

Melatonin protects from hepatic reperfusion injury through inhibition of IKK and JNK pathways and modification of hepatocyte proliferation

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Background

Reactive oxygen species (ROS) are involved in the pathophysiology of ischemia/reperfusion (I/R) injury. Melatonin is a potent scavenger of ROS. This study was designed to elucidate its effects in a combined hepatic warm ischemia / resection model.

Methods

The right lateral and caudate lobes (30% of liver) of female Sprague-Dawley rats (200-220 g) underwent warm ischemia for 30 minutes followed by resection of the non-ischemic liver tissue (70% of liver). Some rats were gavaged with 50 mg/kg melatonin 2 hr before experiments. Controls received the same volume of vehicle. Transaminases, histology, immunohistochemistry, and fresh tissue flowcytometry were used to assess hepatic injury, expression of molecular markers for IRI (iNOS), NF-kappaB signaling (IKK), oxidative stress and apoptosis (JNK, cJUN), and cell proliferation (PCNA, Ki67). One-way analysis of variance (ANOVA) or Fisher's exact test were used as appropriate. Log rank test of Kaplan-Meier analysis was used for survival. Results are presented as mean ± SEM.

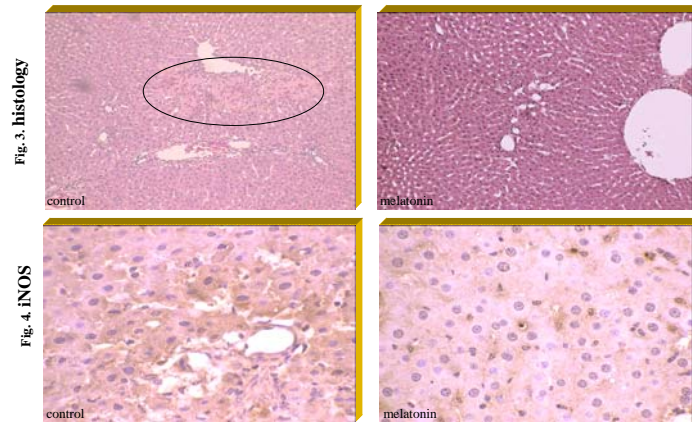
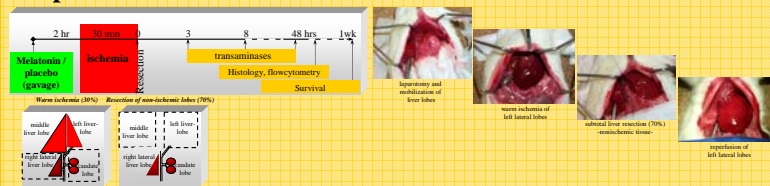


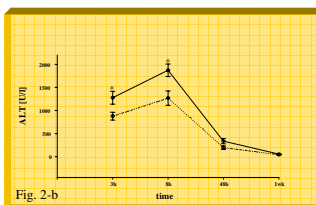
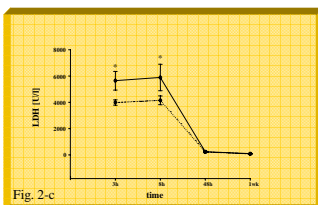
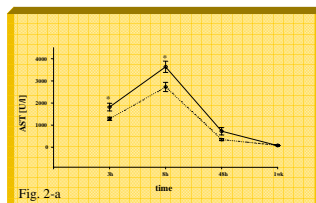
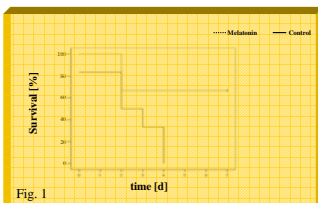
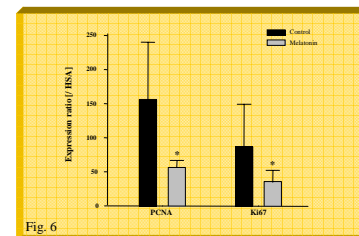
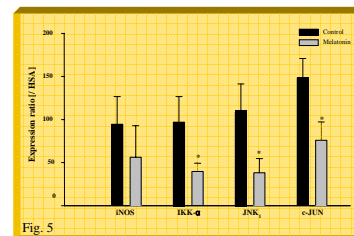
Table. Melatonin decreased liver injury and inflammation

	Control	Melatonin
<i>8 hr after reperfusion</i>		
Necrosis [%]	14.7±1.62	6.8±1.20*
Index of Liver damage ¹	1.6±0.22	1.1±0.07*
Leukocyte infiltration ² [%]	20.9±1.53	17.2±1.02*
<i>1 wk after reperfusion</i>		
Leukocyte infiltration ³ [%]	38.9±1.18	21.9±0.91*

Conditions as described in Materials and Methods; n=6 in each group. Index of liver damage and inflammatory response (index of leukocyte infiltration) were assessed early (8 hr) and late (1 wk) after warm ischemia / resection. ¹ Index of liver damage was assessed in 40 areas of 0.15 mm² and evaluated per slide with a point counting method: grade 0, minimal or no evidence of injury; grade 1, mild injury consisting in cytoplasmic vacuolization and focal nuclear pyknosis; grade 2, moderate to severe injury with extensive nuclear pyknosis, cytoplasmic hyperosinophilia, and loss of intercellular borders; and grade 3, severe necrosis with disintegration of hepatic cords, hemorrhage, and neutrophil infiltration. ² Index of leukocyte infiltration: grade 1, <10 leukocytes / field (focal infiltration); grade 2, 10-20 leukocytes/field (mild infiltration); grade 3, 21-50 leukocytes/field; grade 4, >50 leukocytes / field. Values are mean ± SEM. *, p<0.05 for comparison to control by one-way ANOVA with Student's-Newman-Keuls post hoc test.

Results

Melatonin led to significantly improved 1-week survival rates after resection (p=0.03, Fig. 1), while significantly decreasing ALT, AST, LDH (Fig. 2a-c), and the indices for necrosis, liver damage, and leukocyte infiltration (Fig. 3, Table); which was associated with 35%-50% decreased expression of IKKα, JNK1, and cJUN in liver (p<0.05) and a trend toward reducing iNOS expression (Fig. 4, 5). In parallel, significantly reduced expression of both PCNA and Ki67 was documented (Fig. 6).



Conclusion

Melatonin is hepatoprotective after warm I/R and resection most likely via mechanisms including inhibition of IKK and JNK signaling pathways and regulation of hepatocyte proliferation. The reduced expression of proliferative markers was not associated with any detrimental effect to liver recovery, as shown by lower transaminases, subtle histopathologic changes, and improved survival.

*, p<0.05, n=6 in each group