SUJMS
Sana'a University Journal of Medical Sciences

Original Article

The Effect of the Cyclosporine and Other Immunosuppressive Drugs on some Immune Responses among Renal Allograft Recipients.

Arwa M. Othman [1], Ammar A. R. Jabbar [1], Ahmad Y. al-Jaufy [1], Nagib W. Abu-Asba, Mohammed A. Nasher[1] and Yahia A. Rajaá[2]

Department of Microbiology1, Department of Community Medicine2

Abstract

The aim of this study was mainly to investigate certain aspects of the cellular immunity of the renal allograft recipients - under two regimens of triple maintenance therapy - in comparison with healthy individuals. Sixty six renal allograft recipients and 120 healthy individuals (controls) were enrolled. According to the immunosuppressive regimens, patients were subdivided into two groups. Group I comprised 30 renal recipients taking the triple immunosuppressive therapy of cyclosporine A, prednisolone and azathioprine. Group II consisted of 36 renal recipients, also, under triple immunosuppressive therapy with cyclosporine A, prednisolone and mycophenolate mofetil.

Counts of total white blood cells (WBCs), absolute polymorphnuclear (PMN) cells, and absolute lymphocyte, phagocytosis function tests as well as the percentage of T lymphocytes were measured for both the renal transplant recipients and the controls.

The study showed that the absolute PMNs counts was significantly higher in patient groups (I and II) than in the control groups; however, the absolute lymphocyte counts were significantly lower in the renal transplant recipients than in the control group. Comparing the patient groups and the control group, there was no significant difference in the WBCs counts. In addition, the engulfment activity of the phagocytes (PMNs and monocytes), as assessed by internalization of S. aureus, was similar in the three groups. In contrast, the intracellular killing activity of the phagocytes, as measured by nitroblue tetrazolium (NBT) reduction test, was significantly lower in the patients groups (I and II) than in the control group.

Within the renal transplant patients (groups I and II), it was found that the results of all the above tests were similar in both group I and II

Introduction:

Kidneys have been the most frequently transplanted organs for many years. Success of renal transplantation is a function of several variables. However, the major determinant of the graft survival depends on the immune system's ability to recognize foreign substances (antigens) expressed on the graft and to respond to them. Although, this defence mechanism is the base to survive in a hostile world of microorganisms, this defence system becomes a major obstacle in renal and other organs transplantation. The likelihood of acceptance or rejection of the graft is closely related to the extent of genetic differences between the donor and the recipient of the graft. The major histocompatibility complex (MHC) genes encode proteins that are essential to immune recognition: MHC class I and II antigens. T lymphocytes interact through cell surface receptor known as T cell receptor (TCR) with MHC antigens found on the surface of

_Corresponding Author: prof . Ammar A. R.Jabar , Division of applied sciences ,Faculty of medicine and health sciences P.O. Box 13078 sana'a.

antigen presenting cells (APCs).

This interaction triggers both cellular and humoral immune responses resulting in graft rejection 1,2.

The major breakthrough in human renal transplantation began with the introduction of azathioprine in 1962. Azathioprine was soon combined with prednisone, and the combination became the foundation for clinical immunosuppressive therapy, but the event that changed the practice of transplantation was the discovery of the drug cyclosporine A (CSA) in 1980. The initial combination of CSA with low-dose prednisone and the subsequent addition of azathioprine for triple-drug therapy has provided an exceptionally effective regimen in terms of both patient and graft survival Patients:

A total of 186 individuals were enrolled in this study. Sixty-six were adult renal allograft recipients (patients) and the remaining 120 were apparently healthy individuals (controls). The patients were 50 males and 16 females. Their ages ranged between 18 and 57 years. All renal recipients were primary transplant patients and were more than 8 months post-transplantation. The patients constituted two groups. Group I patients were given low doses of the triple maintenance therapy of cyclosporine A, prednisolone and azathioprine and consisted of 30 patients. Group II, which consisted of 36 patients, received also low doses of the triple maintenance therapy of cyclosporine A, prednisolone and mycophenolate mofetil.

The control group consisted of 120 individuals. They were aged between 18 and 57 years. The control group consisted of 90 males and 30 females. The females were not pregnant and were not under contraceptive therapy.

Blood specimens from each individual of the renal transplant patients (groups I and II) were collected in the last five minutes - of the twelve hours - that preceded immediately administration of the next dose of the immunosuppressive drugs (ISDs).

Methods:

White blood cell, absolute PMN cells and lymphocytes counts:

The white blood cell, the absolute PMN cells and lymphocytes counts were made automatically using the Cell-Dyn 3700 Full Automated Blood Counter, USA.

Evaluation of phagocytic functions in vitro

1- Phagocytosis test

The engulfment activity of the phagocytes was assessed by mixing them with the S. aureus, after 30 minutes two blood films were made for each sample. After drying the blood films were fixed, and stained with Giemsa stain. To differentiate between S. aureus that were engulfed by the phagocytes and those which were only attached to them, two to three drops of tannic acid (1% w/v) were added 6.

2- Evaluation of the intracellular killing activities by the phagocytes using the nitroblue tetrazolium (NBT) test:

It is based on that phagocyte (PMN and monocyte) cells ingest and reduce the yellow NBT dye intracellularly to a blue derivative (insoluble crystals) of formazan. The phagocytes' enzymes of the oxidative burst, which are responsible for bacterial killing, are involved in the reduction of NBT dye to deep blue colour. The blue insoluble crystals of formazan were counted directly under a light microscope. The dye is only reduced in activated cells. Therefore, the number of cells reducing the dye gives an estimate of the proportion of the activated cells in vivo7.

The determination of the percentage of T-lymphocytes by the E-rosette test:

The principle of the E-rosette test involves the attachment of sheep red blood cells (SRBCs) to

certain membrane markers (CD2) on the T-lymphocytes when incubated together8.

The statistical analyses of the results:

The Social Package of Statistical Science (SPSS) program (version 11.5; 2002) was used in the statistical analysis of the data.

Results

The effect of the ISDs on the range and mean of WBC counts:

The range (minimum and maximum limits) of the WBC counts among the study groups and their respective means are shown in Table 1. There was no significant difference between the mean WBC counts of the renal recipients and the control group, P = 0.16. Similarly, no significant difference was found between the WBC counts within the renal transplant patients of groups (I and II) (P = 0.30), Table 1.

The effect of the ISDs on the absolute counts of PMNs:

The absolute counts of the PMNs from the patients in both groups (I and II) were significantly higher than those of the control group (P < 0.001). However, within the renal transplant patients taking the two regimens of ISDs, there was no statistically significant difference between their absolute counts of PMNs (P = 0.79), Table 2.

The effect of the ISDs on the absolute counts of total lymphocytes:

The absolute counts of the total lymphocytes in the renal recipients (groups I and II) were significantly lower than those of the control group, P < 0.001, Table 3. On the other hand, there was no statistically significant difference between the lymphocytes counts within the patient groups (I and II), P = 0.41, Table 3.

The effect of the ISDs on the engulfment activities by the patients' phagocytes:

The difference between the mean percentages of the engulfment activities of S. aureus by the PMNs and monocytes - as assessed by the tannic acid staining - from both groups of the renal transplant patients and the non-renal recipients was not significant, P = 0.29, Table 4. Similarly, comparing the two renal transplant patient groups, there was no significant difference between their respective mean percentages engulfment activities of S. aureus by their phagocytes, P = 0.56, Table 4.

The effect of the ISDs on the killing activities by the patients' phagocytes:

Comparing the renal transplant patient and the non-renal recipient groups, there was a significant difference, P < 0.001, between the mean percentages killing activities by their phagocytes - as evidenced by the lower reduction rate of the NBT dye from yellow to blue by the transplant patients' phagocytes as compared with the non-renal recipients, Table 5.

However, there was no significant difference, P = 0.55, between the mean percentages killing activities by the two groups' phagocytes, Table 5.

The effect of ISDs on the percentages of T-lymphocytes:

The determination of the percentage T-lymphocytes was performed, in vitro, by the E-rosette (the rosette formation) test. The two mean percentages of the rosette forming T-lymphocytes from the renal transplant patient groups (I and II) were significantly lower than that from the non-renal recipients, P < 0.001, Table 6. On the other hand, the difference in the mean percentages of the rosette-forming T-lymphocytes between the patients' groups I and II was not statistically significant, P = 0.38, Table 6.

Discussion

The total WBC counts, in this work, were found to be similar in both the patient groups (I and II) and the individuals of the control group. This is in agreement with the fact that cyclosporine A and corticosteroids

have no suppressor effects on bone marrow cells. Also azathioprine and mycophenolate mofetil, at non toxic doses, usually do not cause bone marrow suppression9. Only three cases, one from group I and two from group II, had WBC counts less than 4X109 cell/L. Their WBC counts returned to normal with the reduction in the doses of azathioprine or mycophenolate mofetil that they received. This is in concordance with the results obtained by another study which found that the administration of azathioprine and mycophenolate mofetil even at non-toxic doses was associated in some patients with bone marrow suppression 10.

The absolute counts of PMNs from both patient groups were significantly higher than that obtained from the control group. This result is supported by another study, which reported that prednisolone causes neutrophilia. This was due to an increase in the release of PMNs from the bone marrow, on the one hand, and that prednisolone, on the other, also increases de-margination of PMNs from the inner walls of the blood vessels. These factors therefore increase, indirectly, the PMN numbers in the blood circulation11. Furthermore, another study found that prednisolone inhibited the expression of adhesive molecules which are necessary for the margination of PMNs to the tissues. This leads in turn, to their accumulation in the peripheral blood 12.

The absolute counts of total lymphocytes in the patient groups were significantly lower than those of the control group. Although cyclosporine A has no effect on the lymphocyte progenitors, prednisolone, azathioprine or mycophenolate mofetil have been reported to reduce the lymphocytes number and distribution. In one report, predinsolone caused lymphopaenia due to the redistribution of lymphocytes from the circulation back to the lymphoid tissue13. In addition, another study found that there was a general lymphopaenia, especially in the CD4+ and CD8+ lymphocytes as a result of treatment with methylprednisolone14. Furthermore, it was found also that the use of long-term low-doses of corticosteroids decrease, reversibly, the B-lymphocyte counts and their specific antibody responses15. Our results are also supported by the results from another study which indicated that mycophenolate mofetil creates selective immunodeficiency in both the T and B lymphocytes and inhibits their proliferation 16, 17.

The ability of phagocyte cells, from both the patients and the control group, to engulf S. aureus was similar. However, the killing activity of S. aureus, by the patients' phagocytes was significantly lower than by the controls phagocytes as shown by the NBT reduction test. These findings are consistent with the results obtained from other studies which reported that corticosteroids did not alter the internalization of microorganisms by the phagocytes but impaired their intracellular killing activity 18, 19. In addition, although cyclosporine A has no direct effect on the phagocytes functions, it acts indirectly on the T lymphocytes by inhibiting their IFN-γ production, which in turn, is responsible for phagocytes activation20.

Regarding the subset of T lymphocytes it was found that the percentages of T lymphocytes from the patients - as determined by the percentages of rosette-forming cells in vitro - were significantly lower than those from the control group. This is supported by the findings from other reports that prolonged corticosteroid therapy decreases the total T lymphocyte count because they have cytotoxic effect on the CD4+ lymphocytes 21. Another support was provided by the evidence that renal transplant patients treated with prednisolone with cyclosporine A and azathioprine or mycophenolate mofetil displayed a significant decrease in both CD4+ and CD8+ T cells

and it attributed this significant reduction to prednisolone22. It is also in agreement with other studies, which reported that mycophenolic acid enhanced the rate of apoptosis in T cells after renal transplantation23, 24.

This study showed that under the two ISDs maintenance protocols, the immune responses in the form of total WBCs count, absolute PMNs and lymphocytes counts, phagocytosis – i.e. engulfment and intracellular killing - and total T lymphocyte percentages were similar in the patient groups I and II. Our results were similar to those obtained by another study which assessed immune function in renal transplant recipients under six different maintenance immunosuppressive drug regimes including those used in our study 25.

Table 1: Total white blood cell counts of the renal transplant recipients and the controls.

	Range	Mean	Standard deviation	P value	
				Between the patients and control group	Group I vs * group II
Group I (n = 30)	**(3.0 – 10.7) X 10^9 cell/L**	**7.36 X 10^9 cell/L**	**1.89 X 10^9 cell/L**		
Group II (n = 36)	**(2.1 – 14.0) X 10^9 cell/L**	**7.92 X 10^9 cell/L**	**2.43 X 10^9 cell/L**	**P = 0.16**	**P = 0.30**
Control group (n = 120)	**(4.1 – 10.2) X 10^9 cell/L**	**7.26 X 10^9 cell/L**	**1.54 X 10^9 cell/L**		

vs * = versus .

Table 2: The absolute counts of the polymorph nuclear (PMN) cells in the renal transplant patient groups and the control group.

	Range	Mean	Standard deviation	P value	
				Between the patients and control group	Group I vs group II
Group I (n = 30)	**2.0 – 7.6 X 10^9 cell/L**	**4.3 X 10^9 cell/L**	**1.34 X 10^9 cell/L**		
Group II (n = 36)	**1.1 – 8.1 X 10^9 cell/L**	**4.6 X 10^9 cell/L**	**1.56 X 10^9 cell/L**	**P = 0.009**	**P = 0.42**
Control group (n = 120)	**2.2 – 6.7 X 10^9 cell/L**	**3.9 X 10^9 cell/L**	**1.01 X 10^9 cell/L**		

Table 3: The absolute counts of the lymphocytes in the renal transplant patient groups and the control group

	Range	Mean	Standard deviation	P value	
				Between the patients and control group	Group I vs group II
Group I (n = 30)	**0.9 – 3.8 X 10^9 cell/L**	**2.3 X 10^9 cell/L**	**0.78 X 10^9 cell/L**		
Group II (n = 36)	**0.8 – 4.5 X 10^9 cell/L**	**2.6 X 10^9 cell/L**	**0.84 X 10^9 cell/L**	**P = 0.01**	**P = 0.25**
Control group (n = 120)	**1.3 – 4.7 X 10^9 cell/L**	**2.8 X 10^9 cell/L**	**0.69 X 10^9 cell/L**		

Table 4: The percentages of the phagocytes, that were able to engulf *S. aureus* in *vitro* from the renal recipient and the control groups.

	Range	Mean	Standard deviation	P value	
				Between the patients and control group	Group I vs group II
Group I (n = 30)	78 – 96 %	91 %	3.26 %	P = 0.29	P = 0.56
Group II (n = 36)	86 – 95 %	91 %	2.22 %		
Control group (n = 120)	80 – 98 %	92 %	3.82 %		

Table 5: The percentages of the phagocytes from the renal recipient and the control groups that reduced NBT dye in vitro.

	Range	Mean	Standard deviation	P value	
				Between the patients and control group	Group I vs group II
Group I (n = 30)	24 – 51 %	31 %	5.44 %	P < 0.001	P = 0.55
Group II (n = 36)	19 – 53 %	32 %	6.20 %		
Control group (n = 120)	85 – 97 %	91 %	2.42 %		

Table 6: The percentages of the lymphocytes (that formed E-rosettes) from the renal transplant patient and the control groups.

	Range	Mean	Standard deviation	P value	
				Between the patients and control group	Group I vs group II
Group I (n = 30)	28 – 54 %	37 %	5.91 %	P < 0.001	P = 0.38
Group II (n = 36)	26 – 54 %	36 %	6.30 %		
Control group (n = 120)	55 – 73 %	65 %	5 %		

References:

1-Johanson AH, Hurly CK and Hartzman RJ. Clinical diagnosis and management by lab methods. 19th edition. London. W. B. Saunders. 1996: 195.

2-Paul WE. Fundamental immunology. 5th edition. New York, Lipincott and Willims, 2003; 1357-1361.

3-Murry JE, Merrill JP, Harrison JH, et al. Prolonged survival of human-kidney homograft by immunosuppressive drug therapy. N Engl J Med 1963; 265: 1315.

4-Starlz TE, Weil R, Iwatsuki S, et al. The use of cyclosporine A and prednisone in cadaver kidney transplantation. Surg Gynecol Obstet 1980; 151: 17.

5-Canafax DM, Sutherland ER, Simmons RL, et al. Combination immunosuppression. Three drugs (azathioprine, cyclosporine, prednisone) for mismatched related and four drugs (+ ALG) for cadaver renal allograft recipients. Transpl Proc 1985; 17: 2671.

6-Brousseau P, Payette Y, Tryphoaus H and Blaeky B. Manual of immunological methods. USA, CRC-Press, 1998; 141.

7-Coates TD and Beyer RL. Laboratory evaluation of neutropenia and neutrophil dysfunction. In Rose NR, DeMacario EC, Fahey JL, Friedman H and Penn GM (eds): Manual of clinical laboratory immunology. 4th edition. Washington, DC American Society for Microbiology, 1992; 409.

8-Stites DP. Clinical laboratory methods for detection of cellular immune function. In stites DP, Stobo JD, Wells JV (eds): basic and clinical Immunology. 8th edition. Norwalk, Appleton and Lange, 1994; 286.

9-Danovitch GM. Handbook of kidney transplantation. 4th edition. USA, Lippincott Williams and Wilkins, 2004; 72-134.

10-Danesi R and Tacca DM. Hematologic toxicity of immunosuppressive treatment. Transplantation Proc 2004; 36(3): 703-704.

11-Chapel H, Hanery M, Misbah S and Snowden N. Essentials of clinical immunology. 4th edition. USA, Blackwell Science, 1999; 129-137.

12-Benjamin E, Coico R and Sunshine G. Immunology: a short course. 4th edition. USA, Wiley-Liss, 2000; 389- 390.

13-Smith SL. Immunosuppressive therapies in organ transplantation. Organ Transplant 2002. © 2002 Medscape. http://www.medscape.com.

14-Tornatore KM, Reed K, Venuto R. 24-hour immunologic assessment of CD4+ and CD8+ lymphocytes in renal transplant recipients receiving chronic methylprednisolone. Clin Nephrol. 1995; 44(5):290-8.

15-Fedor ME, Rubinstein A. Effects of long-term low-dose corticosteroid therapy on humoral immunity. Ann Allergy Asthma Immunol 2006; 97(1): 113-116.

16-Halloran PF. Molecular mechanisms of new immunosuppressants. Clin Transplant 1996; 10(1 Pt 2): 118-23.

17-Pascual M, Theruvath T, Kawai T, Tolkoff NR, and Cosimi BA. Strategies to improve long term outcomes after renal transplantation. N Engl J Med 2002; 346(8): 580-590.

18-Tam PE and Hindsill RD. Evaluation of immunomodulatory chemicals: Alteration of macrophage functions in vitro. Toxicology and Applied Pharmacology 1984; 76(1): 183-194.

19-Philippe B, Ibrahim-Granet O, Prevost MC, Gougerot-Pocidalo MA, Sanchez Perez M, Van der Meeren A and Latge JP. Killing of A. fumigatus by alveolar macrophages is mediated by reactive oxidant intermediates. Infection and Immunity 2003; 71(6): 3034-3042.

20-Sung SA, Ko GJ, Kim JY, Kim MG, et al. Desquamative interstitial pneumonia associated with concurrent cytomegalovirus and Aspergillus pneumonia in a renal transplant recipient. Nephr Dial Transplantation 2005; 20(3): 635- 638.

21-Alexander JM, Akporiaye ET and Hersh EM. Immunopharmacology. In Katzing BG. Basic and clinical pharmacology. 7th edition. USA, Appleton and Lange, 1998; 916-944.

22-Sauma D, Mora JR, Fierro A, Morales J, Herzog C, Buckel E, Rosemblatt M, and Bono MR. Low-dose prednisone accounts for a transient reduction on CD4+ and CD8+T cells in renal transplant patients under triple therapy. Transplantation Proc 2002; 34: 3183-3184.

23-Kasprzycka M, Wierzbicki P, Nowaczyk M, et al. Cellcept enhances the rate of apoptosis in Tcells after renal transplantation. Transplantation Proc 1999; 31(1-2): 328.

24-Allison AC. Immunosuppressive drugs: the first 50 years and a glanceforward. Immunopharmacology 2000; 47(2-3): 63-83.

25-Hutchinson P, Steven JC, Robert CA and Holdsworth SR. Laboratory assessment of immune function in renal transplant patients. Nephrology Dialysis Transplantation 2003; 18(5): 983-989.