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Effects of mycophenolate mofetil in ischemic acute renal failure in rats

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SUMMARY

Mycophenolate mofetil (MMF) is a purine synthesis inhibitor commonly used as immunosupresive agent in transplantation. Kidney grafts undergo more or less prolonged cold ischemia after harvesting which results in variable degrees of ischemia reperfusion injury. To determine whether the inhibition of early events of cellular infiltration may influence the severity of damage induced by ischemic acute renal failure, 45 Sprague Dawley rats were given MMF at a dose of 20 mg/kg/day (MMF-rats) by gavage 2 days before (pre-MMF group, n = 15) or after (post-MMF group, n = 15) clamping the left renal artery for 40 minutes followed by rigt-sided nephrectomy (control group, n = 15) received vehicle. Serum Creatinine (Screat) was measured daily in all groups. On the 2nd post-ischemic day Screat was significantly lower (p = 0.001) in pre-MMF group compared with post-MMF group and control group (4 +/- 2 mg/dl post-MMF group vs *1.7 +/- 1.2 mg/dl pre-MMF group, control group 5 +/-2, p < 0.05). Kidney biopsies shown that the histologic damage was 54 +/- 28% in post-MMF group* vs *34 +/- 22% in pre-MMF group and 61 +/- 25% in control group (pre-MMF* vs *post-MMF, p NS). On the 5th day post-ischemic, MMF-rats showed more severe tubulointerstitial necrosis (pre-MMF group: 17 +/- 20%, post-MMF group: 33 +/- 27%) than controls (4 +/- 5%). The severity of ATN was significantly higher in post-MMF group compared with controls (p = 0.01). Tubulointersticial T-lymphocyte (T CD 5) and monocyte (ED 1) infiltration evaluated on the 2nd post-ischemic day was less intense in group I (T CD5: 3 +/- 3, ED 1: 10 +/- 9, cel/mm2) compared to post-MMF group (T CD 5: 10 +/- 4, ED 1: 55 +/- 40) and to control group (T CD 5: 10 +/- 4, ED 1: 64 +/- 46). However, on the 5th post-ischemia day, ED 1 infiltration was significantly higher in post-MMF group (24 +/- 18%) compared to pre-MMF group (5 +/- 5, p NS) and also in pre-MMF group* vs *control group (31 +/- 33, p < 0.05). Our results suggest that MMF given before a renal ischemic insult may reduce the severity of histologic damage resulting from ischemia reperfusion injury.*

Key words: ***Renal failure acute. Immunosupression. Immune system. Lymphocytes T. Mycophenolate mofetil.***

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EFECTOS DEL MICOFENOLATO MOFETIL EN LA INSUFICIENCIA RENAL AGUDA ISQUÉMICA EN RATAS

RESUMEN

El micofenolato mofetil (MMF) es un inhibidor de la síntesis de las purinas comúnmente usado como inmunosupresor. Previo al trasplante, el riñón es sometidos a períodos variables de isquemia, resultando en daño tisular por isquemia-reperfusión. Para determinar si la inhibición de infiltración celular temprana están implicados en la evolución de la IRA isquémica, esta se indujo en ratas Sprague Dawley por oclusión del pedículo renal izquierdo durante 40 minutos con nefrectomía derecha, se le administró MMF (20 mg/kg/día por gavage) 2 días previos a la isquemia (MMF-pre, n = 15), post-isquemia (MMF-post, n = 15) o vehículo (control, n = 15). La creatinina sérica (CS) fue medida diariamente en todos los grupos. Al segundo día postisquemia la CS fue significativamente más baja en el grupo MMF-pre con respecto al grupo MMF-post y control. El análisis histológico reveló que el daño histológico fue (34 ± 22 en el MMF-pre vs *54 ± 28% MMF-post, p NS). Al quinto día las ratas pre-tratadas y post-tratadas con MMF presentaron mayor necrosis tubulointersticial que en el control (17 ± 20* vs *33 ± 27, en el control 4 ± 5%). La infiltración de linfocitos T y monocitos (CD5 y ED 1 respectivamente) fue menor en animales pre-tratados con MMF, mientras en el grupo post-tratado y control fue mayor al segundo y quinto día. Así, en el intersticio a los 2 días, las células T CD5 fueron 10 ± 4 cel/ mm^2 en el MMF-post* vs *3 ± 3 en el MMF-pre (p < 0,01), en el control 10 ± 4. A los 5 días no hubo diferencias significativas entre los grupos. Las células ED1 fueron 55 ± 40 cel/ mm^2 en el MMF-post* vs *10 ± 9 en e MMF-pre, en el grupo control 64 ± 46 (p < 0,05). A los 5 días se mantuvo menor infiltración en el MMF-pre (5 ± 5 cel/ mm^2* vs *24 ± 18 en el MMF-post, grupo control 31 ± 33). Estos resultados sugieren que el tratamiento con MMF previo a la inducción de isquemia-reperfusión puede mejorar el daño renal temprano (2 días).*

Palabras clave: ***Insuficiencia renal aguda. Inmunosupresión. Sistema inmunitario. Linfocitos T. Micofenolato mofetil.***

INTRODUCTION

Acute renal failure (ARF) is a common disease with high morbidity and mortality affecting almost 5% of hospitalized patients.[1] Treatment options for ARF are limited and the mortality rate still is 30%-50%.[2] Ischemia-reperfusion (I-R)-induced renal damage is one of the most common causes of ARF and it is characterized by decreased local oxygen, cellular metabolism impairments with decreased levels of ATP metabolic substrate and glucose, inflammation, free radicals production, apoptosis, and necrosis, all of them leading to deterioration of the tubular cells.[4-8] Renal damage produced by I-R generates an inflammatory response causing damage in associated tissues with severe deterioration of epithelial cells, expression of adhesion molecules, leukocyte infiltration, and cytokines production.[9-11] Depending on ischemia severity and tissue susceptibility, ischemic damage may result in permanent impairment of renal function due to cellular death, or to temporary function compromise due to sub-lethal damage of renal cells with subsequent recovering.[11-13] Renal ischemia leads to epithelial cell damage at the S3 segment of the proximal tubule and its consequences greatly depend on the regeneration of these specific cells.[5,6,14,18] The outer portion of the renal medulla is particularly susceptible to ischemic injury and is primarily responsible of the clinical and pathophysiologic presentation of ARF.[6,18] Some investigators have suggested that inflammatory cells, particularly lymphocytes, macrophages, and neutrophils play an important role in ischemic ARF. Lymphocytic infiltration has been observed in human kidneys after ischemia, as well as expression of the adhesion molecule ICAM-1, which is also related with neutrophilic infiltration into ischemic renal tissue.[14,16,17,19,20]

Other authors have shown that the administration of the anti-ICAM-1 monoclonal antibody protects against ischemic ARF.[21,22] Another route that contributes to I-R injury is the cytotoxic component of infiltrating cells such as macrophages, lymphocytes, and neutrophils that comprises oxygen reactive species and nitric oxide; thus, inhibition of the in-

ducible nitric oxide synthase (iNOS) by antisense oligonucleotides protects proximal tubular cells against ischemic/hypoxic damage *in vivo*.[23,24] Renal transplantation implies that kidneys are submitted to ischemia during variable periods leading to ischemic damage.

Different immunosuppression protocols have been used in renal transplant with excellent results, although transplant survival is unpredictable. Some of these immunosuppressive agents are also nephrotoxic, such as cyclosporin A and tacrolimus, and may cause renal injury through non-immunological mechanisms such as ischemia, hypertension or hyperlipidemia. The response to renal injury is characterized by leukocytic infiltration, production of inflammatory cytokines, and renal function impairment.[25,26]

Mycophenolate mofetil (MPM) is an immunosuppressant commonly used in transplanted patients; it is a non-competitive reversible inhibitor of 5′-mono phosphate inosine dehydrogenase, which controls the synthesis of guanosine triphosphate; its mechanism of action is by depletion of intracellular levels of guanosine triphosphate (GTP) and deoxyguanosine triphosphate (dGTP), which leads to suppression of DNA synthesis in T and B lymphocytes stimulated with antigens or mitogens. It does not inhibit early events of lymphocytes activation including cytokine production. It also inhibits antibody formation and production of adhesion molecules on the cellular surface. It has been used to prolong transplant survival in animal and human models, and to treat inflammatory conditions such as rheumatoid arthritis, also in 5/6 nephrectomy to reduce cellular infiltration within the tubule and interstitium with decreased renal damage been observed in the remnant kidney.[27,34] Recent investigations have shown that MPM therapy decreases interstitial infiltration by macrophages and lymphocytes as well as myofibroblasts and inhibits cellular repair and regeneration in the proximal tubule, aggravating renal dysfunction.[34] It was recently reported that MPM therapy significantly damages renal function and reduces compensatory hypertrophy in the remnant kidney, cellular proliferation, myofibroblasts infiltration, and collagen III deposition in subtotal nephrectomy in rats.[36]

Recovering from ARF requires replacement or regeneration of epithelial tubular cells. This process is accompanied by changes in the expression of genes encoding for growth modulating factors.[37,38] It has conventionally been suggested that tissular damage is repaired by means of proliferation of surviving parenchymal cells.[39]

Since it has been shown that rats treated with MPM after unilateral I-R induction and contralateral nephrectomy have higher number of normal tubules and an increase in the number of tubules with total necrosis with lymphocytes and monocytes reduction, the aim of this study was to determine whether previous administration of MPM to inhibit early events of cellular infiltration might be implicated in the course of ischemic ARF. With this goal, it was decided to use MPM two days before the induction of I-R in order to inhibit cellular infiltration and proliferation.

MATERIAL AND METHODS

Experimental design

Male Sprague Dawley rats weighting 320-400 g were used. Three groups of rats were used, 2 experimental groups (n = 15 in each group) and a control group (n = 15). Both experimental groups, MPM-pre and MPM-post, and the control group kept at room temperature within the physiological range were submitted to medial laparotomy under anesthesia with ketamine (75 mg/kg IM) and diazepam (5 mg/kg IM)[40] with dissection of both renal pedicles. Ischemia was induced by occluding the left renal pedicle for 40 minutes by means of a non-traumatic clamp, followed by right nephrectomy. The MPM-pre group received MPM (Roche. New York. USA) 48 hours before ischemia induction and the MPM-post group approximately 18 hours after at a dose of 20 mg/kg/day, by gravity, until the end of the experiment (5 days). The control group received water. All animals were allowed to take food and water *ad libitum*. Blood was drawn daily by puncturing the caudal vein to determine serum creatinine. Rats from each group were sacrificed at days 2 and 5 after I-R induction and the kidney was extracted for histological study.

Histological studies

Tissue preparation

Three-millimeter wide renal tissue samples were taken from the superior and inferior poles. One portion was fixed in 10% formalin and Methyl Carnoy's for hematoxylin-eosin and PAS study and then were included in paraffin, and the other portion was fixed in Tissue Freexing Medium preservation media and were frozen in a mixture of dry ice and acetone and stored at –70º C for immunohistological study.

Peryodic acid Schiff (PAS) staining for paraffin samples

Biopsies in paraffin were stained with peryodic acid Schiff or PAS.[41] Between 200 and 250 tubules of each tissue sample were examined under light microscopy with 60¥ and the 10¥ lenses. All the tubules in the cortical and yuxtaglomerular areas of each biopsy sample were examined and classified according to the percentage of the cross sectional area showing histological damage.[42]

The severity of the tubular lesion was classified by the following criteria: Normal or no injury = intact tubules with normal cells and preservation of the brush border; Tubular cell damage = loss of the brush border; Focal tubular necrosis = loss of the brush border with intratubular detritus and preservation of the tubular basal membrane; Complete necrosis = tubule necrosis and rupture of the tubular basal membrane with cytoplasm extrusion.[6]

Fluorescein indirect immunofluorescence staining for frozen samples

Immunohistological studies were done on 4-mm wide frozen sections obtained from renal tissue of the studied rats with appropriate monoclonal antibodies (murine anti-rat CD5) (Biosource; USA) to detect lymphocytic infiltration, which bind to the CD5 antigen, which is mainly expressed in T lymphocytes and barely in a subpopulation of B lymphocytes. A second anti-mouse IgG fluorescein-conjugated monoclonal antibody from rat is added to detect the former reaction (Accurate Chemical, USA). Monocytes-macrophages determination is based on the detection of monoclonal antibodies were the former (mouse anti-rat ED-1) (Biosource; USA) binds to the antigen. A second fluorescein-conjugated anti-mouse IgG monoclonal antibody from rat is added to detect the former reaction (Accurate Chemical; USA).

Creatinine determination

0.5 mL of peripheral blood without anti-coagulant were extracted from each study rat by puncturing the caudal vein. Baseline serum creatinine values and daily for the experimental 5 days were determined. The measurement was done according to Jaffé's methodology.[43]

Statistical methodology

The results are expressed as mean and standard deviation for the study groups. The results at days 2 and 5 were compared with the Turkey's post-test ANOVA test.

RESULTS

The administration of MPM before ischemia-reperfusion slightly increases the creatinine level

I-R induction markedly increased creatinine levels from the first post-ischemia day, being higher during the second day (Fig. 1). Serum creatinine during the second day was significantly lower in the pre-treated group than in the other two groups (Fig. 1). At day 5, creatinine came back to normal in all groups (Fig. 1).

By studying the changes in serum creatinine levels, we observed that pre-treatment with MPM results in functional protection from I-R injury since the MPM pre-treated group kept low creatinine levels from the first post-ischemia day as compared with the post-treated group and the control group in which creatinine levels raised from the beginning, with higher values during the second day, and all three groups achieving normal values at day 5.

Histological changes in the renal tissue at days 2 and 5 after ischemia-reperfusion

A number of rats in MPM-pre (n = 8), MPM-post (n = 5) and control (n = 3) groups were sacrificed

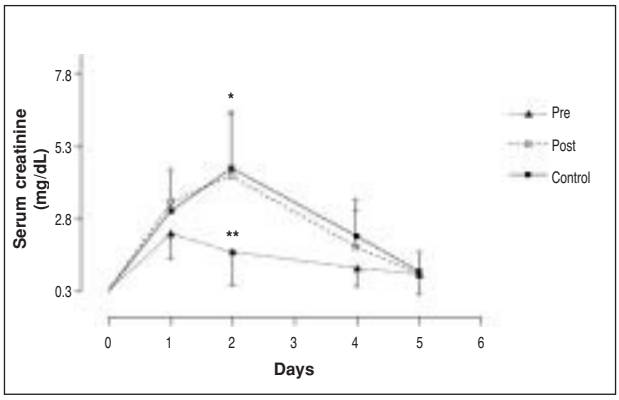


Fig. 1.—Serum creatinine levels in MPM-treated rats with ischemic ARF and controls. *The rats of the MPM-pre and MPM-post groups received MPM (20 mg/Kg/d); 2 days before the induction of ischemia-reperfusion and 1 day after, respectively; rats of the control group received the vehicle (distilled water). The results represent the mean ± standard deviation for each day. * p < 0.01 ** p < 0.001. ARF: Acute renal failure, MPM: Mycofenolate mofetil.*

at day 2 after ischemia–reperfusion to assess renal tissue, and at day 5: MPM-pre (n = 7), MPM-post and control (n = 9) groups. Tubulointerstitial damage was present in all 3 groups being higher in non-treated rats as compared with those treated with MPM were less histological changes and less tubulointerstitial damage were observed. Lymphocytes and macrophages infiltration was determined in renal biopsies taken at days 2 and 5.

Histological quantification of degree of necrosis (PAS) at days 2 and 5

The degree of histological damage was determined with PAS. The damage extent was assessed in terms of percentage of cross sectional area of tissue showing some degree of histological damage. Table I shows the histological findings; at day 2 tubular necrosis was more intense in the control group where 61% ± 25% of the tubules showed necrosis (Fig. 2). Although tubular necrosis was present in MPM-pre (34% ± 22%) and MPM-post (54% ± 28%) groups, the difference was not statistically significant. At day 5, tubular necrosis was more intense in the MPM-post group; it was observed that rats in this group presented necrosis of epithelial tubular cells with intratubular detritus and in some cases with preserved basal membrane. It was determined that necrosis in the MPM-post was 33% ± 27% vs. 4% ± 5% in the control group, the difference being statistically significant ($p < 0.05$). In the MPM-pre group, it was 17% ± 20%. Although it showed a decreasing trend, there were no significant differences when comparing to MPM-post and control groups.

Lymphocytic infiltration (CD5) at days 2 and 5 after ischemia-reperfusion

Lymphocytic infiltration was demonstrated by the detection of the CD5 molecule.

The presence of CD5-positive cells in the rats of the MPM-post group at day 2 was 10 ± 4 vs. 3 ± 3 cells/mm^2 in the MPM-pre group. Previous therapy with MPM decreased infiltration. The difference was statistically significant ($p < 0.01$). In the control group it was 10 ± 4 cells/mm^2; when compared with the MPM-pre group the differences were statistically significant ($p < 0.05$), whereas they were not when compared with the MPM-post group (Fig. 3A and 4). Infiltration of CD5-positive lymphocytes at day 5 was increased in the control group as compared to day 2. In the MPM-pre group, the number of CD5-positive cells slightly increased (6 ± 5 cells/mm^2) and in the MPM-post it was slightly decreased (8 ± 8 cells/mm2) as compared with day 2. In the control group it markedly increased (19 ± 23 cells/mm^2). However, the differences were not statistically significant between the experimental groups and the control group (Fig. 3B).

Monocytic infiltration (ED1) at days 2 and 5 after ischemia-reperfusion

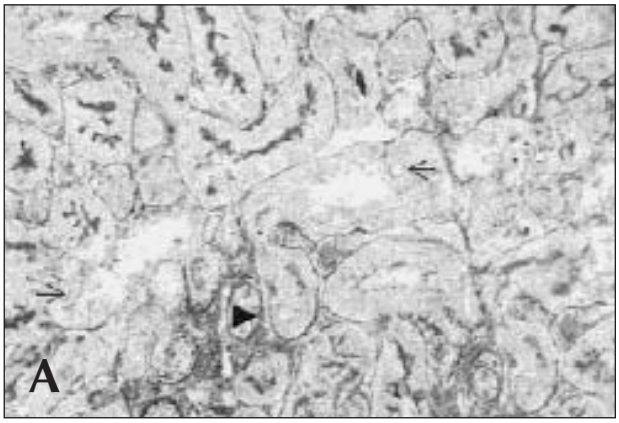
The presence of monocytes was demonstrated by detection of the ED1 molecule. At day 2, there was a higher number of ED1-positive cells (64 ± 46 cells/mm^2) in the control group then in the MPM-pre group (10 ± 9 cells/mm^2). The difference was statistically significant ($p < 0.05$). In the MPM-post group, it was 55 ± 40 cells/mm^2. When comparing MPM-pre and MPM-post groups, significant differences ($p < 0.05$) were observed (Fig. 5A and 6). The differences between the control group and the MPM-post group were not significant.

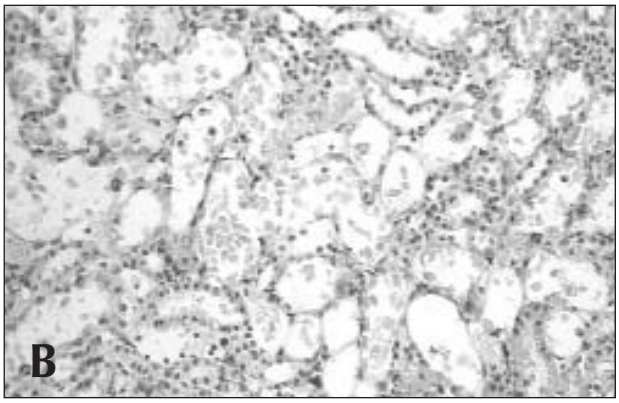
At day 5, it was observed that the number of ED1-positive cells was decreased as compared to day 2. Infiltration of ED1 cells persisted higher in the control group (31 ± 33) and in the MPM-post group (24 ± 18 cells/mm^2) than in the MPM-pre group (5 ± 5 cells/mm^2). There were no significant differences between groups (Fig. 5 B).

Table I. Histological findings in renal tubular cells

	MPM-pre		MPM-post		Control	
	Day 2	Day 5	Day 2	Day 5	Day 2	Day 5
No injury %	65.6 ± 7.0	82.9 ± 20.1	46.0 ± 27.7	66.7 ± 26.8	40 ± 25	96.0 ± 5.3
Focal necrosis %	7.5 ± 2.6	7.8 ± 6.4	9.0 ± 6.9	16.2 ± 11.2	26.0 ± 2.0	3.3 ± 4.4
Complete necrosis	34.4 ± 22	17.2 ± 20.3	54.0 ± 27.7	33.3 ± 26.8*	61 ± 25.0	4.0 ± 5.3*

Values are expressed as mean ± standard deviation; n = 3-9 biopsies per group, for each sacrifice set. Tubular injury is expressed as percentage of the examined area. *Corresponds to statistical significance between groups *$p < 0.05$.





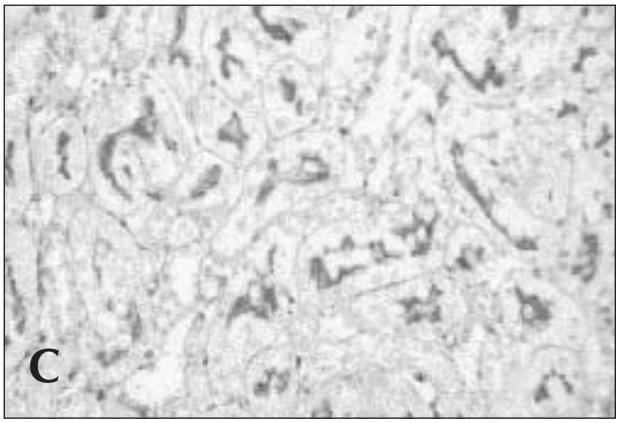
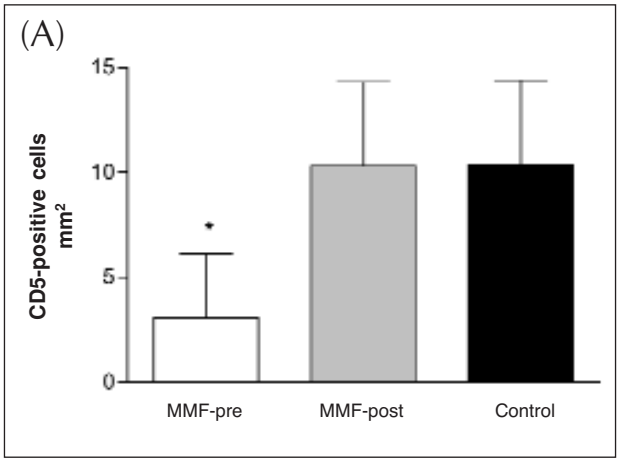


Fig. 2.—Structural changes in the renal túbulo-interstitium at days 2 and 5 after ischemia-reperfusion. *(A) PAS staining showed at day 2 in the control group tubulointerstitial necrosis with tubular dilation (arrows) and damaged areas with sclerosis and dilation (arrowhead). (B) At day 5, necrosis of tubular epithelial cells with intratubular detritus was shown in the MPM-post group. (C) The biopsy at day 5 in the control group shows normal appearance. Magnification: 400¥. PAS: Peryodic acid Schiff.*



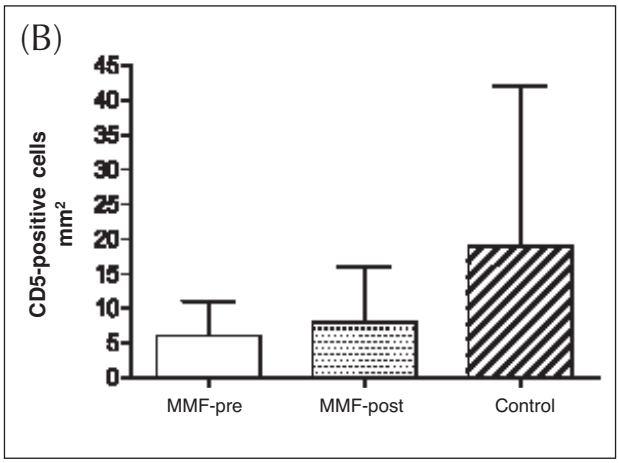


Fig. 3.—CD5-positive t lymphocytes in the renal tubule-interstitium at days 2 and 5 after ischemia-reperfusion. *The biopsies from the three groups of rats sacrificed at days 2 and 5 were treated with monoclonal antibody (murine anti-rat CD5) and then with a second fluorescein-conjugated rat anti-mouse IgG antibody. The values are expressed as mean ± standard deviation for rats from each group; positive cells were evaluated in at least 200 fields per rat. (A) At day 2, biopsies from the MPM-pre (n = 8), MPM-post (n = 5) and control (n = 3) groups were evaluated; the difference between the MPM-pre and MPM-post groups, and the MPM-pre and control groups were statistically significant, $p < 0.01$ and $p < 0.05$, respectively. (B) At day 5, biopsies from MPM-pre (n = 7), MPM-post (n = 9) and control (n = 9) groups were studied; there were no significant differences.*

DISCUSSION

Ischemia-reperfusion injury is a common cause of renal dysfunction in transplanted patients and is an additional risk factor for the development of acute renal failure.[25,44] The kidneys, which are normally highly perfused, are particularly vulnerable to hipop-

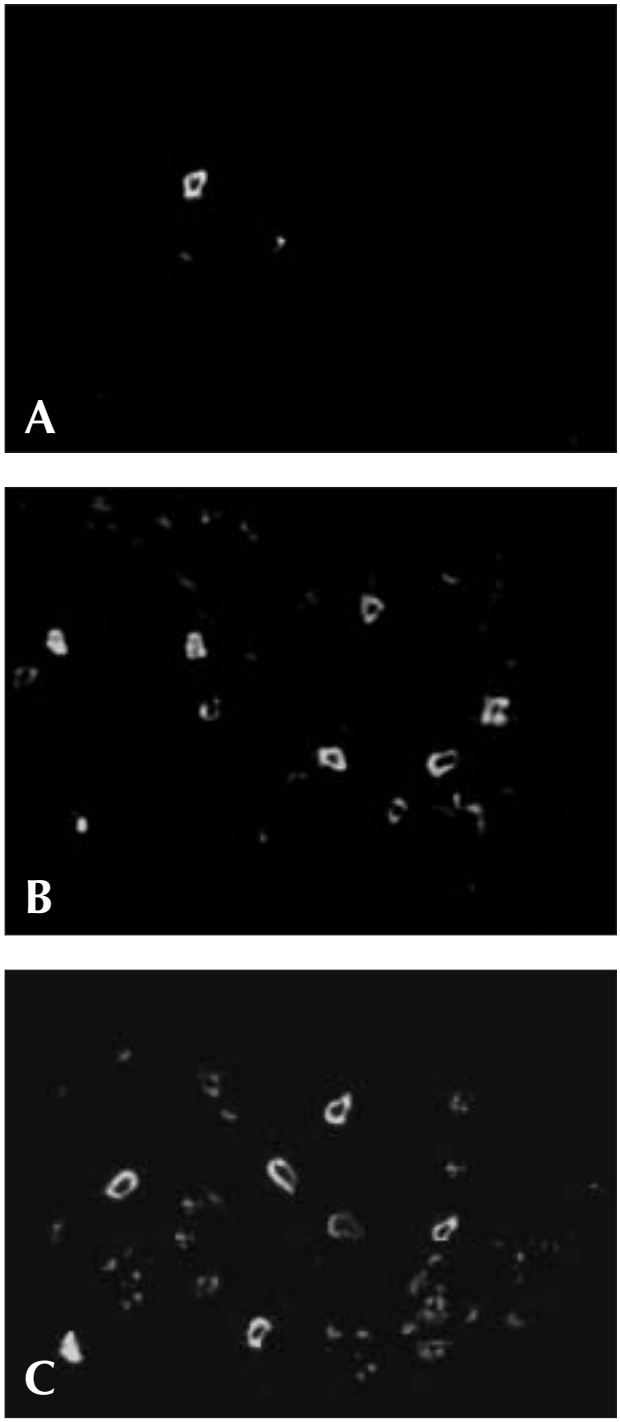


Fig. 4.—CD5-positive t lymphocytes infiltration at day 2 in MPM-pre, MPM-post and control groups. *(A) The biopsies treated with anti-CD5 monoclonal antibody and a second fluorescein-conjugated antibody showed decreased lymphocytic infiltration at day 2 in the MPM-pretreated group. (B) The biopsies at day 2 in the MPM-post group and in the control group (C) show lymphocytic infiltration; Magnification: 400¥. MPM: Mycofenolate mofetil.*

erfusión- or ischemia-induced impairment.[45] The injury due to ischemia–reperfusion generates an inflammatory response causing damage to the renal tissue, associated with severe deterioration of epithelial cells, endothelium activation, proteases activation, cytokines production, and adhesion molecules expression with the subsequent leukocytes infiltration

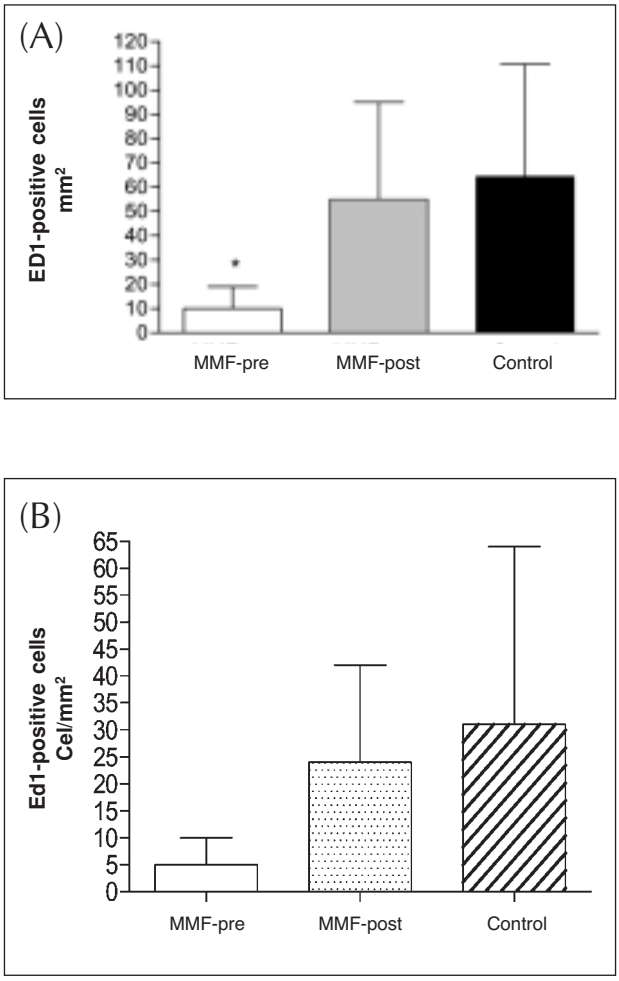


Fig. 5.—ED1-positive monocytes in renal tubule-interstitium at day 2 after ischemia-reperfusion. *The biopsies from the three groups of rats sacrificed at days 2 and 5 were treated with monoclonal antibody (murine anti-rat CD5) and then with a second fluorescein-conjugated rat anti-mouse IgG antibody. The values are expressed as mean ± standard deviation for rats from each group; positive cells were evaluated in at least 200 fields per rat. (A) At day 2, biopsies from the MPM-pre (n = 8), MPM-post (n = 5) and control (n = 3) groups were evaluated; the difference between the MPM-pre and MPM-post groups, and the MPM-pre and control groups were statistically significant, p < 0.05. (B) At day 5, biopsies from MPM-pre (n = 7), MPM-post (n = 9) and control (n = 9) groups were studied; there were no significant differences.*

within the kidney.[3,9,11] Cellular recovering after ARF is the only way to recover from damage, the mechanisms mediating on this recovery being complex and, so far, poorly understood.[35] The rat model of I-R injury-induced ARF has recently been used to assess its effects on the kidney, showing that it is associated with renal damage.[3,46] There is evidence of the delay in functional and structural recovering from post-renal transplantation ischemia, as compared to acute renal failure from a different etiology, which has been linked with the use of immunosuppressants.[3,46,47] The most frequently used immunosuppressant drugs in renal transplantation are calcineurin inhibitors, among which are cyclosporin A and tacrolimus that are associated with adverse effects, especially nephrotoxicity.[48] Also MPM, a non-competitive reversible inhibitor of de novo synthesis of purines, with low nephrotoxicity suppresses T and B lymphocytes and monocytes proliferation.[32,49,50] MPM has been used in rat ischemia-induced ARF model to assess its effects on the kidney, showing that it decreases the proliferation of tubular cells.[3] We have previously reported that MPM, administered I-R induction, aggravates renal damage; however, there are differences between both models: in the first one, ischemia induction was on both kidneys for 45 minutes; in the present work, ischemia induction was for 40 minutes an only on the left kidney, then contralateral nephrectomy was performed. It is likely that ischemia induction for longer time could explain higher damage in the MPM post-treated group previously reported.[51] In the present study, it was planned to administered MPM before in order to inhibit early effects of cellular infiltration and assess their implication on the course of ischemic ARF.

Recently, some investigators have reported a rat model of I-R and contralateral nephrectomy, with MPM therapy given after ischemia, and functional recovering after ischemia was not influenced by MPM.[3] This feature is in agreement with what has been observe in our study, where 2 days after I-R, creatinine levels in the post-treated group and the control group were similar, normalizing within 5 days. The difference with our model lies on previous administration of MPM, which is given 48 hours before I-R induction; in this group, creatinine levels were significantly reduced at day 2 as compared with post-treated and control groups. Similar results were reported in previous studies using bilateral ischemia in rats with pre-treated MPM 2 days before the ischemia, observing functional protection of I-R renal injury at day 2, which supports the cytoprotective effect of MPM by decreasing the hemodynamic and tubular function changes.[46] Studies on I-R models suggest that renal leukocytes infiltration is related with tissular damage, and many of these studies point towards neutrophils, lymphocytes, and macrophages as important mediators of this process, with significant changes in the cellular infiltrate in this pathology being reported.[10,41,52] It has been published that mononuclear leukocytes start showing up within the renal interstitium as soon as ischemic ARF takes place.[19] It has previously been suggested that MPM does not directly protect against ischemic injury in rat models but that it acts through reduction of RANTES gene expression, which is important in macrophages and T cells chemotaxis, and of the expression of a macrophage-associated inflammatory factor, so that there is a decrease in renal damage,

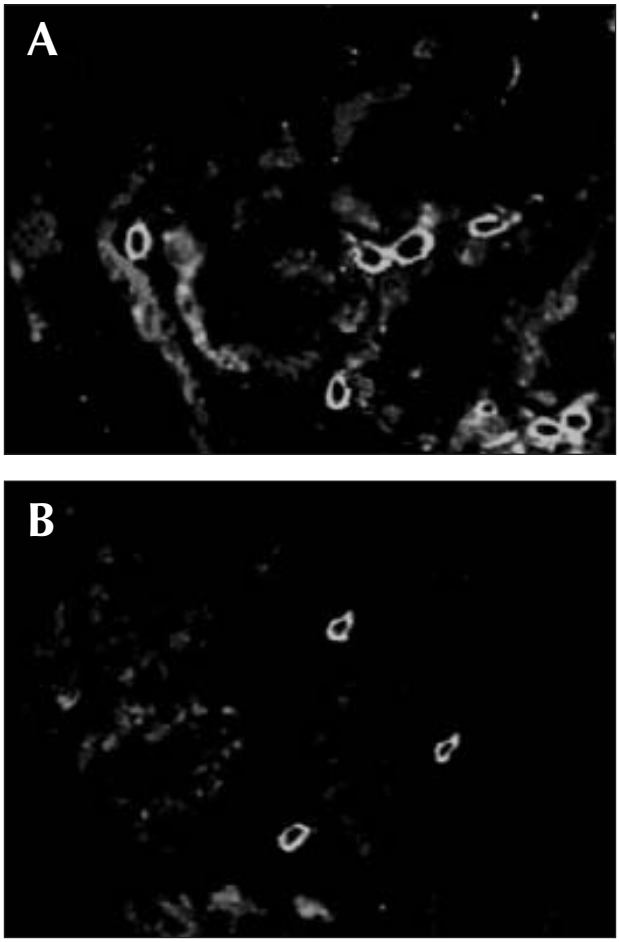


Fig. 6.—ED1-positive monocytes infiltration at day 2 in the control group and the MPM-pretreated group. *(A) The biopsies treated with anti-ED1 monoclonal antibody and a second fluorescein-conjugated antibody showed decreased monocytic infiltration at day 2 in the control group. (B) The biopsies at day 2 in the MPM-pre group show decreased lymphocytic infiltration; Magnification: 400¥. MPM: Mycofenolate mofetil.*

which highlights the contribution of leukocytes infiltration in the model of I-R renal damage.[35] This may explain the results of our study where the highest macrophages infiltration was seen at day 2 in the control group occurring at the same time that the highest histological damage seen as necrosis. Both MPM-treated groups presented less macrophage infiltration with less necrosis, the lowest degree of tissular damage associated with lower macrophage infiltration seen in the MPM pre-treated group. It has been previously reported that MPM decreases initial proliferation of tubular cells and consequently decreases tubular regeneration.[3] Other authors reported similar observations of MPM adverse effects on cell growth and cytokines release by tubular epithelial cells.[53] This could be related with the outcomes of our model regarding 5 days after ischemia, where we observed minimal tubular necrosis in the control group as compared to MPM-treated groups that showed higher degree of necrosis, being higher in the post-treated group. The differences observed in necrosis degree between both MPM-treated groups, the pre-treated group showing lower histological damage, suggest that MPM administered before I-R induction both inhibits initial proliferation of tubular cells, and therefore their regeneration, and inhibits early cellular infiltration since MPM blocks the expression of adhesion molecules in the vascular endothelium, which are required to initiate migration of inflammatory cells; this explains less infiltration of macrophages and lymphocytes in this group, whereas in the post-treated group MPM administration did not occur before 18 hours ischemia induction, which may explain the higher level of tissue damage and the higher number of infiltrating macrophages and lymphocytes in this group. That is to say, MPM differently affects ARF and I-R damage depending on the time at which we assessed renal function. We may explain it as follows: MPM inhibits cell migration to the kidney and cell proliferation, and even proliferation of renal cells themselves; these cells may be both harmful cells that migrate at an early stage causing damage, such as neutrophils, and those migrating and proliferating after the damage has occurred and having regenerative functions; this is why MPM-treated animals have less damage at the beginning (lower infiltration of aggressing cells) but also less regeneration after 5 days post-ischemia (lower infiltration of regenerating cells. Exactly the opposite would occur in the control group. Previous investigations have suggested that damaged tissue is repaired by proliferation of surviving parenchymal cells.[39] The origin of cellular regeneration taking place in the kidney after renal injury is not well defined. It is not known whether there are stem pluripotential cells or not in the adult kidney able to differentiate into renal epithelial cells.[54] The observation of the adverse effect of MPM on epithelial cells growth seems interesting assuming it is considered an anti-proliferative selective agent on lymphocytes due to the potent inhibition of de novo synthesis of the 5′-guanosine triphosphate dehydrogenase required for its synthesis.[55] Apparently there exist the possibility that MPM may affect high-activity cells and proliferative capacity similar to that observed in tubular epithelial cells and in smooth muscle cells in rats with aortic allograft, which are inhibited by MPM.[56] These findings corroborate the lack of exclusivity in the anti-proliferative action of MPM on lymphocytes. Previous studies showed that transplantation of hematopoietic progenitor or stem cells (HSC) may contribute in renal tubular regeneration after ischemia-reperfusion injury. These investigations showed that mouse HSC may be integrated within regenerating kidneys and that integration increases after ischemia–reperfusion injury, with the observation of decreased expression of the common leukocyte marker CD45.2 and increased expression of renal cell markers; this shows that HSC can differentiate into renal tubular cells during the regeneration that takes place after ischemia-reperfusion injury in a murine model.[57] It seems interesting to speculate on the possibility of an MPM-mediated injuring mechanism by acting on the integration of these stem cells in renal tissue and inhibiting their proliferation and differentiation into renal tubular epithelial cells.

To conclude, this work supports that MPM administered after the induction of ischemia-reperfusion does not modifies renal damage and that previous therapy with MPM improves early renal damage (two days).

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