Histological analysis and the blood flux in kidneys submitted to different periods of ischemia/reperfusion

Análise histológica e do fluxo sanguíneo em rins submetidos a períodos distintos de isquemia/reperfusão

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ABSTRACT

PURPOSE: Evaluate the renal blood flow by using a laser flow meter, Laserflow Vasamedics®, after the ischemia/reperfusion in two different times of the arterial renal vessel clamping.

METHODS: The renal blood flow was evaluated by using a laser flow meter after two different times of ischemia/reperfusion procedure, 30 and 60 minutes. It was used 20 Wistar male rats, divided in two groups of 10 rats: Group A (30 minutes of ischemia on the left kidney, with later analysis of the blood flow in 1, 5 and 20, after the renal reperfusion start) and Group B (60 minutes of ischemia on the left kidney, with later analysis of the blood flow in 1, 5 and 20 minutes, after the renal reperfusion start).

RESULTS: In the first minute, there were not significant differences between the two groups (p=0.789). In the 5th minute there were not significant differences, but there was a tendency (p=0.068). In the 20th minute, there was a significant difference between the 2 groups (p=0.022). When the means are observed, it is possible to notice that Group A has a larger flux than Group B.

CONCLUSION: The kidneys submitted to 30’ of ischemia/reperfusion are subject to a larger restitution of the blood flow in comparison to the Group which had a longer time.

Keywords: Ischemia. Reperfusion. Kidney. Rats.
Introduction

The ischemia/reperfusion injury (IR) can be defined as a whole of alterations due to privation and re-establishment of the oxygen supply to tissues and organs. It is a complex phenomenon which involves not only intracellular effects, but also an inflammatory response to the injury.

The pathogenesis of the ischemia/reperfusion is not completely understood. There are evidences which relates it with the decrease of mitochondrial ATP production, loss of the selective permeability of the cell membranes, increase of intracellular sodium and calcium leading the renal cell to injury or death.

A renal injury caused by ischemia makes relation with different medical areas, being essential to the Renal Surgery, Vascular and Transplantation.

The lateness in re-establishing the renal function after the transplantation results in oliguria, increase of the immunogenicity of the graft and episodes of acute rejections. These situations might be related to the donator and the kidney receptor (pre-renal, renal and post-renal factors associated to the transplanted kidney).

Experimental studies evidenced that both the ischemia and blood flow restitution in ischemically injured kidneys after hypothermic preservations, activate a complex sequence of events which sustains the renal injury and have a central role in the late kidney’s function development.

Previous researches in animal models had demonstrated that the consequences of ischemia/reperfusion depend on the duration, resulting in biochemical alterations and even cellular necrosis. Thompson et al., in a retrospective human study, had evaluated the impact of ischemia time in patients with only one kidney submitted to open partial nephrectomy. They concluded that 20 to 35 minutes are, respectively, the maximum time for the kidney submitted to open partial nephrectomy. They concluded their efforts to maintain the ischemia time as little as possible. We developed our project aiming to appraise the differences between the renal blood flow after distinct times of ischemia.

Methods

This article has the approval of the Pontifical Catholic University of Parana Ethical Committee.

In this experimental study, 20 male Wistar (Rattus norvegicus albinus, Rodentia mammalia) rat were used. They had 90 days of life and 250 to 300 g of weight in mean. They were divided in two groups of 10 rats: Group A (30 minutes of ischemia on the left kidney, with later analysis of the blood flow in 1, 5 and 20 minutes, after the renal reperfusion start) and Group B (60 minutes of ischemia on the left kidney, with later analysis of the blood flow in 1, 5 and 20 minutes, after the renal reperfusion start).

The work had initiated with Group A. It was done an anesthetic induction, followed by a median laparotomy, exploring the abdominal cavity, indentifying and dissecting the left renal pediculus. After that, the venous and arterial left vessels were occluded with a Bulldog type clamp for 30’. Later on the ischemia time, the clamp was taken out and the reperfusion was started, after 1 minute the laser flow meter was positioned in the superior,
medium and inferior regions of the left kidney to measure the respective blood flux. The measures were repeated in the 5th and 20th minutes (Figure 1).

The same procedures were done in Group B, however the clamping time of the left renal arterial vessel was 60 minutes.

After this process, the rats were submitted to euthanasia by giving the anesthetic in a three times higher dose. The data obtained were statistically analysed.

**FIGURE 1** - Position of the flow meter to measure the renal blood flux after ischemia period.

**Results**

The results obtained consisted in the values of the renal flow from 10 rats from Group A and 9 rats from Group B. During the anesthetic procedure, one rat from the second Group deceased.

The results were expressed in means, medians, minimal values, maximum values and standard deviations. To compare the 2 Groups in relation to the mean flow, it was used the t Student test for independent samples. The comparison of the evaluation moments inside each Group was done by using the variance analysis with repeated measures. The normality condition of the variables were evaluated with the Shapiro-Wilks test. The values p<0.05 indicated statistical significance. The data were organized in Excel worksheet and analysed with the computer program Statistica v.8.0 (Table 1).

**TABLE 1** - Group A and B renal blood flow in the 1st, 5th and 20th minute.

<table>
<thead>
<tr>
<th>Moment</th>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Standard Deviation</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLOW_1'</td>
<td>A</td>
<td>10</td>
<td>28.7</td>
<td>22.6</td>
<td>9.7</td>
<td>51.6</td>
<td>14.3</td>
<td>0.789</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>9</td>
<td>27.0</td>
<td>28.1</td>
<td>13.0</td>
<td>45.1</td>
<td>13.1</td>
<td></td>
</tr>
<tr>
<td>FLOW_5'</td>
<td>A</td>
<td>10</td>
<td>28.8</td>
<td>31.3</td>
<td>4.9</td>
<td>43.2</td>
<td>13.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>9</td>
<td>18.9</td>
<td>18.3</td>
<td>8.2</td>
<td>34.4</td>
<td>7.4</td>
<td>0.068</td>
</tr>
<tr>
<td>FLOW_20'</td>
<td>A</td>
<td>10</td>
<td>33.8</td>
<td>34.4</td>
<td>6.8</td>
<td>73.0</td>
<td>19.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>9</td>
<td>16.7</td>
<td>14.7</td>
<td>8.7</td>
<td>34.0</td>
<td>7.3</td>
<td>0.022</td>
</tr>
</tbody>
</table>

In the first minute (Figure 2), there was not significant difference between the Groups (p=0.789). In the 5th minute (Figure 3), there were not significant differences between the Groups (p=0.068), but it was observed a tendency. In the 20th minute (Figure 4), there was a significant difference between the groups (p=0.022). By analyzing the means, it is possible to note that Group A has a larger flow than Group B.

**FIGURE 2** - Flow in the 1st minute Group A x Group B.

**FIGURE 3** - Flow in the 5th minute Group A x Group B.
**Discussion**

The ischemia plays an important role in many different per operative clinical situations. Although the blood revascularization to the ischemic organ is essential to prevent the irreversible cellular injury, the reperfusion might worsen the damage caused by the isolated ischemia. The ischemia period itself is sufficient to cause a significant renal loss of function, but the injury caused by the reperfusion showed to be more harmful than the isolated ischemia because it is produced a greater local inflammatory response leading to systemic damages\(^2\).

The rat’s kidney exposed to chronic ischemia develops diffuse tubular atrophy, interstitial fibrosis and good glomerular preservation. The only rare injury in humans but frequent in rat is the interstitial inflammation\(^6\).

The kidneys are more resistant to ischemia than other organs, in opposite to the liver which its histology is precociously affected. Many studies in animals demonstrated the renal tolerance to ischemia for more than 30 minutes, but there is a concerning about the total recuperation of the renal function after all this period of time\(^2\).

In a study with dogs it was observed that a period longer than 30’ could lead to a renal recuperation\(^7\). In study with pigs, Humphreys *et al.*\(^8\) observed a renal function deterioration in kidneys submitted to a ischemia period of 75 minutes.

The time of ischemia could influence in the animals’ survival. In our study, the Group A showed 100% of survival and Group B gave the evidence of 90%. Similar results were found in a study which the rate of rat survival submitted to a mean ischemia period of 37.5 minutes while the animals who had died were proceeded to a mean time of 81 minutes\(^1\).

It was observed in our research that after the first and 5\(^{th}\) minute of reperfusion, there were not found significant results in both groups. Although in the flux analysis after the 20\(^{th}\) minute of the left arterial renal clamp withdrawal, significant evidences were observed. The rat submitted to 30’ of ischemia showed a greater flow in comparison to the animals submitted to 60 minutes. This information demonstrates that the longer is the ischemia time, the greater is the renal damage, as the blood flux to the organ survival is lower.

The absence of significant results at 20’ may be due to the technique found to adapt the flow meter to the kidney, mainly in the first minute.

It is not possible to conclude what was the most harmful mechanism to the kidney: the prolonged ischemia or the reperfusion.

The longer is the ischemia time, the intensive are the histological changes, increasing the percentage of cortical necrosis and the damage of the acute tubular necrosis. This situation was described by Alvaréz *et al.*\(^2\) which the Group A, of 30’ of ischemia, showed a case of cortical necrosis with 100% of damage and the other Group, 10%. There were observed four cases of cortical necrosis in the Groups of 45 and 90 minutes.

In the initial project, one of the objectives was to analyse more than the renal blood flow, but to evaluate the possible histopathological alterations in different times of ischemia. However, due to the difficulties in interpreting the laminas, the lack of comparative models and the unavailability of the lab pathologist, we chose to change the initial theme of the project. We observed that in the majority of the studies, the renal injuries are measured by biochemical parameters (creatinine and diuresis), energetical supply (ATP, ADP, AMP), histological alterations and others.

The results found in our project were compatible to literature and statistically significant, nevertheless the flow meter is not the ideal method to evaluate de vitality of the organs because it is subjected to many external bias, such as the rat movement, difficulty to adapt the equipment on the kidneys and delayed in measuring the stabilization of the flux. Moreover, the laser flow meter is not frequently mentioned in the literature as a method of analysis.

Even with the greater experience of the surgeons, the time limit of 30 minutes is still a safe border between irreversible injuries to the kidney when it has no proper cooling preservation.

It is necessary to make future studies to define which are the better parameters to measure the ischemia/reperfusion injury,
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Moreover it is indispensable the development of methods to protect the kidneys during the lesions.

Conclusion

The 30 minutes of renal occlusion establishes a larger flux in comparison to the kidneys submitted to 60 minutes of ischemia. The kidneys submitted to more than 30’ are subject to a reduced organ survival.

References


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