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Contribution of mTOR to oxidative/nitrosative stress leading to organ injury in a rat model of hindlimb ischemia/reperfusion

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Introduction: Ischemia/reperfusion (I/R) injury that is essential for ischemic limb recovery exacerbates the damage and remote organ injury leading to multiple organ dysfunctions due to the generation of free radicals[1]. The involvement of mTOR pathway on oxidative/nitrosative stress in hindlimb I/R remains unclear. Therefore, the aim of this study was to investigate whether or not mTOR contributes to organ injuries induced by I/R.

Method: I/R-induced distant and target organ injuries were performed by using hindlimb tourniquet model in male Wistar rats. Control (n=8) or rapamycin (n=8) groups received saline (4 ml/kg, i.p.) or rapamycin (1 mg/kg; i.p.; 1 h before reperfusion). I/R or I/R+rapamycin groups were subjected to latex tourniquets which were applied to the root of the hindlimbs for 4 h to induce ischemia, and the tourniquet was removed to initiate hindlimb reperfusion for 4 h[2]. After that all rats were sacrificed at the end of the reperfusion under anesthesia with ketamine (90 mg/kg, i.m.) and xylazine (10 mg/kg, i.m.) and the kidney and gastrocnemius muscle were collected from all animals. NADPH oxidase subunit $gp91^{phox}$ protein expression was measured by immunoblotting[2,3], nitrotyrosine, a marker of peroxynitrite formation by ELISA kit, MDA, a marker of lipid peroxidation and catalase activity as described previously[4,5] in these tissues. All data were given as means±SEM.

Results: I/R caused an increase in $gp91^{phox}$ expression and catalase activity as well as MDA and nitrotyrosine levels. The increase in these parameters was prevented by rapamycin (Table 1). Rapamycin alone had no significant effect on the parameters measured.

Table 1 Effect of rapamycin on I/R-induced changes leading to oxidative/nitrosative stress

Gastrocnemius muscle	**Vehicle**	**I/R**	**Rapamycin**	**I/R+ Rapamycin**
$gp91^{phox}$ expression	1.00±0.00 (4)	1.70±0.19* (4)	1.08±0.12 (4)	1.13±0.12$^{\#}$ (4)
Nitrotyrosine levels	83.82±2.79 (8)	116.70±4.28* (8)	87.91±3.13 (8)	82.38±3.20$^{\#}$ (8)
MDA levels	4.81±0.15 (8)	7.63±0.70* (8)	5.72±0,17 (8)	5.81±0,21$^{\#}$(8)
Catalase activity	4.28±0.49 (8)	12.10±3.32* (8)	6.95±1,31 (8)	5.05±0,46$^{\#}$ (8)
Kidney				
$gp91^{phox}$ expression	1.00±0.00 (4)	1.66±0.18* (4)	0.94±0.10 (4)	0.87±0.05$^{\#}$ (4)
Nitrotyrosine levels	1088.35±88.11 (8)	1486.76±44.60* (8)	1112.25±63.12 (8)	1192.77±47.20$^{\#}$ (8)
MDA levels	7.36±0.424 (8)	11.67±1.49* (8)	6.75±0,41 (8)	8.12±1.15$^{\#}$ (8)
Catalase activity	46.57±4.64 (8)	66.91±5.04* (8)	49.36±4.32 (8)	52.24±5.36$^{\#}$ (8)

*($P < 0.05$) *vs* control group, $^{\#}$ ($P < 0.05$) *vs* I/R group.

Conclusion: Our results demonstrate that I/R-induced oxidative/nitrosative stress presumably due to enhanced expression of $gp91^{phox}$, production of peroxynitrite, the activity of catalase, and lipid peroxidation are mediated by mTOR. Our data indicate that rapamycin could be useful in reducing oxidative stress in the acute hindlimb I/R.

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