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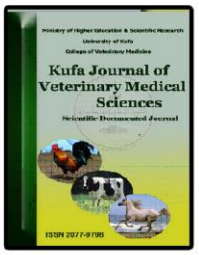

Cytogenetic Effects Of Tacrolimus After Chronic Oral Administration In Male Albino Rats

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Abstract

This study was carried out to investigate cytogenetic effects of chronic exposure of an immunosuppressant Tacrolimus in male albino rats. Thirty albino male rats were divided into 3 equal groups, the first one (T1) administrated orally therapeutic dose 70µg/kg.BW of Tacrolimus. The second group(T2) dosed double dose 140 µg/kg.BW of Tacrolimus, while the third group dosed distilled water with drops of ethanol as solvent like in treated groups and considered as control one(C). cytogenetic study included mitotic index, chromosomal aberration and blast index, the mitotic index showed significant decrease in both treated groups compared with the control one, the blast index showed significant decrease in T2 group compared with T1 and the control group**.** Chromosomal aberration in the stem cells of bone marrow represented by ring chromosome observed in T2 group which received double dose of drug. In conclusion Tacrolimus chronic exposure in rats indicate some genetic effect which needed more investigations in other species and even man.

Key words: Tacrolimus, cytogenetic effect, chronic toxicity.

الخلاصه:

اجريت هذه الدراسة لتقييم بعض التاثيرات السمية للتعرض المزمن للمثبط المناعي (التاكروليمس) في ذكور الجرذان المهقاء. استخدم في هذه الدراسة ثلاثون من ذكور الجرذان المهقاء .قسمت هذه الجرذان الى ثلاث مجاميع متساوية حيث جرعت المجموعة الاولى بجرعة علاجية قدرها 70 مايكرو غرام من وزن الجسم في حين جرعت المجموعة الثانية بجرعة مضاعفه قدرها 140 مايكرو غرام لكل كيلو غرام من وزن الجسم ,اما المجموعة الثالثة فجرعت بالماء المقطر مع قطرات من الايثانول المخفف كمذيب كما هو الحال للمجاميع المعالجة وعدت مجموعه سيطرة, تمت دراسة التأثيرات الخلوية عن طريق حساب معامل الانقسام الخلوي ومعامل انقسام الخلايا المولدة للخلايا اللمفاوية اظهر معامل الانقسام الخلوي نقصانا معنويا في كلا المجموعتين الاولى والثانية المعالجة بالعقار مقارنة بمجموعة السيطرة وكذلك اظهر معامل انقسام الخلايا المولدة للخلايا اللمفاوية نقصانا معنويا في حيوانات المجموعة الثانية التي جرعت بضعف الجرعة العلاجية فقط مقارنة بحيوانات المجموعة الاولى التي جرعت بجرعه علاجيه ومجموعة السيطرة . وقد سجلت بعض الزيخ الصبغي في الخلايا الجذعية لنخاع العظم في حيوانات المجموعة الثانية متمثلة بصبغيات حلقيه. نستنتج من هذه الدراسة وجود بعض التاثيرات السمية الجينية لهذا العقار بعد التعرض المزمن حيث تحتاج المزيد من الدراسات على حيوانات اخرى او حتى على الانسان.

Introductions:

Tacrolimus (formerly FK506) selectively inhibit transcription of interleukin (IL)-2 and several other cytokines, mainly in T-helper lymphocytes[(1)]. The introduction of Tacrolimus in the 1990s has significantly improved the survival of transplanted organs[(2)]**.** Tacrolimus binds to an intracellular protein FKBP-12, formed a complex with calcium, calmudlin and calcineurin which inhibit phosphorylation activity[(3)] Tacrolimus occasionally used in the treatment of various immune-mediated diseases[(4)]**.** This prevent the effect of dephosphorylation of nuclear factor of T-lymphocytes which necessary to initiate gene transcription of interlukin IL-2 synthesis, so the net outcome inhibit T-lymphocyte activation [(3)] Tacrolimus uses include Prophylaxis of transplant rejection in liver, kidney or heart allograft recipients.(0.15-0.20 mg/kg.BW)orally[(5)],treatment of Eczema and dermatitis in concentration(0.1%-0.3%)topically[(6)].A number of studies have found a close correlation between the pharmacokinetic parameters of tacrolimus and the clinical outcome[(7,8)]. However, despite the long use of the drug in clinical practice, the best way to use tacrolimus is still a matter of intense debate[(9,10)]

Materials α Methods:

Animals:

Thirty male albino rats, aged 8-10 weeks with weight range (150-170g), supplied by the animal house of the College of veterinary medicine of Baghdad university. They were housed and maintained in a conventional animal facility, with controlled conditions of temperature (20 ± 5ºC). Standard pellet and diet were produced *ad libitum*.

Experiential desing:

Thirty males albino rat randomly divided equally into 3 groups as follow:

1- 1St group (**T1**) was administrated orally with (70 μg /kg B.W) of Tacrolimus daily for 90 days as therapeutic dose.

2- 2nd group (**T2**) was administrated Tacrolimus orally with (140 μg/ kg.BW) as tow fold dose.

3- 3th group(**C**) was considered control group given distilled water orally.

Tacrolimus dosed orally by gastric gavage with overnight fasting. This work was carried at approved of College of veterinary medicine of Baghdad University in accordance with international ethical standard of research of work with laboratory animals.

Cytogenetic analysis in rat bone marrow:

Chromosomal analysis(abberations), mitotic index and blast index of experimental animals carried out according to(Allen, *et al*., 1977), by obtaining the bone marrow from bone of animals by anesthetized by ketamine+ xylazine and slide preparation by Giemsa stain. Mitotic index and blast index used as the following equations respectively.

$$\text{MI\%} = \frac{\text{Cells of Metaphase}}{1000} \times 100 \quad ( )$$

$$BI\% = \frac{\text{Number of lymphoblast}}{1000} \times 100 \quad (\quad)$$

1000=Total number of observed cells.

Statistical analysis:-

Data subjected to analysis of Varian (ANOVA) one way , least significant difference (LSD) and to compete between mean at level of 0.05 significances by using(SPSS), Version 10.

Results:

The results revealed significant decrease($P<0.05$) in both mitotic index and blast index in bone marrow of exposed groups (T1 and T2) depending on received doses of drug comparing with control one which received distilled water (Table 1 and Fig. 1) .

Table (1) The effect of different doses of Tacrolimus given orally for 90 days on mitotic index and blast index of mal albino rats.

parameter / groups	Mitotic index M±S.E	Blast index M±S.E
C N=5	4.27±3.00 A	3.45±20.90 A
TI N=5	2.22±1.50 B	3.31±21.10 A
T2 N=5	1.30±0.90 C	2.21±12.20 B

LSD of MI%=0.3 LSD of BI%=0.4

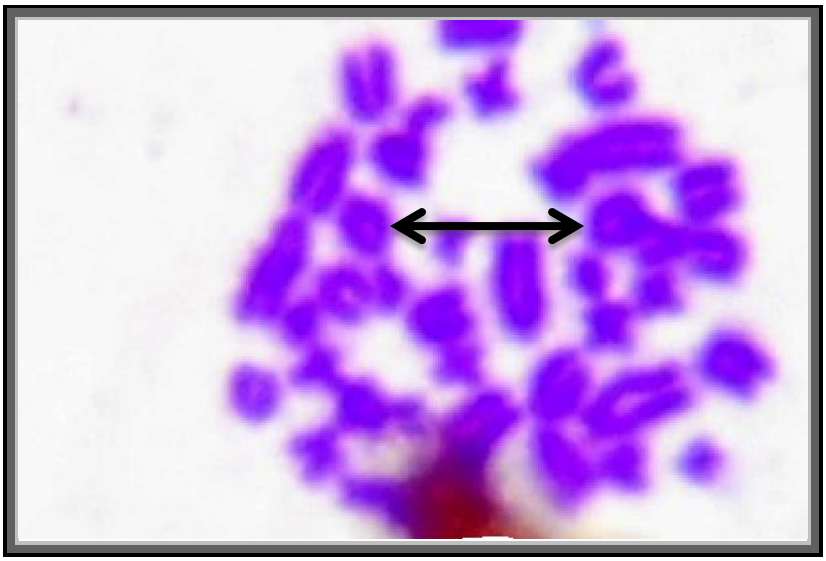
-Different capital letters denote siginficant differences ($p<0.05$) between groups.

The decreasing in MI% is more significant in animals of T2group which received double dose of Tacrolimus (140µg/kg.BW) than the T1 group which received therapeutic dose (70µg/kg.BW). The mitotic index is simply a measurement to determine the percentage of cells undergoing mitosis. Durations of the cell cycle and mitosis vary in different cell types. An elevated mitotic index indicates more cells are dividing, and thus obvious in

cancer cells, The mitotic index may be elevated during necessary processes to life, such as the normal growth of plants or animals, as well as cellular repair of an injury[11]. The values of mitotic index are an indication of the degree of cytotoxicity [12]. The decrease in mitotic index indicates the loss of dividing cells, which may be attributed to Tacrolimus interference in the normal sequences of mitosis leading to reduction in T and B lymphocyte**.** Kurtoglou and Yuksel [13] Determine the possible genotoxic effects of Tacrolimus which is by using sister chromatid exchange (SCEs), chromosome aberration (CAs), micronuclei tests (MN) and cell growth kinetics such as mitotic index (MI) and replication index (RI) in human lymphocytes. Tannuri *et al* [14] who observed administration of immunosuppressants did not change the mitotic index of the regenerating liver in newborn animals submitted to heptectomy. Astellas Pharma [15] who reported that Tacrolimus decreased the mitotic index dose-dependently mannar started from 70 µg/ml in Chinese hamster models.

Chromosomal aberrations:

The results of chromosomal analysis revealed there was a certain chromosomal aberration represented by ring chromosome in stem cells of bone marrow in animals of T2group which received double dose of Tacrolimus (140µg/kg.BW) for 90 days (Fig.4-14).while other groups (T1) which received therapeutic dose (70µg/kg.BW) didn't show any chromosomal aberration in stem cells bone marrow.



Figure(1):**A-(↔)** Ring chromosome in stem cells bone marrow of male albino rat received double dose (140µg/kg.BW)of Tacrolimus for 90 days (Geimsa stain× 100).

Chromosomal aberrations (CA) are one of the important biological consequences of human exposure to ionizing radiation and other genotoxic agents. Hagmar, *et al*.[16]. Many types of cancers are associated with specific types of CA which are etiologic for the cancer in question[17]. The chromosomal aberration which observed in the present study mean there is chromosomal mutation in somatic cells of bone marrow, this genetic damage if fixed and transmitted to the2nd cellular

generation by cells division might lead to carcinogenic effect in certain organs or tissue occur during pregnancy since tacrolimus transmitted through placenta[18]. The result of chromosomal aberration are in agreement with Kurtoglou and Yuksel, [13] who found the possible genotoxic effects of Tacrolimus which is by using sister chromatid exchange (SCEs), chromosome aberration (CAs), micronuclei tests (MN) and cell growth kinetics such as mitotic index (MI) and replication index (RI) in human lymphocytes. The cells were treated with 5, 25, 50, and 100 ng/ml concentrations of Tacrolimus, for 24 and 48 h treatment periods. Tacrolimus induced CA and MN frequency at all concentrations for 24 and 48 h. Also the result of this study are in agreement with Abou-shaaban, *et al* [19] who reported that Tacrolimus sub acute(7 days) treatment in mice with doses (4, 8 and 16 mg/kg.days) significantly increased the aberrations in chromosomes and abnormal spermatozoa in epididymis and vas deferens, indicating it to be a germ cell mutagen. Blast index (BI) represents a ratio of blast cells that are stimulated by mitogens[20]. Blastogenesis (lymphocyte transformation) refers to the process of formation of large polymorphic blast like cells in culture of lymphocyte stimulated by either nonspecific mitogens or antigens, which is considered as a result of immunological recognition. Blast index used to assess cellular immunity in patients with immunodeficiences, autoimmunity, infectious disease and cancer[21]. Takeuchi.H. *et al* [22] founded The relative pharmacodynamic potency of tacrolimus which examined by the mean ratio of drug concentrations giving 50% inhibition of blastogenesis of lymphocytes(blast index).

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