Effects of methylprednisolone pulses on renal function of guinea pigs

Dimitrios Gourgiotis, Lydia Nakopoulou*, Emmanuel Kavazarakis, Maria Moustaki, Marios P Zeis, Theano Tsoukatou & Petros M Zeis

Second Department of Pediatrics and Department of Pathology*, University of Athens, Greece
“P&A Kyriakou” Children’s Hospital, Thibon and Levadias, 115 27 Athens, Greece

Received 22 October 2001; revised 8 July 2002

Methylprednisolone produced a dose dependent significant increase in glomerular filtration rate, a significant decrease in sodium excretion, and a significant increase in urinary alkaline phosphatase activity in guinea pigs. The renal histology in groups with 4, 5 and 6 doses revealed mild degenerative changes in the tubular epithelial cells. The results suggest the beneficial effects of methylprednisolone pulse therapy on renal function mainly by increasing glomerular filtration rate with only minimal side effects.

Methylprednisolone (MP) pulse therapy has been broadly used to reverse acute rejection in renal transplant recipients1,2, to treat renal diseases of immunological origin3,4 and focal and segmental glomerulosclerosis4,5,6. However, the direct effects of repeated administration of pharmacological doses of glucocorticoid hormones on renal function remain controversial. Either a significant increase5,7,8 or a reduction in glomerular filtration rate (GFR)9,10 by MP have been reported.

In the present study the effects of MP pulses have been investigated on renal function of normal guinea pigs including the histological changes following repeated MP pulses.

Materials and Methods

Adult guinea pigs (49) weighing 440-860g were used. They had free access to food and water. Seven guinea pigs were used as controls (group C), while 42 were divided into 6 groups of 7 animals each (groups I, II, III, IV, V, VI). Every second day a dose of 30mg/kg body weight of MP was given ip, as it was given iv in pediatric patients with severe renal disease5,7,9. The animals of group I received one dose (1st day); group II received two doses (1st, 3rd day); group III, three doses (1st, 3rd, 5th day); group IV, four doses (1st, 3rd, 5th, 7th day); group V, five doses (1st, 3rd, 5th, 7th, 9th day) and group VI received six doses (1st, 3rd, 5th, 7th, 9th, 11th day). All animals were kept in metal cages for 24hr urine collection following the last ip MP injection. Urine collections were also made in the controls. The animals were then anesthetized with pentothal; blood was drawn by cardiac puncture, after which the animals were sacrificed and kidneys removed for histological examination by light microscopy. The body weight of each animal was determined before and at the end of the experiment. Serum sodium11,12, serum creatinine13 and blood urea14 were measured using standard laboratory techniques. In a sample from the total 24hr urine collected, sodium11,12, creatinine13 and urea14 levels were measured together with the urinary activities of alkaline phosphatase (AP)15 and lactic dehydrogenase (LDH)16,17 using kits of Bio-Merieux. The enzyme activities are expressed in μmol/min of urine excreted in 24 hr. Creatinine clearance was estimated for every animal in each group by applying the formula Cr=U/P, where Cr=creatinine clearance, U=creatinine in a sample from the 24hr urine collection, V=total 24hr urine volume divided by 24hr and P=plasma creatinine. The Cr for every experimental animal was corrected for body weight and expressed in g body weight, as ml/hr/g. Using the levels of serum and urine creatinine (Scr and Ucr) and serum and urine sodium (SNa and UNa) for every animal, the fractional excretion of sodium (fENa) was estimated using the formula fENa= [(UNa x Scr) / (SNa x Ucr)] x 100. The total sodium in the 24 hr urine collection was expressed in μEq/hr/g. Sections (3μm thick) from the kidneys were cut with microtome, fixed and embedded in paraffin, stained with hematoxylin and eosin, PAS, Masson trichrome and silver methenamine and examined by

1*Correspondent author
Phone: +3010-7793000
Fax: +3010-7774383
E-mail: march193@hol.gr
light microscopy. Statistical analysis was performed using ANOVA for comparisons among groups and Student's t-test for unpaired observations between groups.

Results
The body weight of the guinea pigs increased by 5-10% following MP administration, especially in the groups V and VI. The mean GFR as measured by creatinine clearance increased (from 0.050±0.005 to 0.106±0.010) following MP pulses (Table 1, Fig. 1a) and this difference was statistically significant among groups (P<0.05). When compared to controls (group C) a significant increase was observed in all groups except from group I. Similar results were obtained for urea clearance. The mean fractional excretion of sodium (fENa) gradually fell from 1.03±0.09 to 0.15±0.03 after MP pulse (Table 1, Fig. 1b) and the observed difference was statistically significant (P<0.05). Compared to controls (group C) a significant decrease was observed in all groups, except from group I. Mean total sodium excretion in the 24hr urine collection expressed in μEq/hr/g fell significantly from 372.28±35.30 to 155.73±25.43 after each MP pulse (Table 1). Comparison among groups revealed a significant difference (P<0.05) whereas comparing to controls (group C) a significant decrease was observed in all groups, except from group I (group I P>0.05, group II P<0.05, group III P<0.05, group IV P<0.01, group V P<0.01, group VI P<0.01).

Urinary AP activity increased from 14.00±7.00 to 104.00±6.00 in all groups following MP administration (Table 1, Fig. 1c) and this difference reached a significant level among groups (P<0.05). Compared to controls (group C), however, this increase was observed in all the groups except in group I and II. In all groups treated with MP, LDH activity was not significantly different either among groups or between each treated group and (group C) controls (data not shown), indicating that MP did not produce extensive renal cell damage. The histological changes corresponded with the functional observations. In groups IV, V and VI which received 4, 5 and 6 MP pulses respectively, light microscopy revealed mild degenerative changes in the brush borders of tubular epithelial cells (Fig. 2).

Discussion
In spite of the fact that MP has minimal sodium retaining activity, repeated doses may be responsible for sodium and water retention and therefore for the increased body weight of the experimental animals. In the present study, the gradual fall in fENa and total sodium excretion, which indicates a parallel rise of sodium reabsorption, appears to be the result of the repeated doses of MP. Indeed, chronic MP administration (more than 4 pharmacological doses) leads to a significant increase in tubular reabsorption of sodium.

However, despite this effect of multiple doses, the present results are in line with the observations of other investigators, indicating that chronic administration of pharmacological doses of glucocorticoid hormones causes an increase in GFR. In cortisone-treated dogs, glucocorticoids act directly, causing either vasodilation of renal arterioles or a shift of fluid from the intracellular to extracellular compartments. An alternative possibility, namely extracellular volume expansion due to salt retention that may provide the basis for the increased GFR seen with glucocorticoid hormones, appears unlikely, since this increase is noticed even in MP treated rats and cortisone treated

<table>
<thead>
<tr>
<th>Groups</th>
<th>Creatinine clearance (ml/hr/g)</th>
<th>fENa (μEq/hr/g)</th>
<th>Total Na excretion/24hr (μEq/hr/g)</th>
<th>AP (μmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (C)</td>
<td>0.039±0.006</td>
<td>1.110±0.080</td>
<td>416.670±51.420</td>
<td>6.000±4.000</td>
</tr>
<tr>
<td>I</td>
<td>0.050±0.005</td>
<td>1.030±0.090</td>
<td>372.280±35.300</td>
<td>14.000±7.000</td>
</tr>
<tr>
<td>II</td>
<td>0.065±0.007</td>
<td>0.900±0.080</td>
<td>316.200±50.060</td>
<td>20.000±10.000</td>
</tr>
<tr>
<td>III</td>
<td>0.075±0.008</td>
<td>0.820±0.060</td>
<td>267.720±35.140</td>
<td>43.000±6.000</td>
</tr>
<tr>
<td>IV</td>
<td>0.084±0.008</td>
<td>0.580±0.060</td>
<td>206.760±35.240</td>
<td>66.000±12.000</td>
</tr>
<tr>
<td>V</td>
<td>0.093±0.014</td>
<td>0.330±0.040</td>
<td>176.490±22.830</td>
<td>83.000±9.000</td>
</tr>
<tr>
<td>VI</td>
<td>0.106±0.010</td>
<td>0.150±0.030</td>
<td>155.730±25.430</td>
<td>104.000±6.000</td>
</tr>
</tbody>
</table>
dogs, when sodium balance is negative. It seems therefore that the increased GFR is mainly the result of glucocorticoid action on the vascular smooth muscles. Glucocorticoids decrease the resistance of afferent and efferent arterioles and increase renal plasma flow rate. Although the present are in line with the aforementioned observations regarding the increase of GFR, it was beyond the scope and the design of this study to elucidate the mechanism through which the increase of GFR is achieved.

Although in clinical practice corticosteroid pulse therapy has been used for the management of severe renal diseases with beneficial effect, there have been cases mainly of hypoproteneemic patients who suffered a deterioration of their renal function attributed to MP pulse therapy. These observations somehow dictate that the impact of MP pulses on renal function need further clarification. The present results support the view that at least experimentally MP pulse administration increases GFR, an action which is beneficial in the clinical practice beyond doubt.

The increase of urinary AP activity in all groups following MP administration indicates only damage of the epithelial cells of the proximal tubule, more extensive with more MP pulses. In contrast urinary LDH activity increases when renal cells are extensively destroyed. In our groups LDH activity was not significantly different from that in controls, indicating that MP did not produce extensive renal cell damage, findings compatible with the renal histology on light microscopy.

It is concluded that MP pulse therapy increases the GFR, a favourable action in the acute phase of many renal parenchymal diseases while the side effects are negligible.

References


18 Chaney A L & Marbach E P. Modified reagents for determination of urea and ammonia, Clin Chem, 8 (1962) 130.


25 Davis J O & Howell D S. Comparative effect of ACTH, cortisol and DOCA on renal function, electrolyte excretion and water exchange in normal dogs, Endocrinology, 52 (1953) 245.


29 Lash L H, Tokarz J J & Pegoudek D M. Susceptibility of primary cultures of proximal tubular and distal tubular cells from rat kidney to chemically induced toxicity, Toxicology, 103 (1995) 85.