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Melatonin impact on cyclosporine-induced liver damage: an update

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Cyclosporine A (CsA) is the elective immunosuppressive drug in transplantation and autoimmune disorders, even if among its multiple side effects, liver damage limits clinical application. Chronic CsA delivery induces in the liver a hypermetabolic state and oxidative damage, ROS production, associated with a stress response [1]. Melatonin, the indolamine produced by the pineal gland, has been proven to be particularly effective in the amelioration of respiratory balance in health and diseases [2].

Nevertheless the contribution of autophagy (or macroautophagy), the pivotal process that removes abnormal proteins or organelles, like mitochondria and peroxisomes, to preserve homeostasis during CsA therapy is still emerging.

This microscopic study aimed to further demonstrate the beneficial melatonin role in the liver treated with CsA focusing on autophagosomes, mitochondria, ER morphology and chaperones expression in the rat.

Sprague-Dawley rats were s.c. injected with CsA (15 mg/Kg/day), melatonin alone (1 mg/Kg/day), or with melatonin and CsA, at the above dosage, for 30 days. The liver was extracted and processed for histopathological, enzymatic, immunohistochemical and ultrastructural analysis.

Mitochondria damage was evaluated by citochrome c-oxidase histochemistry, ER stress by immunostaining of resident chaperones (GRP78, GRP94), autophagic flux by the presence of autophagosomes and autophagolysosomes by TEM analysis and by immunostaining of ubiquitin and p62/sequestome-1 protein, a marker of autophagic flux. To best characterize an efficient autophagic flux in the liver and mitochondria ultrastructure we inserted, as positive control, 24h fasted rats [3].

Melatonin supplementation restored cytochrome c oxidase-positive brown signal in the cytoplasm of hepatocytes whereas it almost disappeared in CsA-treated group.

Moreover during immunosuppressive regimen, RER fragmentation (Figure 1) and chaperones overexpression, late autophagosomes, often mitophagosomes, together with intense ubiquitin and p62-positive aggregates were seen in hepatocytes.

By contrast, in rats given CsA plus melatonin, RER morphology was restored and autophagosomes almost absent (Figure 2), p62 and ubiquitin immunostainings became weak. In 24h-fasted rats ER dilatation, perinuclear elongated mitochondria and autophagic vacuoles were detected in hepatocytes (Figure 3).

These novel observations suggest that melatonin alleviates CsA-induced hepatotoxicity by restoring proper mitochondria, ER organization and stimulating autophagic flux, so keeping an adequate cellular detoxification ability.

In conclusion, in this chronic in vivo model melatonin, acting in the recovery of adaptive autophagy and hampering ER stress-driven apoptosis, further confirms its efficacy to contrast hepatic damage.

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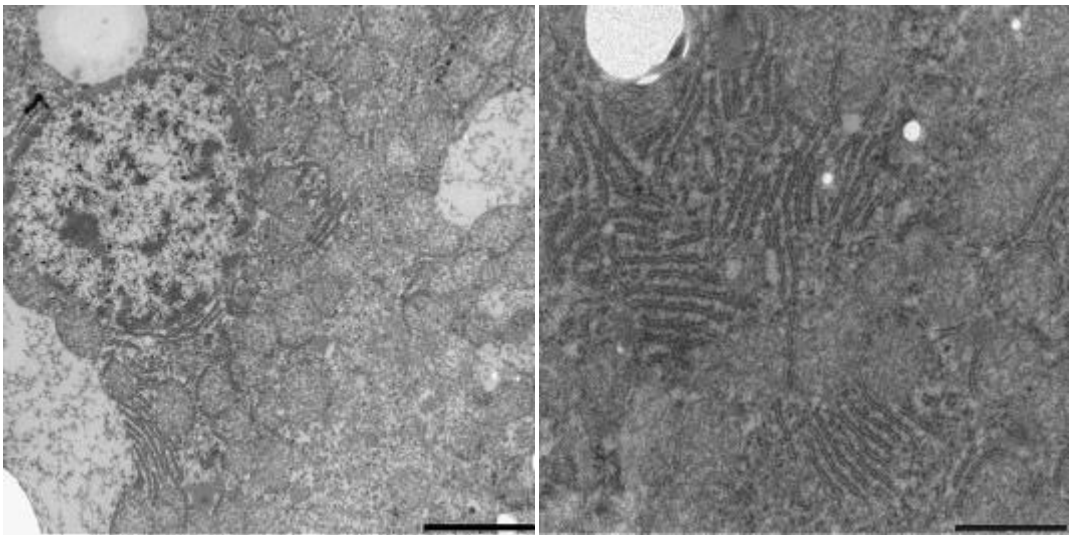

Figure 1. Electron micrographs of CsA-treated liver: scattered RER stacks and amorphous mitochondria. TEM FEI Tecnai G2Spirit; bar = 2µm and 1µm respectively

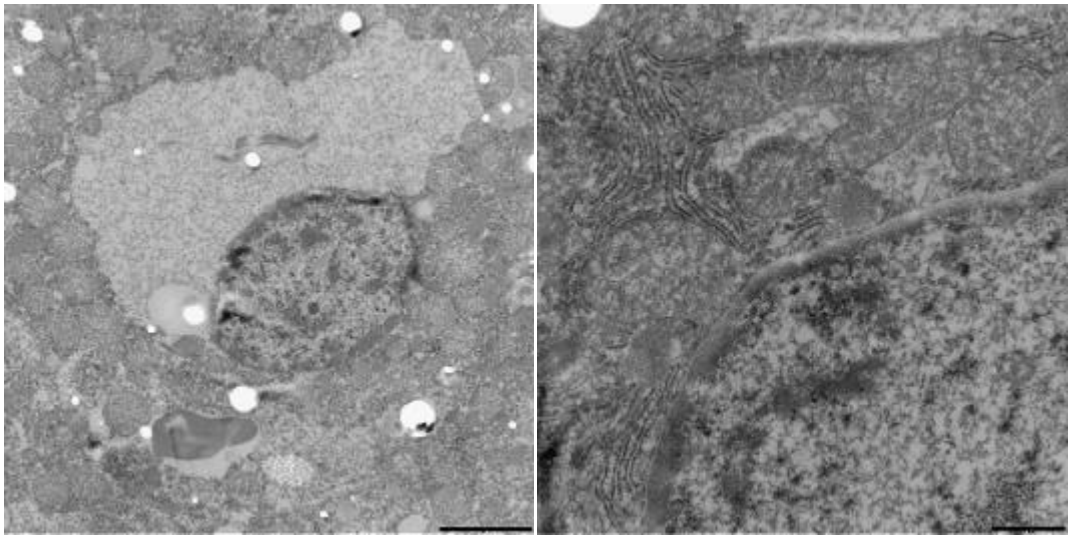

Figure 2. Electron micrographs of CsA plus melatonin-treated liver: regular RER, round and elongated mitochondria. TEM FEI Tecnai G2Spirit; bar = 2µm and 500nm respectively

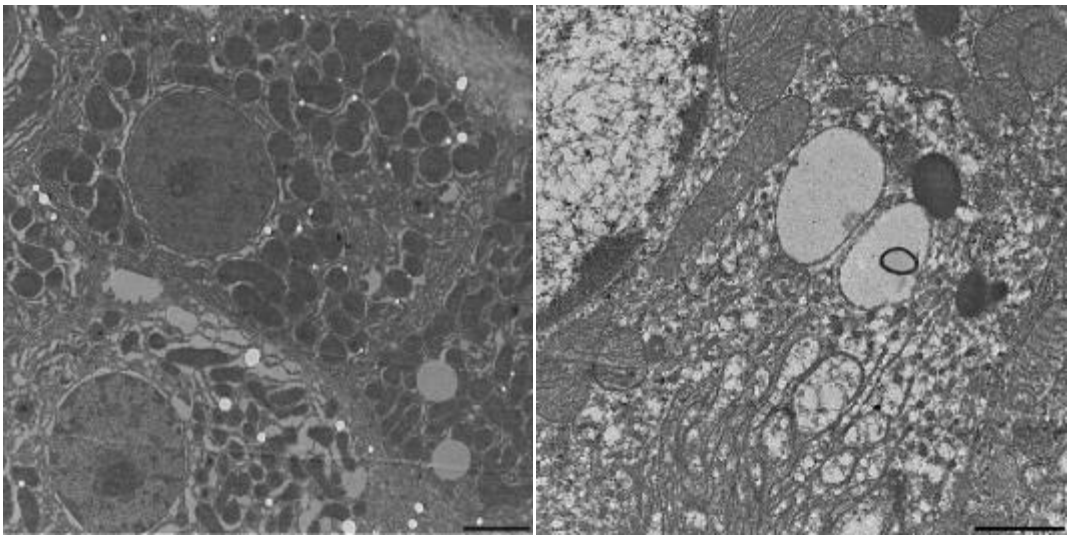

Figure 3. Electron micrographs of 24h fasted rat liver: dense elongated mitochondria and RER dilation, autophagic vacuoles. TEM FEI Tecnai G2Spirit; bar = 2µm and 1µm respectively