فروق معنوية في النسب المئوية لخلايا الكبد ذات الهالة مقارنة بمجموعة السيطرة. بينت نتائج دراسة تأثير العقار سيفوتاكسيم في الشكل الظاهري للنطف الناضجة بعد المعاملة مع الجرعة 600 ملغم\ كغم وزن جسم زيادة معنوية في تشوهات النطف مقارنة بمجموعة السيطرة ولم تظهر فروق معنوية في نسبة التشوهات مع الجرعتين 150 و 300 ملغم\ كغم وزن جسم مقارنة بمجموعة السيطرة.

تشير نتائج هذه الدراسة إلى أن العقار سيفوتاكسيم له تأثير وراثي في الخلايا الجسمية والخلايا الجنسية في ذكور الفئران البيض.

Abstract

Cefotaxim is a semi synthetic antibiotic. Its bactericidal activity results from inhibition of cell wall synthesis. Cefotaxim has activity against wide range of gram- positive and gram- negative organisms.

This study conducted in the laboratories of Dep. Of Biology. College of science. University of Tikrit and the Iraqi Research Center of Cancer and Medical Genetics- Iraq.

Genotoxicity of cefotaxim was evaluated in mammal cells by:
1- Evaluation of chromosome aberration in bone-marrow cells (somatic cells).

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2- Evaluation of chromosome aberration in primary spermatocytes (germ cells).

3- evaluation of the effect in sperm morphology (sexual cells).

4- Evaluation of genetic damage in liver cells by comet assay.

Male mice have been treated with different doses by intra peritoneal injection for 3, 5 and 10 successive doses. The used doses were 150, 300 and 600 mg.kg^{-1}.bwt.

The results showed that cefotaxim caused significant dose related increase in percentages of chromosome aberration in bone-marrow cells of white male mice after treatment with 300 and 600 mg.kg^{-1}.bwt. While it has no effect in mitotic index of bone-marrow cells compared with control group. Cefotaxim caused significant increase in chromosome aberration in primary spermatocytes after treatment with 600mg.kg^{-1}.bwt. and significant increase in DNA damage (Olive Tail Moment OTM) in liver cells associated with the dose 600mg.kg-1.bwt. of cefotaxim and significant differences in percentages of liver cells with comet tail. The results of the present study to sperm morphology showed significant increase of abnormalities compared with control group with 600mg.kg^{-1}.bwt.

The results of this study refer that cefotaxim has genetic effect in somatic cells, gem cells in white male mice.

Introduction

Attention has been focused recently on the biological and cytogenetic activity of drugs and chemicals (1). The ability of grisofulvin to induce damage in the mitotic spindle and aneuploidy in various somatic cells has been reviewed (2).

Antibiotics have been used in treatment of bacterial infections in human (3) and animals (4).

Cefotaxime sodium is a semi synthetic, broad spectrum cephalosporin antibiotic, the bactericidal activity of cefotaxime results from inhibition of cell wall synthesis. Cefotaxime has activity against a wide range of gram-positive and gram-negative organisims. Cefotaxime is active against some strains of pseudomonas aureginosa. Anearobs: bacteroids spp, Closridium spp, Peptococcus spp, and Peptostreptococcus spp.(5).

This study was carried out to evaluate the possible genotoxic effects of cefotaxime using metaphase chromosome analysis of bone marrow

cells and primary spermatocytes, sperm abnormalities of male mice, and OTM analysis of liver cells.

Material and methods

Animals:-

Mature malls of white Swiss Balb mice 9-12 weeks old, 25-30 g were used in all experiments. Animals were obtained from a closed random-bred group from Iraqi Research Center of Cancer and Medical genetics-Baghdad- Iraq , maintained under controlled conditions of humidity and receiving food and water *ad labitum*.

Chemicals:-

Cefotaxim sodium is a semisynthetic, broad spectrum cephalosporin antibiotic manufactured by IBN HAYAN PHARMA-HOMs-SYRIA.

Treatment and cytological preparations:-

Chromosome aberrations: For bone marrow and primary spermatocytes, groups of 5 animals were intra peritoneal injected with repeated daily doses of 150, 300 and 600mg cefotaxim Kg^{-1} b wt for 5 days. These doses are equivalent to half, once and twice therapeutic dose of serious infections respectively and are calculated according to the body surface area of man and mice as described by Paget and Barnes (6). Various concentrations of cefotaxim were prepared by dissolving with sterilized water prior to administration via intra peritoneal injection. Samples were taken after 22 h of last treatment.

Bone marrow chromosomes were prepared following the method of Yoshida and Amano (7). Primary spermatocytes at diakinesis metaphase l were prepared according to the air drying technique of Evans *et al* (8).

Slides were stained with Geimsa in Sorenson solution (pH 6.8). 75-100 well spread metaphases were analyzed per animal for scoring different types of aberrations.

Mitotic activity:

The effect of cefotaxim in mitotic activity of bone marrow cells was investigated by analyzing 1000 cells for each mouse. Dividing cells scored and the mitotic index was calculated (No. of dividing cells/ 1000 cells).

Primary spermatocyte chromosomes were prepared following the method of Evans *et al* (8) as following: The testes were removed, transferred to a sterile petri-dish containing isotonic sodium citrate solution (2.2%) and cut into small pieces, minced, then teased out the tubular contents with. The resulting cells were centrifuged, at 1500 rpm for 5 min.. Hypotonic sodium citrate solution (1%) was added drop by drop and left for 20 min. at 3 7°C. Centrifuge at 1500 rpm for 5 min. Fixed with 3:1

methanol: glacial acetic acid. Drops were dropped onto dry slides which were air dried. Five-seven slides were prepared for each mouse.

Sperm abnormalities:

The protocol recommended by Wyrobek and Bruce (9) was followed.

Comet assay:

Comet assay was conducted after the protocol of Tice *et al* (10) with some modifications as following: the liver minced into large pieces in 5ml of cold HBSS containing 20 mM EDTA/10% DMSO, When settled, aspirating mincing solution, adding fresh mincing solution, mincing into small pieces, remove and mixing 5 µL of the cell suspension with 75 µL LMPA.

Slides covered with first layer of NMA were used. After removing of cover slide 80 µL LMPA with cell suspension applied . when solidified, third layer of 85 µL of LMPA placed and applied cover slip. The cover slip removed and slides immersed in ice-cold fresh lyses solution (2.5 M NaCl, 100mM Na_2EDTA, 10mMTris- 10% DMSO, pH10). After 1hr at 4ºC in dark, placed the slides in the tank of horizontal electrophoresis unit side by side, and filled the tank with the electrophoresis buffer (1mM Na_2EDTA, 300 mM NaOH, pH, 13.5). after 20 min, the unit was operated for 30 min at 25V and 300 mA and then switched of the unit. The slides were immersed three times with neutralization buffer (0.4 Tris-HCl, pH 7.5) then rinsed the slides in cold absolute ethanol to fix the unwind DNA, then the slides was stained with 5 µL of ETBr 10% and covered with cover slips. The slides was analyzed within 2hr and dimmed in cold absolute ethanol to remove the stain and stored for archive. The Olive tail moment (OTM) was computed as a measure of DNA damage.

Statistical analysis:

Student t- test for calculating the significant of the experimental versus control data of the chromosome aberrations, sperm abnormalities, and OTM. The results of mitotic index were analyzed using F-test (6).

Results

Bone marrow chromosomes:-

Figure 1 shows metaphase chromosomes of mice with types of aberration.

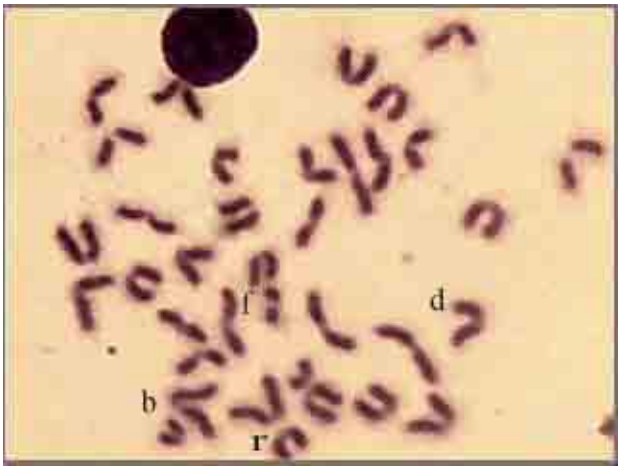

Figure 1 metaphase chromosomes of mice shows some of detected aberrations. B: break. D; deletion. F: fragment. R: robertsonian translocation. 1000X Geimsa stain.

Table 1 and 2 show types of chromosome aberration and mitotic indices in mice bone marrow cells 22 h after IP injection of cefotaxim doses of 150, 300, and 600 mg.kg^{-1} b wt. for 3, 5, and 10 days.

Gaps were recorded and excluded in total aberrations. Breaks and fragments, deletions, Robertsonian translocations and numerical aberrations were recorded. The dose 150 mg.kg^{-1} bwt. had no significant effect in chromosomal aberrations even after 10 days of treatment. The dose 300 mg.kg^{-1} bwt. induced significant increase in the percentage of chromosomal aberrations (P<0.05) 22 hr post treatment with 10 successive daily doses. The highest tested dose 600 mg.kg^{-1} bwt. induced significant percentage of chromosomal aberrations with all treated groups. The maximum percentage of chromosomal aberrations reach 5.00± 0.29 (P<0.01) 22hr after 10 days successive days treatment with the dose 600 mg.kg^{-1} bwt. compared with the control group (Table- 1).**

Table 1 chromosomal aberration in bone marrow cells of male mice induced after intra peritoneal injection with different doses of cefotaxim.

Treatment Dose: mg.kg^{-1}.bwt.	Mice killed After hrs	No. of metaphases	No. of metaphases with						No. of abnormal metaphases	Mean% of abnormal metaphases
			Gaps		Frag. and/or breaks	Del (1)	R.T(2)	Endomit(3)	Exclude gaps± S.E.	Include gaps± S.E.
			Chromat	Chromos						
Control (N.T.)(4)		400	8(2)	-	3(0.75)	-	-	-	3	0.75± 0.40
Cefotaxim 3 successive doses	24 24	400 400	12(3.0) 17(4.5)	1(0.25) 2(0.50)	4(1.0) 4(1.0)	- 1(0.25)	- -	- -	4 6	1± 0.40 1.50± 0.29

150 300 600	24	400	20(5.00)	3(0.75)	13(3.25)	-	1(0.25)	-	14	3.50± 0.64*
5 successive doses 150 300 600	24 24 24	400 400 400	12(3.0) 28(7.0) 40(10.0)	5(1.25) 9(2.25) 14(3.50)	5(1.25) 9(2.25) 14(3.50)	1(0.25) - -	- 1(0.25) 2(0.50)	- - 1(0.25)	6 11 18	1.50± 0.60 2.75± 0.48 4.50± 0.25**
10 successive doses 150 300 600	24 24 24	400 400 400	23(5.75) 31(7.75) 41(10.25)	3(0.75) 3(0.75) 3(0.75)	8(2.0) 11(2.75) 14(3.50)	1(0.25) 2(0.50) 4(1.0)	- 1(0.25) -	- 2(0.50) 1(0.25)	9 16 20	2.25± 0.25 4.0± 0.40* 5.0± 0.29**

(1) Deletion (2) Robertonian translocation (3) Endomitosis(4) Non treated * significant at 0.05 level (t-test) ** significant at 0.01 level (t-test).

This study showed no effect of cefotaxim in mitotiuc activity (Table-2).

Table 2 Mitotic activity in bone marrow cells of mice after treatment with different doses of cefotaxim.

Treatment and doses	Harvest time after the last treatment	No. of mice	Total No. of examined cells	No. of Dividing cells	MI
Control	24hr	5	5000	160	32.0
Cefotaxim 3 successive doses 150 mg.kg^{-1} b. wt 300 mg.kg^{-1} b. wt 600 mg.kg^{-1} b. wt	 24hr 24hr 24hr	 5 5 5	 5000 5000 5000	 161 163 156	 32.2 32.6 31.2
5 successive doses 150 mg.kg^{-1} b. wt 300 mg.kg^{-1} b. wt 600 mg.kg^{-1} b. w	 24hr 24hr 24hr	 5 5 5	 5000 5000 5000	 170 155 151	 34.0 31.0 30.2
10 successive doses 150 mg.kg^{-1} b. wt 300 mg.kg^{-1} b. wt 600 mg.kg^{-1} b. w	 24hr 24hr 24hr	 5 5 5	 5000 5000 5000	 155 160 150	 31.0 33.0 30.0

Spermatocytes chromosomes:-

Figure 2 shows metaphase I chromosomes of mice .

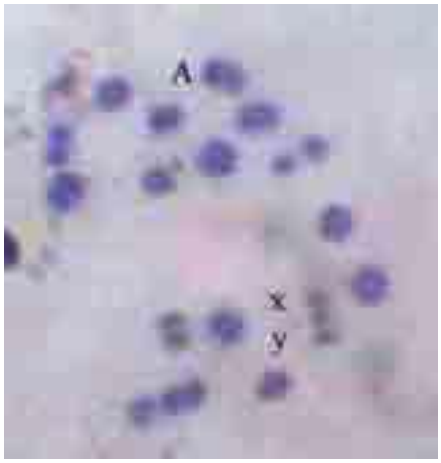

Figure 2 Metaphase I chromosomes shows A: autosomal bivalents and X-Y bivalent. 100X Giemsa stain.

Table- 3 shows the frequencies and distribution of chromosome rearrangements observed at diakinesis metaphase I of mice spermatocyrtes after treatment with repeated doses of cefotaxim. The types of the induced abnormalities were mainly X-Y, and autosomal univalents, breaks, fragments, and robertsonian translocations in the form of chain IV. The doses 300 and 600 mg. kg^{-1} b wt. induced significant increase in the percentage of chromosomal aberrations in mice spermatocytes. The maximum percentage of the induced aberrations reached 8.0± 0.0* and 9.6± 0.0* for 300 and 600 mg. kg^{-1} b wt. respectively for 5 days of repeated treatments 9.6± 0.0* and 12.00± 0.0 for 300 and 600 mg. kg^{-1} b wt. respectively for 10 days of repeated treatment compared with the control group.**

Table 3 Percentage of the different types of metaphases with chromosomal abnormalities in mouse spermocytes after treatment with Cefotaxim

Dose mg/kg.bwt.	Mice Killed after(hr)	No. of metaphases	No. of metaphases with					Mean% of metaph. With chromosome aberration
			X-Y univalent	Autosomal univalents	X-Y + A.U.	X-Y + breaks	Frag. And/or breaks	
0	24	375	7	2	3	1	-	3.5± 0.48
150	24	375	12	6	4	-	-	5.9±0.50
300	24	375	17	6	4	3	-	8±0.65*
600	24	375	25	13	2	-	-	10.6±0.49**

*** Significant at 0.05 level (t-test) ** Significant at 0.01 level (t-test)**

Morphological sperm abnormalities:

Figure 3 shows sperm morphology in mice.

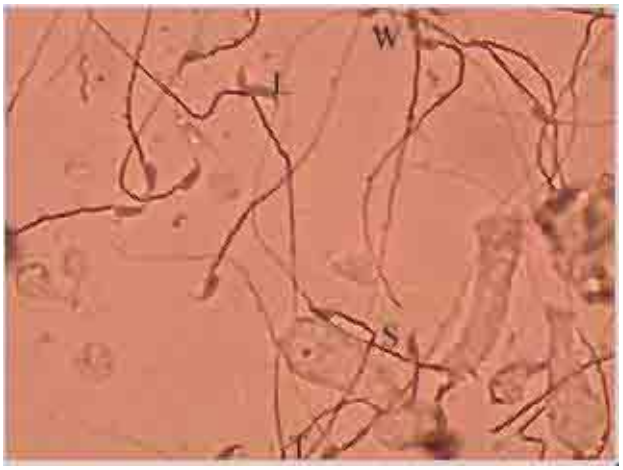

Figure 3 sperms of white mice. L: large. S: small. T: triangle. W: with out hook. Spermin stain. 1000X.

Table 4 shows mean percentage of sperm head abnormalities in control and treated mice with the different doses of Cefotaxim.

Various morphological sperm abnormalities were scored. Head abnormalities were more prominent. The head might acquire an unusual shape or might have a reduced or big size. Animal treated with the two doses 300 and 600 mg. kg^{-1} b.wt. showed significant increases in total abnormalities which reached 6.34± 0.66 and 9.28± 0.29 respectively compared with 2.72± 0.40 for control group.

Cefotaxim induced head sperm abnormalities in sperms of mice.

Table 4 Mean percentage of sperm head abnormalities in control and treated mice with different doses of Cefotaxim.

Dose mg .kg^{-1}.bwt	No. of Examined sperms	No. of Abnormal sperms	Mean % ± S.E.	Types of sperm head abnormalities					
				Amorph.	With out hock	Triangle	Banana shape	Small	Big
0	2503	101	4.04± 0.15	24	34	19	9	7	8
300	2625	202	8.2± 0.8*	45	51	63	14	13	16
600	2536	281	11.1± 0.36**	50	78	66	43	16	28

*** Significant at 0.05 level (t-test) **Significant at 0.01 level (t-test)**

DNA damage in liver cells:

Table 5 shows mean percentage of DNA damage in liver cells of control and treated mice with different doses of cefotaxim. All used doses caused significant DNA damage. The damage was dose dependant.

Table 5 mean percentage of DNA damage and cells with damaged DNA from liver of control and treated mice with different doses of cefotaxim.

Dose mg. kg^{-1}.b wt	OTM	Cells with damaged DNA
0	0.051±0.012	11.92±2.96
150	0.186±0.031*	23.18±4.23
300	0.426±0.120**	48.34±6.46
600	0.699±0.221**	62.77±6.66

OTM : Olive Tail Moment. All values in means ± S.E. * Significant at 0.05 level (t-test) ** Significant at 0.01 level (t-test)

Discussion

The study showed that cefotaxim has effect in both somatic and germ cells of male mice. This can be clarified by the increased incidence of chromosomal abnormalities in bone marrow cells and primary spermatocytes after sub acute treatment with the two higher doses of cefotaxim 300, and 600 mg. kg^{-1} b wt. the percentage of induced aberrations increased with the increasing of dose. The results also showed increased rate of damage in DNA of liver cells, OTM values was highly increased in the treatment groups when compared with control group, percentage of cells with tails increased also. These increases were related with dose increasing.

This may be explained by the fact that cefotaxim is metabolized in liver and substantial amounts are excreted in the bile and about 10% in urine. For this reason the dose must no exceeds 12g daily in human divided in 4- 6 equal doses even with sever or serious infections such as meningitis.

High incidence of metaphases with chromatid and chromosome gaps were scored in bone marrow cells after the treatment with cefotaxim. Scoring of gaps importance in assessing the mutagenic potential of compounds is controversial (12) and can be considered as separated category of damage (13). So the gaps were scored in this work but excluded from total aberrations. The maximum percentage of the induced aberrations excluding gaps was 5.00± 0.29 ($P<0.01$) 22hr after 10 days successive days treatment with the dose 600 mg.kg^{-1} b.wt. of cefotaxim.**

Cefotaxim induced significant percentage of fragments/breaks which represent the principle type of induced aberrations excluding gaps. The maximum percentage of fragments/breaks was 3.50% after sub acute treatment for 10 consecutive days with the highest tested dose compared with 0.75% for the control. Nichols (14) correlates chromosomal fragment/break formation to genetic mutation. More over fragments, which may lost at cell division, will be deletion of genetic material in progeny cell. This genetic loss may be not tolerated in actively dividing cell populations (15). The majority of chemical

mutagens/clastogens are capable inducing fragments/breaks (16, 17). Deletions and centric fusions were also scored but it was in low percentage. Numerical aberrations in the form of endomitosis also scored but it was rare too.

Cefotaxim induced chromosomal abnormalities in mouse primary spermatocytes after sub acute treatment with the doses 300 and 600 mg.kg^{-1} b wt. The most common type of aberrations were univalent which is the separation of chromosomes bivalents. X-Y univalent was more often than autosomal univalent, Imai *et al* (18); Hu and Zhu (19) observed this phenomenon. Fragments/breaks were also scored in mouse primary spermatocytes after treatment with cefotaxim, while it was not observed in the control group.

Some antibiotics e.g. refampicin, induced chromosomal aberrations in both somatic (20) and germ cells (21) of male mice. Other give weak or negative result (22, 23).

The two higher doses 300 and 600 mg/kg b. wt. induced significant percentage of sperm abnormalities which reach 6.34± 0.66 and 9.28± 0.29 respectively compared with 2.72± 0.40 for the control. Head abnormalities were more prominent. The results emphasize the positive correlation between cytogenetic damage and sperm abnormalities that was reported previously in humane (24) and in mice (25, 26). The regular process of sperm development involves the activity of several genes and the formation of normal sperm head involving intricate synchronous morphological and biochemical steps (27). Sperm morphology is the genetically controlled by numerous autosomal and sex-linked genes (28, 29, 30).

Cefotaxim induced damage in DNA of mice liver cells. The percentage of damage and cells with damaged DNA increased dependently with increasing of dose. This may refer to the serious effect of using higher dose and in agreement with recommendation of the manufacturer of the drug. Trimethoprim induced a dose dependent increase in level of damaged DNA in humane lymphocytes (31).

In conclusion cefotaxim have significant effect on DNA. Using of this antibiotic must be under wise guidance.

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