Tikrit Journal of Pure Science
ISSN: 1813 – 1662 (Print) --- E-ISSN: 2415 – 1726 (Online)
Journal Homepage: http://tjps.tu.edu.iq/index.php/j

The ability of Mycophenolate Mofetil to induce chromosome aberration and DNA damage in *Mus Musculus* Mice

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https://doi.org/10.25130/tjps.v24i7.453

ARTICLE INFO.
Article history:
-Received: 2 / 7 / 2019
-Accepted: 5 / 9 / 2019
-Available online: / / 2019
Keywords: Immunosuppressive therapy, Mycophenolate Mofetil, genotoxicity, chromosome aberrations, comet assay.
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ABSTRACT

Mycophenolate mofetil (MMF, cellcept) is widely used in maintenance immunosuppressive therapy for prevention refractory rejection in sold organ transplant recipients. MMF is orally used, and rapidly metabolized to its active constituent Mycophenolic acid. MPA is an inhibitor of Inosine Monophosphate Dehydrogenase II (IMPDH II) in lymphocyte causing in reduction in intracellular guanine nucleotide pools and leads to inhibition of lymphocyte proliferation. MMF is considered to be an effective and safe immunosuppressive agent compared with other medicins. MMF lacks the nephrotoxicity. But it has important side effects, gastrointestinal and heamatological adverse effects are the most common. The results of this study show the high significant in total chromosome aberrations in treatment groups compared with control. Also there is high significant differences in total damage DNA in treatment groups than control. In conclusion, MMF may has a high genotoxicity by induced chromosome aberration and DNA damage.

Introduction

Mycophenolate mofetil (MMF, cellcept) is widely used in maintenance immunosuppressive therapy for prevention refractory rejection in sold organ transplant recipients. MMF is orally used, and rapidly metabolized to its active constituent Mycophenolic acid (MPA) [1].

MPA a reversible, noncompetitive inhibitor of Inosine Monophosphate Dehydrogenase II (IMPDH) in activated lymphocytes, causes a reduction in intracellular guanine nucleotide pools and leads to an suppression of lymphocyte proliferation [2]. The potential of immunosuppression of MMF may depend on other mechanisms. It eliminates clones of activated lymphocytes by induction apoptosis of these cells and suppresses responding to stimulation of antigen. It inhibits glycosylation and adhesion molecules, thus decrease recruitment of lymphocytes and monocytes to sites of inflammation, and prevents tissue damage by nitric oxide via depletion of nitric oxide synthase [3].

Lymphocytes are more dependent on the de novo pathway for purine synthesis, while most cells are able to use both salvage and de novo pathways, thus MPA exerts selective anti-proliferative effect. MPA is a potent inhibitor of type II isoform of IMPDH fivefold compared with the type I isoform expressed in resting cells [4].

Heamatological toxicity is among the side effects of MMF administration, and while anemia is the most commonly, due to bone marrow suppression or heamolysis, Leukopenia is the most important adverse effect. These are usually mild and dose-related [5]. Myelotoxicity may appears with MMF treatment [6]. Carcinogenic effects data of MMF are contradictory. Some studies determine the mutagenicity of MMF *in vitro* and may enhance cancer invasiveness, on the other hand, MMF associated with suppress tumor dissemination *in vitro* [7].

Gastrointestional (GI) side effects are the most commonly complication in patients treated with MMF, and are dose- dependent occurring in up to 20% of patients at doses of 2g daily [8], and the most common GI complication is diarrhea, which may caused by induction at villous atrophy or by inhibition of mitosis in intestinal epithelium [9].

The present work aims to investigate the genotoxic potential as the ability of MMF to induce and assess DNA damage by chromosome aberration and comet assay.

Materials and Methods

The study is conducted in 40 males of laboratory white mice weighting 20-25g, 6-8 weeks age. Animals were maintained under controlled ambient temperature 25 C°, and a 12\ 12 hrs. light\ dark cycle for two weeks prior to commencement of the experiments

The medicine :

MMF (cellcept) was a film kapli tablets of 500 mg, which provided by Roche. It was freshly prepared via dissolving in adequate volume of sterile distilled water to obtain the desired concentration.Treatments were for 5 consecutive days for all groups except positive control (mitomycin C) which was single injected dose, after 24 hours of the last dose the animals were sacrificed in both tests.

Chromosome aberration:

In this test 20 males of mice were used, it was hard to obtain chromosomal spreads with therapeutic doses, therefore less doses (4.16 and 8.33 $mg.kg^{-1}$.bw) were used in this test, 5 animals for each group and treated as follow:

1. 5 animals as negative control treated with distilled water.
2. 5 animals as positive control treated with mitomycin C 0.33 $mg.kg^{-1}$.bw.
3. 5 animals treated with cellcept 4.16 $mg.kg^{-1}$.bw.
4. 5 animals treated with cellcept 8.33 $mg.kg^{-1}$.bw.

The experiment was conducted according to the method described by Proudlock, 2016. [10].

Comet assay:

20 males were used in this test, Therapeutic doses (16.6 and 33.3 $mg.kg^{-1}$.bw) were used for impact assessment in patients who use this medicine doses and treatment as follow:

1. 5 animals as negative control treated with distilled water.
2. 5 animals as positive control treated with mitomycin C 0.33 $mg.kg^{-1}$.bw.
3. 5 animals treated with cellcept 16.6 $mg.kg^{-1}$.bw.
4. 5 animals treated with cellcept 33.3 $mg.kg^{-1}$.bw.

The experiment was conducted according to the method described by Tice *et al*., 2000. [11].

Results and Discussion

Numerical and structural aberrations were evaluated in chromosomes of bone marrow cells of mice, and the results of numerical aberration reveals significant differences in 4.16 and 8.33 $Mg.Kg^{-1}$.bw treated groups compared to negative control. MD ± S.E was 16.60 ± 1.10, and 16.80 ± 1.10 respectively, table (1).

With respect to structural aberration, our results showed a significant differences in both groups of treatment compared to negative control, MD ± S.E was 36.40 ± 2.16, and 47.40 ± 2.16 respectively, table (1).

Table (1): Cellcept induced numerical and structural chromosome aberrations in white mice bone-marrow cells.

D.V	T/ D $mg.kg^{-1}$.bw		M.D ± S.E
Numerical	NC	Mitomycin C 0.33	25.00 ± 1.10*
		Cellcept 4.6	16.60 ± 1.10*
		Cellcept 8.33	16.80 ± 1.10*
Structural	NC	Mitomycin C 0.33	103.40 ± 2.16*
		Cellcept 4.6	36.40 ± 2.16*
		Cellcept 8.33	47.40 ± 2.16*

Tukey HSD, * mean difference is significant at 0.05 level, D.V= dependant variables, NC= Negative control, T= Treatment, D= Dosage, M.D= mean difference, S.E= standard error mean.

In chromosomal aberration test numerical aberration can be scored, and it may be aneuploidy or polyploidy, Whereas structural aberration such as gaps, breaks, deletions, fragments Robertsonian translocations and centromerice attenuations. One of the in vivo genetic toxicology assays is chromosome aberrations test in mammalian bone marrow cells, it determines whether substances induce types of chromosome aberration in bone marrow cells. Depending on the mechanism of action, there are two type of structural aberrations, these include chromosome type and chromatid type aberration. In chromosomal aberration test (*in vivo*) aneuploidy and polyploidy could arise as a numerical abnormalities. Although increasing in polyploidy indicates for numerical aberration but not necessary induction for aneugenic potential, simply it may be cytotoxic potential or cell cycle perturbation [12].

The current study reveal a high significant in structural aberrations in treatment groups than negative control, this indicates the ability of MMF to induction genotoxicity. At the same time a high significant aroused in numerical abnormalities in compared to negative control, which indicates that MMF possess cytotoxic potential or its toxic effect on cell systems, figure (1) Shows chromosome spreads of white mice.

Structural chromosome aberrations result mostly from double strand DNA damage which can be induced directly or indirectly, (in the most cases at genotoxins) due to errors in repair or replication DNA system leading to double strands breaks (DSB). Increasing at incidence of structural abnormalities after treatment indicates genotoxic potential. Breaks and deletions results from DSB that are not repaired [10].

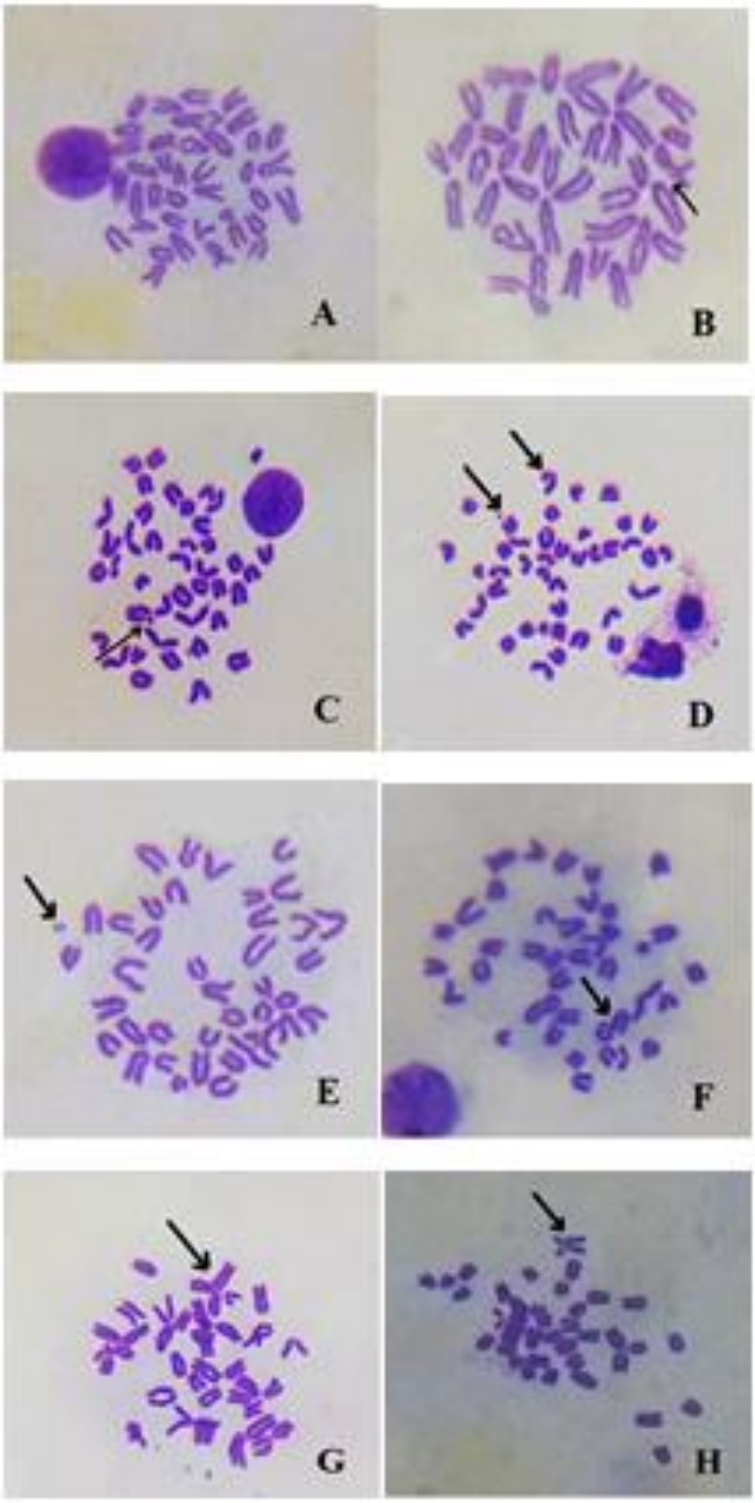

Fig. 1: Metaphase chromosome of mice bone marrow, 100X n=40. (A) normal chromosomes, (B) gap, (C) break, D) deletion & fragment, (E) fragment, (F) tail to tail convergence, (G) head convergence, (H) robertsonian translocation.

In vivo comet assay contribute to genotoxic potential identification of agent and assessment of dose response, and understanding substances mechanism of action [13]. In the here current study DNA migration was evaluated in bone-marrow (BM), liver, and kidney cells of white mice treated with 16.6 and 33.3 $mg.kg^{-1}$.bw. of cellcept, and the results showed a damage levels in negative and treated groups, Figure 2.

The comet assay results showed a high significant increase in bone marrow (BM) damaged cell in both treatment groups (5.60 ±0.97 and 12.00 ±0.97) compared to negative control. There was a significant increase in BM total damage in treatment groups

(10.60 ±1.60 and 16.60 ±1.60) compared to negative control. Liver damaged cell increased significantly in both treatment groups (9.80 ± 1.40, 22.20 ±1.04) related to negative control, also a significant arise in liver total damage in treatment groups (16.80 ± 1.68, 32.00 ± 1.68) than control. In addition, there was a significant differences in kidney damage cell in treatment groups (15.20±1.13, 19.60±1.13) compared to negative control, and there was significant increase in kidney total damage in both treatment groups (18.40 ± 4.29, 30.80 ±4.29), tables 2,3,4 explain these results.

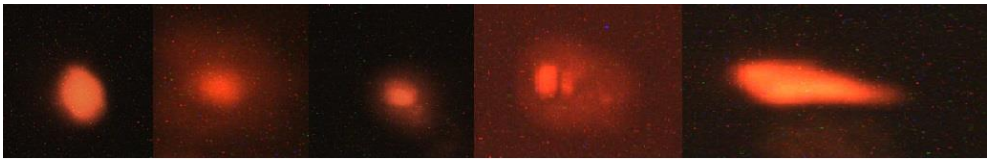

Fig. 2: five classes of DNA, the first one normal cell, other are different levels of DNA damage.

Table (2) DNA damage in bone marrow cells of white mice.

D. V.	T/ D mg.kg^{-1}.bw		M.D ± S.E
Cells with damaged DNA	NC	Mitomycin C 0.33	19.60 ± 0.97 *
		Cellcept 16.6	5.60 ± 0.97 *
		33.3	12.00 ± 0.97 *
Total DNA damage	NC	Mitomycin C 0.33	27.80 ± 1.60 *
		Cellcept 16.6	10.60 ± 1.60 *
		33.3	16.60 ± 1.60 *

Tukey HSD, * mean difference is significant at 0.05 level, D.V= dependant variables, NC= Negative control, T= Treatment, D= Dosage, M.D= mean difference, S.E= standard error mean.

Table (3) DNA damage in liver cells of white mice.

D. V.	T/ D mg.kg^{-1}.bw		M.D ± S.E
Cells with damaged DNA	NC	Mitomycin C 0.33	27.80 ± 1.04 *
		Cellcept 16.6	9.80 ± 1.04 *
		33.3	22.80 ± 1.04 *
Total DNA damage	NC	Mitomycin C 0.33	41.40 ± 1.68*
		Cellcept 16.6	16.80 ± 1.68 *
		33.3	32.00 ± 1.68 *

Tukey HSD, * mean difference is significant at 0.05 level, D.V= dependant variables, NC= Negative control, T= Treatment, D= Dosage, M.D= mean difference, S.E= standard error mean.

Table (4): DNA damage in kidney cells of white mice.

D. V.	T/ D mg.kg^{-1}.bw		M.D ± S.E
Cells with damaged DNA	NC	Mitomycin C 0.33	27.60 ± 1.13 *
		Cellcept 16.6	15.20 ± 1.13 *
		33.3	19.60 ± 1.13 *
Total DNA damage	NC	Mitomycin C 0.33	40.20 ± 4.29 *
		Cellcept 16.6	18.40 ± 4.29 *
		33.3	30.80 ± 4.29 *

Tukey HSD, * mean difference is significant at 0.05 level, D.V= dependant variables, NC= Negative control, T= Treatment, D= Dosage, M.D= mean difference, S.E= standard error mean.

The alkaline comet assay (PH>13) can be used to determine DNA damage such as alkali_labile sites (ALC), strand breaks, DNA-DNA or DNA-protein crosslink's. Increased incidence of DNA migration indicates DNA strand breaks and/or ALS. Although DNA repair reduces DNA migration by elimination DNA lesions, excision repair may induces DNA migration by incision- related DNA strand breaks. Positive results in comet assay can not only indicates strand breaks which may lead to chromosome aberrations formation, but also a basic sites (Ap sites) modifications and induction of gene mutations [13].
Researchers suggested several different methods to evaluate comet formation and assessment of the results and images of Single Cell Gel Electrophoresis (SCGE), The distance of DNA migration is most commonly used when dealing with low damage levels. But this technique is not useful with relatively high DNA damage, as increasing extent of the damage paired with increasing extent of the tail with increasing intensity of Fluorescent staining but not in length only. A scoring method was recommended by Collins [14], which may be useful for a laboratory without previous experience because of its relative case of application. This method includes five classes of damage ranging from no tail (0 shape) to almost all DNA in tail (4 shape), this system gives quantitative analysis which is adequate for many purposes. Another method for comet assessment is known as tail moment, that calculated as: measure of tail length

by the measure of DNA in the tail, it was introduced by olive *et al* [15] The method chosen depends on the resources investigator and study design [16]. Results of this study agreed with Dridi *et al*, 2016 concluded, and showed a significant increase in DNA damage particularly in class 3 and 4 according to both treatment and dosing_time in MMF_treated rats [5].

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قابلية مركب mycophenolate mofetil على احداث التشوهات الكروموسومية وضرر الDNA في الفئران

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الملخص

يستخدم عقار MMF بشكل واسع في كبح المناعة لمنع الرفض المعاكس عند المرضى الذين تم نقل الاعضاء اليهم. اذ يعطى MMF فموياً ويمتص ويتأيض بشكل سريع في القناة الهضمية وفي الكبد الى شكله الفعال mycophenolic acid (MPA) . يعمل مركب MMF على تثبيط الشكل الثاني من انزيم(Inosine Monophosphate Dehydrogenase II (IMPDH II) في الخلايا اللمفاوية الفعالة والذي يدخل في مسارات تصنيع الكوانين, لذلك فان تثبيط هذا الانزيم يؤدي الى انخفاض محتوى الخلية من الكوانين وبالتالي تفقد الخلية القدرة على التكاثر. يعد MMF عقار فعال وامن ككابح مناعة مقارنة مع العقاقير الاخرى. ولكن في نفس الوقت له العديد من التأثيرات الجانبية وخاصة على القناة الهضمية والتأثيرات الدموية. وجد من خلال الدراسة الحالية وجود فروقات معنوية عالية في التشوهات الكروموسومية العددية والتركيبية لدى جميع مجاميع المعاملة مع MMF مقارنة مع مجموعة السيطرة. كما وجد زيادة معنوية عالية في حجم الضرر الكلي في DNA الخلية لجميع الانسجة في جميع مجاميع المعاملة مقارنة مع مجموعة السيطرة السالبة. يستنتج من هذه الدراسة ان مركب MMF يعمل على تحفيز التشوهات الكروموسومية و الضرر في DNA وهذا مؤشر على قابيلتة على احداث السمية الوراثية.