

## EFFECTS OF THE CYCLOSPORINE ON SOME HEMATOLOGICAL INDICES IN BONE MARROW TRANSPLANTED-RABBIT

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### ABSTRACT

This study was conducted to evaluate the effects of cyclosporine on some hematological indices in male rabbits after bone marrow transplantation operation. Thirty male rabbits were used in the present study which divided into three groups equally (ten rabbit each group).Rabbit of first group were give 0.5 ml of normal slain from Day zero till Day 10 of experiment then applied to intra bone marrow-Bone marrow transplantation (IBM-BMT) at Day 11 then after, these rabbit give normal slain from Day 12 till Day 20 and considered as shame group. Rabbits of second and third groups were applied to same protocol that used in first group except that the rabbit were give 12.5 (T1) and (T2) 25 mg/kg/BW of cyclosporine respectively instead of normal slain.

blood was collected At Day10 and Day 21 from all rabbit of the experiment via heart puncture and Red blood cell (RBCs)count , white blood cell (WBCs) count, Deferential leukocyte count, Packed cell volume(PCV) and hemoglobin concentration(Hb) were measured.

Results of the present study showed significant decrease ( $p < 0.05$ ) WBCs count, Neutrophils, Lymphocytes, in groups treated with 12.5 and 25 mg/kg/BW in compare to shame group at Day 10 and Day 21 while RBCs count, PCV and Hb values were significantly decrease ( $p < 0.05$ ) T1 and T2 groups compare with that in shame group at Day 10 only then return to normal at Day 21.

Results of the present study suggested that the cyclosporine at both doses act as immune suppresser in bone marrow transplanted- rabbits.

## INTRODUCTION

Allogeneic bone marrow transplantation (allo-BMT) is a potentially curative therapy for certain diseases of the hematopoietic system, immunodeficiency, autoimmune diseases, solid malignant tumors, and so on (1, 2). Some researchers have developed a new and powerful bone marrow transplantation (BMT) method It's Intra bone marrow-BMT (IBM-BMT) (3), in which donor bone marrow cells (BMCs) are directly injected into the recipient's bone marrow cavity, Therefore, a much greater number of donor hematopoietic stem cells and mesenchyme stromal cells (including mesenchyme stem cells) can be inoculated into the recipient bone marrow by IBM-BMT than by conventional i.v. BMT, This result in the rapid reconstitution of donor hematopoietic cells and permits a reduction in the doses of irradiation used as a conditioning regimen (4,5).The introduction of cyclosporine 20 years ago to the repertoire of immunosuppressive drugs constitutes one of the major breakthroughs of modern medicine. It led to a significant improvement in the outcomes of organ transplantation (6, 7).Cyclosporine is a lipophilic cyclic undecapeptide with one unique amino acid in its structure. It was originally derived from a filamentous fungus *Tolypocladium Inflatum* Gams in the laboratories of the Sandoz Company in Basel, Switzerland. In 1971, the antibiotic screening process, which also included testing of various compounds for their immunosuppressive properties, Drs J. Borel and H. Stahelin observed that a fungal extract containing cyclosporine displayed not only a considerable immunosuppressive activity but also absence of any significant cytotoxic properties (8).In 1976 the biological properties of cyclosporine, the first immunosuppressive agent with a specific anti-T-lymphocyte activity, were described in detail, (9) leading the way to its use in animal models of transplantation. (10, 11 and 12)Thirteen years after its discovery, in November of 1983, the US Food and Drug Administration (FDA) approved cyclosporine for treatment and/or prevention of transplant rejection. Currently, cyclosporine is used for prevention of graft rejection in kidney, liver, heart, lung, and combined heart-lung transplantation. In addition, it found its place in bone marrow transplantation in prevention of graft-versus-host disease as well as in treatment of autoimmune conditions,

like psoriasis, atopic dermatitis, rheumatoid arthritis, and a variety of glomerular disorders. (13)The present study conducted to evaluate effects of .

## **MATERIALS AND METHODS:**

### **Experimental design**

Thirty male rabbits were randomly divided into three groups as following:

1. Group one (sham group) consist of ten male rabbits, two of them were used as donor and other eight male rabbits were administered 12.5ml/kg/BW normal slain (N.S) orally for ten day, At Zero time experimental the Collection of Blood samples (10ml) were collect from each animal by the heart (cardiac puncture)by sterile syringe 22 Gage needle.

The blood was used to performed hematological exam to measurement the white blood cells account and there differential, red blood cells account, Hb, P.C.V.

At DAY10 of experimental was collecting the blood as same procedure at zero time and did same examination before operation.

At DAY11 of experimental allogeneic Intra–bone-marrow injection of bone marrow transplantation Aspiration method (IBM-BMT) AM was done and rabbits were Re administered with normal slain after surgical operation until DAY20 of experimental.

At the end of this experimental study of experimental was collecting the blood as seam procedure at zero time and At DAY10 than made the same examination to Estimation of blood values

2. Group two (treatment with 12.5ml/kg/BW cyclosporine orally administration) and seam protocol of sham group was applied except of the use (treated with 12.5ml/kg/BW cyclosporine orally administration) replacing of normal slain.

3-Group three (treatment with 25ml/kg/BW cyclosporine orally administration) and same protocol of sham group was applied except of the use (treated with 25ml/kg/BW cyclosporine orally administration) replacing of normal slain.

## **Preparation of rabbit's bone marrow cells (BMCs) and intra bone-marrow (IBM) injection of BMCs**

At the first were prepared the animals one as a donor and other rabbits as a recipients

### **Anesthesia**

Each rabbit was general anesthetized with Ketamine (20 mg/Kg/BW) and xylzine (9 mg/Kg/BW) intra muscular.

### **Harvesting of bone marrow cells by Aspiration method (IBM by AM)**

The hair is clipped and the skins wash and disinfect with Anti septic povidone iodine 10%. The rabbit fixed in lateral recumbence following anesthesia a tiny skin incision should be made to facilitate entry of biopsy needle. The site of skin penetration is mid-way between the head of femur and the greater trochanter. By placing the thumb on the greater trochanter, the location of the trochanter fossa may determine by palpation the needle advanced toward the trochanteric fossa with its long axis parallel to the long axis of the moderate flexion of the limb may aid in determination of the position of the femur by palpation. The needle should be directed along medial aspect of greater trochanter for a distance of approximately 8-10mm until it encounters resistance imported by bone of trochanter fossa, an intra-modularly pin of about 16 gauges was directed through the skin until it contact the cortical bone of fossa. The shaft of pin is thin aligning with the shaft of femur, and slight drilling motions open the modularly cavity figures (2) and (3).

Care is taken to prevent the pin to enter cavity the pin is removed quickly and replaced with 16gauge needle attached to a (5ml) syringe, slightly suction is applied to the syringe and is discontinuous as soon as marrow appears in the syringe. No anticoagulant used in the syringe, smear prepared within seconds after bone marrow collections, because bone marrow clot rapidly, after taken samples of bone marrow injection of bone marrow inside a cavity of bone in other recipients animal (3) .



**Figure (2) shows Harvesting of bone-marrow cells (BMCs) by Aspiration**



**Figure (3) shows Harvesting of bone marrow cells (BMCs) by Aspiration method**

#### **Procedure for Intra bone marrow–bone marrow transplantation (IBM-BMT)**

As the seam procedure did to in harvested of bone marrow for preparation of animals expiated. A trans-needle was inserted into the bone after inserting the needle; whole BMCs harvested using the PM was slowly injected into the bone marrow cavity as in Figure (4)



**Figure (4) shows injected of bone marrow in bone**

## RESULTS AND DISCUSSION

### White blood cells WBCs (Leukocyte)

Effect of cyclosporine on bone marrow transplantation. Regarding to the effect of bone marrow transplantation on total WBCs there was significant increase in WBCs in rabbit that have intra bone –bone marrow transplantation (IBM-BMT) without treated by immunosuppressive drugs (cyclosporine) in sham group (G<sub>1</sub>) in table (1) in 14 agreement with (14, 15) .

There have been several reports in the rapid recurrence or persistence of autoimmune disease after auto or allogeneic BMT (16).Therefore increase of WBCs in Day20 after operation in this study may be result from autoimmune disease.

In the present study, cyclosporine caused decreased in WBCs count related two IBM- BMT in dose and time depended manner this result in agreement with other (17).

CSA inhibits calcineurin via binding to the immunophilin, cyclophilin. It is this step that prevent the dephosphorylation of nuclear factor of activation T-lymphocyte (NFATs).and its subsequent translocation from cytoplasm to the nucleus in an IL2 mediated process to (6, 18).

So the decreased of total WBCs after treatment with CSA, may be due to inhibition activation of promotes of cell activation as mentioned above and over all immune response. And these results supported by significant decrease of IL-2 in the present study.

**Table (1) Show the Effect of cyclosporine and bone marrow transplantation on total leukocytes (WBCs)  $\times 10^9/L$  in Rabbit**

<b>Time Groups</b>	<b>Zero</b>	<b>In Day10 Before operation</b>	<b>In Day20 After operation</b>
<b>sham group (G<sub>1</sub>)</b>	Ba 9.73 $\pm$ 0.63	Ba 9.49 $\pm$ 0.66	Aa 32.82 $\pm$ 4.05
<b>Treated with CsA 12.5mg/kg/bw (G<sub>2</sub>)</b>	Aa 9.42 $\pm$ 0.84	Cb 5.48 $\pm$ 0.24	Bb 6.89 $\pm$ 0.52
<b>Treated with CsA 25mg/kg/bw (G<sub>3</sub>)</b>	Aa 9.69 $\pm$ 0.77	Cc 3.97 $\pm$ 0.15	Bc 5.58 $\pm$ 0.68

### **Differential Leukocyte count**

Results of the present study revealed significant increases of Neutrophils ,Lymphocytes , Eosinophils, Basophiles and monocyte in rabbit of sham group (1) and these result came in match will results reported by (19,20)

IBM-BM has been used as a potentially curative therapy for patient with wide variety of disease including hematological disease disorder and autoimmune disease Allogeneic bone marrow transplantation (BMT) has been used as a potentially curative therapy for patients with a wide variety of diseases, including hematological disorders, congenital immune Deficiencies, metabolic disorders, autoimmune diseases and solid tumors (21,22).

Therefore the increases of all leukocytes in the present study likely due to improve immune hematopoietic reconstitution as mention by (3).

Influence of bone marrow and inconsistence with increase of total WBCs in the present study.

On the other hand ,orally doses with dose 12.5mg/kg.BW and 25 mg/kg.BW cyclosporine caused to decrease Neutrophil, and lymphocyte this decrease may be explained by inhibitory effect of CAs on produce of many cytokines such as motional by (6,18).and supported by decrease of IL2 that observe in the present study .

**Table (2) Show the Effect of cyclosporine and bone- marrow transplantation on (Neutrophils  $\times 10^9/L$ ) in Rabbit**

<b>Time</b> <b>Groups</b>	<b>Zero</b>	<b>In Day10 Before operation</b>	<b>In Day20 After operation</b>
<b>sham group (G<sub>1</sub>)</b>	Ba 4.11 $\pm$ 0.43	Ba 4.57 $\pm$ 0.36	Aa 7.59 $\pm$ 0.57
<b>Treated with CsA 12.5mg/kg/bw (G<sub>2</sub>)</b>	Aa 4.25 $\pm$ 0.68	Cb 3.04 $\pm$ 0.28	Bb 3.68 $\pm$ 0.17
<b>Treated with CsA 25mg/kg/bw (G<sub>3</sub>)</b>	Aa 4.35 $\pm$ 0.57	Cc 2.34 $\pm$ 0.14	Bc 3.07 $\pm$ 0.41

**Table (3) Show the effect of cyclosporine and bone marrow transplantation on (Lymphocytes  $\times 10^9/L$ ) in Rabbit**

<b>Time</b> <b>Groups</b>	<b>Zero</b>	<b>In Day10 Before operation</b>	<b>In Day20 After operation</b>
<b>sham group (G<sub>1</sub>)</b>	Ba 2.84 $\pm$ 0.06	Ba 2.49 $\pm$ 0.17	Aa 11.75 $\pm$ 1.65
<b>Treated with CsA 12.5mg/kg/bw (G<sub>2</sub>)</b>	Aa 2.55 $\pm$ 0.03	Bb 1.56 $\pm$ 0.87	Cb 1.12 $\pm$ 0.14
<b>Treated with CsA 25mg/kg/bw (G<sub>3</sub>)</b>	Aa 2.74 $\pm$ 0.04	Bc 1.02 $\pm$ 0.69	Bb 0.63 $\pm$ 0.12

### **RBCs, Hb and PCV**

Concerning with effect of BMT on RBCs, Hb and PCV the result revealed presence of significant increase of RBCs, Hb and PCV in Day 20 after operation of IBM-BMT as shown in table (4.5,6and 7).

The bone-marrow transplantation is well established treatment for patient with sever aplastic anemia (23).

Therefor increase of RBCs, Hb and PCV may be result from increase of hematopoietic tissue.

CAS causes decrease of RBCs, Hb and PCV in both doses in Day 10pefor operation as in table (4.5, 6and7).

This result may be due to inhibitory effect of CAS on erythropoietin level as reported by (23).

In other hand its well-known Low testosterone has been shown to be independently associated

with anemia in older individuals ( 24,25) So it's likely that CAS caused decrease RBC, Hb and PVC in the present study by its inhibitory effect on testosterone as mentioned by (26).

**Table (4) Show the Effect of cyclosporine on total Erythrocytes (RBCs)  $\times 10^{12}/L$  in Rabbit**

<b>Time Groups</b>	<b>Zero</b>	<b>In Day10 Before operation</b>	<b>In Day20 After operation</b>
<b>sham group (G<sub>1</sub>)</b>	Aa 5.17 $\pm$ 0.13	Aa 5.09 $\pm$ 0.23	Bb 7.65 $\pm$ 0.03
<b>Treated with CsA 12.5mg/kg/bw (G<sub>2</sub>)</b>	Aa 5.14 $\pm$ 0.27	Bb 3.79 $\pm$ 0.09	Aa 4.86 $\pm$ 0.07
<b>Treated with CsA 25mg/kg/bw (G<sub>3</sub>)</b>	Aa 5.05 $\pm$ 0.15	Bc 3.01 $\pm$ 0.04	Aa 4.78 $\pm$ 0.05

**Table (5) Show the effect of cyclosporine and bone marrow transplantation on total (Hb g/l) in Rabbit**

<b>Time Groups</b>	<b>Zero</b>	<b>In Day10 Before operation</b>	<b>In Day20 After operation</b>
<b>sham group (G<sub>1</sub>)</b>	Aa 128.4 $\pm$ 4.12	Aa 117.3 $\pm$ 2.49	Bb 92.43 $\pm$ 2.71
<b>Treated with CsA 12.5mg/kg/bw (G<sub>2</sub>)</b>	Aa 127.9 $\pm$ 4.33	Bb 105.51 $\pm$ 3.63	Aa 124.45 $\pm$ 3.11
<b>Treated with CsA 25mg/kg/bw (G<sub>3</sub>)</b>	Aa 127.7 $\pm$ 4.16	Bc 92.84 $\pm$ 2.31	Aa 123.19 $\pm$ 3.84

**Table (6) Show the effect of cyclosporine and bone-marrow transplantation on total (P.C.V %) in Rabbit**

Time Groups	Zero	In Day10 Before operation	In Day20 After operation
sham group (G <sub>1</sub> )	Aa 38.57 ± 2.65	Aa 37.76 ± .2.04	Bb 19.89 ± 1.21
Treated with CsA 12.5mg/kg/bw (G <sub>2</sub> )	Aa 38.13 ± 2.81	Bb 29.43 ± 1.78	Aa 32.78 ± 2.38
Treated with CsA 25mg/kg/bw (G <sub>3</sub> )	Aa 37.21 ± 2.09	Bc 21.86 ± 2.02	Aa 33.63 ± 2.13

تأثير عقار السايكلوسبورين على بعض المعايير الدمويه المستخدمة في عملية نقل نخاع العظم في الأرانب

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### الخلاصة

اجريت هذه الدراسة في مختبرات قسم الجراحة والتوليد كلية الطب البيطري جامعة البصرة حيث صممت لمعرفة تأثير عقار السايكلوسبورين على عملية نقل نخاع العظم في ذكور الارانب . استخدمت فيها 30 ارنب ذكر قسمت الى ثلاثة مجاميع كل مجوعه تحوي على عشرة ارناب اثنان منها عوملت كواهب لنخاع العظم و الثمانية الاخرى كانت مستلمه ،وكانت المجموعه الاولى هي مجموعه سيطرة وقد اعطيت المحلول الملحي الطبيعي ،والمجموعه الثانية اعطيت العقار السايكلوسبورين عن طريق الفم وبجرعه 12.5 ملم/كغم من وزن الجسم،والمجموعه الثالثه اعطيت العقار السايكلوسبورين عن طريق الفم وبجرعه 25.00 ملم/كغم من وزن الجسم. وكانت الية العمل كالاتي :

سحب الدم في وقت الصفر، ثم التجريع لمدة عشرة ايام قبل العملية وفي اليوم 11 اجراء العميلة و أخذ عينات من نخاع العظم للفحص. من ثم معاودة التجريع لعشرة ايام اخرى لغاية اليوم 21 ثم سحب الدم من ثم قتل الحيوان واسيخراج الكبد والكلية ونخاع العظم وفحصهم .

اما فيما يخص فحوصات الدم فهي عد كريات الدم البيض والتفريقي وعد كريات الدم الحمر والهيموكلوبين والتراص لكريات الدم الحمر. اضافة الى فحص المناعة الايلايزا لمعرفة تركيز IL2 في الدم .

وقد اقترحت الدراسة في كلا المجموعتين ان عقار السايكلوسبورين يخفض بشكل ملحوظ المناعة من خلال تاثيره المباشر على كريات الدم البيض و IL2 مما بسبب انخفاض الخلايا اللمفية . كما توضح الدراسة ان طريقة نقل نخاع العظم بواسطة الحقن المباشر مباشرةً اكثر اماناً وفعالية من طريقه الحقن عن طريق الوريد .

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