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Lovastatina protege a função renal e mitocondrial na isquemia/reperfusão renal em ratos

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ABSTRACT

PURPOSE: To investigate the effect of lovastatin on renal ischemia followed by reperfusion.

METHODS: Thirty one Wistar rats submitted to left renal ischemia for 60 minutes followed by contralateral nephrectomy were divided into two groups: A (n =17, control, no treatment), and B (n=14, lovastatin 15 mg/kg/day p.o. ten days before ischemia). The animals were sacrificed at the end of ischemia, after 24 hours and at seven days after reperfusion. Survival, serum urea and creatinine levels and renal mitochondrial function were evaluated.

RESULTS: Mortality was 29.4% in group A and 0.7% in group B. Urea and creatinine levels were increased in both groups, but the values were significantly lower in group B. Mitochondrial function showed decoupling in 83.4% of group A, as opposed to 38.4/% of group B.

CONCLUSIONS: The result shows a protective action of renal function by lovastatin administered before ischemia/reperfusion. Since most of the mitochondrial fraction presented membranes with the ability to maintain ATP production in group B, stabilization of the mitochondrial membrane should be considered as part of the protective action of lovastatin on renal function in ischemia/reperfusion.

Key words: Lovastatin. Mitochondria. Reperfusion Injury. Ischemia. Rats.

RESUMO

OBJETIVO: Investigar a ação da lovastatina na isquemia renal seguida de reperfusão.

MÉTODOS: Trinta e um ratos Wistar submetidos à isquemia renal esquerda durante 60 minutos, seguida da nefrectomia contralateral, foram distribuídos em dois grupos: A (n=17, controle, sem tratamento) e B (n=14, recebendo 15 mg/Kg/dia de lovastatina via oral), durante os dez dias que antecederam a isquemia. Os animais foram mortos ao final da isquemia, e com 24 horas e sete dias após a reperfusão. Foram avaliadas a sobrevida, os valores séricos de uréia e creatinina e a função mitocondrial renal.

RESULTADOS: A mortalidade foi 29,4% no grupo A e 0,7% no grupo B. Os níveis de uréia e creatinina elevaram-se nos dois grupos, mas foram significativamente menores no grupo B. No grupo A a função mitocondrial renal ficou desacoplada em 83,4% dos ensaios, enquanto que no grupo B isto ocorreu em apenas 38,4% dos ensaios.

CONCLUSÕES: Os resultados mostram que a administração de lovastatina antes do episódio de isquemia protege a função renal. No grupo B, como a maior parte da fração mitocondrial isolada apresentou função acoplada à produção de ATP, deve-se também considerar a estabilização da membrana mitocondrial como parte da ação protetora da lovastatina na função renal durante isquemia e reperfusão.

Descritores: Lovastatina. Mitocôndrias. Traumatismo por Reperfusão. Isquemia. Ratos.

Introduction

Warm renal ischemia is the cause of severe kidney injury, possibly resulting in temporary or irreversible renal failure. Ischemia is also an important factor in the determination of renal parenchyma atrophy during urinary obstruction and it can also be involved with the functional deficit observed after kidney transplantation.

The preservation of renal function in order to use a kidney for transplantation starts during the preoperative phase of the donor, with fluid infusion, ventilatory assistance and pharmacological therapy of a patient with brain death being critical elements for the maintenance of hemodynamic stability and the consequent appropriate blood perfusion of the organ to be removed.

Brain dead patients who are donor candidates may become hemodynamically unstable, often requiring the use of vasopressor drugs. Thus, it is common to obtain organs that have suffered a variable period of hypoxia or ischemia with a consequent deleterious action on the metabolism of renal cells that may affect kidney function after transplantation.

Studies by our group have shown that the kidneys of rats submitted to warm ischemia for 60 minutes suffer a fall in their capacity for ATP synthesis, as observed by the values obtained in the evaluation of state III of mitochondrial respiration, a change that lasts over a period of one week. However, it is possible to protect mitochondrial function with the use of chlorpromazine infused before the ischemic episode[1,2]. A protective effect has also been observed with the use of verapamil[3]. However, no variation in ATP production was observed in the analysis of mitochondrial function of rat kidneys obtained during the development of compensatory renal hypertrophy (which causes an increased renal blood flow) followed by ischemia and reperfusion[4].

In the past, some studies have demonstrated that lovastatin has a beneficial effect on the renal circulation, characterized by increased renal blood flow even in normal animals[5-7]. On the basis of the above considerations, the objective of the present study was to evaluate the action of lovastatin in renal and mitochondrial function during warm renal ischemia and reperfusion in rats.

Methods

Thirty one male Wistar rats weighing 200-250g were obtained from the Animal Facilities of the Faculty of Medicine of Ribeirao Preto, University of Sao Paulo.

Warm ischemia

The procedures were carried out under general anesthesia induced with a combination of Ketamine® and Xylazine® administered intramuscularly. The abdominal skin was shaved and disinfected with an iodine solution and a median laparotomy about 2 cm in length was performed. Ischemia was induced with microsurgical vascular clamping of the left renal artery close to its origin in the aorta. The laparotomy incision was sutured and the animals were placed for 60 minutes in individual boxes lined and kept at room temperature. After this period, the animals were again anesthetized, the abdominal cavity was reopened and renal blood flow was reestablished by removing the vascular clamp from the renal artery. Right nephrectomy was then performed. The abdominal cavity was sutured and, after recovery from anesthesia, the animals were kept in cages with free access to water and ration.

Experimental groups

The animals were divided into two groups: group A (control, n=17) and group B (experimental, n=14). Each rat in group B received lovastatin at the dose of 15 mg/kg/day administered p.o. during a period of ten days before the episode of ischemia. Within each group, the animals were divided into three subgroups (I to III) according to the date of sacrifice:

I - End of the period of ischemia (one hour);

II - 24 hours after reperfusion;

III – 7th day after reperfusion.

The kidneys removed at the above time points were used for the study of mitochondrial function.

Blood samples were obtained from all subgroups for the determination of serum urea and creatinine at the end of ischemia. Samples were collected from subgroup II 24 hours after reperfusion and from subgroup III on the 3rd and 7th day after reperfusion.

The survival of control animals (group A) was compared to that of the experimental animals (group B).

Preparation of the mitochondrial fraction

The kidneys were immediately transferred to containers filled with saline at 0°C to 4°C and fragmented. The fragments were transferred to containers filled with homogenization medium (100 mM Tris, pH 7.4, 210mM manitol, 70 mM saccharose and10 mM EDTA in a volume of approximately 10 ml) and triturated three times for three seconds at one minute intervals in a Turrax type homogenizer at 100 to 150 rpm. After cell lysis, the suspension was processed in a refrigerated centrifuge at 4°C (HITACHI – HIMAC CR 21) for three cycles, the first at 750g for five minutes and the other two at 11.200g for ten minutes. The

precipitate resulting from the last centrifugation contained the mitochondrial fraction.

Study of mitochondrial function

The basic parameters of mitochondrial respiration, i.e. state III, state IV and respiratory control ratio (RCR = state III/ state IV), were determined.

Statistical analysis

Serum urea and creatinine data were analyzed statistically by the Kruskal-Wallis method.

Results

Data were available for analysis in 12 animals of group A and 13 of group B.

Survival

Five deaths occurred in the control group (29.4%) during the postoperative period of one week, as opposed to only one death (0.7%) in group B during the same period.

Renal function

Serum urea and creatinine values are presented in Tables 1 and 2. Table 1 shows that at the end of 60 minutes of ischemia there was no significant difference in serum urea concentration between groups ($p>0.05$) as also observed for creatinine concentration (Table 2). After 24 hours there was an increase in serum urea concentration in both groups with significantly higher values in the control group ($p<0.001$) compared to the group pretreated with lovastatin. A similar behavior was observed for serum creatinine ($p<0.01$). The serum urea and creatinine levels of the control group were still significantly higher on the 3rd postoperative day compared to the experimental group ($p<0.001$). On the 7th postoperative day, urea and creatinine concentrations tended to decrease in both groups, with no significant difference between them ($p>0.05$).

TABLE 1 - Mean ± SEM serum urea levels (mg%) of animals submitted to warm renal ischemia for 1 hour followed by contralateral nephrectomy, pretreated or not with lovastatin at the dose of 15 mg/kg/day for ten days.

UREA	1 Hour	24 Hours	3 Days	7 Days	Mortality
Group A	48.3 ± 12	323 ± 51	374.8 ± 32	189.6 ± 97	5 Rats
n	12	7	5	5	
Group B	52 ± 16	165.87 ± 71	179 ± 106	86 ± 29	1 Rat
n	13	8	4	5	

TABLE 2 - Mean ± SEM serum creatinine levels (mg%) of animals submitted to warm renal ischemia for one hour followed by contralateral nephrectomy, pretreated or not with lovastatin at the dose of 15 mg/kg/day for ten days.

CREATININE	1 Hour	24 Hours	3 Days	7 Days	Mortality
Group A	0.61 ± 0.11	3.45 ± 0.69	4.4 ± 1.61	1.78 ± 0.58	5 Rats
n	12	7	5	5	
Group B	0.76 ± 0.17	2.03 ± 0.9	1.6 ± 0.6	0.84 ± 0.25	1 Rat
n	13	8	5	5	

Mitochondrial function

At the different time points studied, evaluation of mitochondrial function showed that ten of the 12 control animals (83.4%) presented decoupling of respiratory function from the function of ATP synthesis, as opposed to only five of the 13 animals pretreated with lovastatin (38.4%).

Discussion

Statins are drugs with a potent anti-inflammatory activity and a broad range of beneficial effects on the renal parenchyma including protection against ischemia/reperfusion injury and obstructive nephropathy and beneficial effects on patients with type 2 diabetes[8,9].

Statins are believed to act on renal hemodynamics by increasing renal blood flow, with an antioxidant and vascular endothelium-regulatory action, regardless of their capacity to reduce serum lipid levels. This was demonstrated in experimental studies with the combination of ischemia and cyclosporine A that has a nephrotoxic and vasoconstricting action side by side with its immunosuppressor effect[10,11]. Another mechanism of action of statins concerns the promotion of angiogenesis, as suggested by Chade *et al*.[12].

More recently, Gottmann *et al*.[13] reported a possible mechanism of action of atorvastatin. Using a renal transplant model in rats the authors demonstrated that animals treated

with atorvastatin showed inhibition of aldose-reductase and concomitant improvement of renal function after prolonged cold preservation.

Analysis of the results obtained in the present study supports the protective action of lovastatin on the renal function of rats pretreated with the drug and submitted to renal ischemia for 60 minutes, followed by reperfusion with a follow-up of seven days. The activity of lovastatin was evident when the survival of the two groups was compared. Whereas mortality was 0.7% over a period of seven days after reperfusion in pretreated animals, it reached a 29.4% rate in the control group over the same period of time.

Another result that supports this effect on renal function is presented in Table 2. After the increase in urea and creatinine levels observed after 24 hours in both groups, on the 3rd postoperative day these values continued to increase in the control group, whereas serum levels were reduced in the animals pretreated with lovastatin compared to the results obtained after 24 hours of reperfusion.

An interesting question raised in the literature concerns the time of administration and the dose of statins. Using a model of ischemia and reperfusion in rats, Nesic *et al*.[14] and Tedorovic *et al*.[15] reported the protective effects on renal function of simvastatin administered as a single dose before ischemia or before reperfusion. In the present study lovastatin was administered by gavage for ten days before the induction of ischemia. The positive results obtained in the two studies within this wide variation in time and dose may be related to the different statins used (simvastatin x lovastatin). This suggests the need for new studies involving more detailed aspects of both the functional and structural renal action of statins.

An original feature of the present study was the evaluation of mitochondrial function. This analysis showed that the isolated mitochondrial fraction presented membranes of better quality in the group pretreated with lovastatin, with the mitochondria being coupled to the production of ATP in most of the animals (61.6%). A different result was observed in the control group, in which functional decoupling was more frequent (83.4%). Decoupling indicates that the integrity of the mitochondrial membrane was affected after the period of ischemia and reperfusion, with difficulty in maintaining ATP production.

The action of ischemia and reperfusion was also manifested in the mitochondrial fraction obtained from animals pretreated with lovastatin, since part of the animals (38.4%) showed decoupling. However, most of the mitochondrial fraction (61.6%) presented membranes with the ability to maintain ATP production. Although it is difficult to compare mitochondrial function between the two groups due to the results obtained for untreated animals, it is possible to consider that the presence of mitochondria of better quality with membranes protected by lovastatin against the deleterious action of ischemia and reperfusion contributed to the rapid functional recovery of these animals. These findings, however, should be confirmed in future studies.

Conclusions

The protective mechanism of lovastatin on renal function after ischemia and reperfusion observed here may have been due to the direct action of the drug on renal vascularization, as previously reported in the literature. However, we should also consider the additional possibility of a protective effect of lovastatin on the stabilization of the mitochondrial membrane.

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