Melatonin protects against cadmium-induced oxidative damage in different tissues of rat: a mechanistic insight

Supplemental Material

Measurement of endogenously produced hydroxyl radical (•OH).

Male Wistar rats (n=24) were equally divided into four groups. In the control group (n=6), rats were intraperitoneally (i.p.) injected with 0.4 mL of 25% dimethyl sulfoxide (DMSO)/100 g body weight. In the melatonin alone group (n=6), animals were treated (orally) with melatonin (10 mg/kg body weight) (46) 30 min prior to DMSO treatment (0.4 mL of 25% DMSO/100 g; i.p.). In the Cd group (n=6), animals were injected (i.p.) with DMSO (0.4 mL of 25% DMSO/100 g) prior to $CdCl_2$ treatment (0.44 mg/kg; subcutaneous) (21). In the treatment group (n=6), rats were treated (orally) with melatonin (10 mg/kg) 30 min prior to DMSO treatment (0.4 mL of 25% DMSO/100 g; i.p.) which was 30 min prior to $CdCl_2$ injection (0.44 mg/kg s.c.). After the treatment period, rats of each group were euthanized by cervical dislocation and the desired organs were collected for estimating the level of endogenously produced •OH. The •OH generated *in vivo* in cardiac, hepatic and renal tissues was measured following the method of Bandyopadhyay *et al*. (30) by using DMSO as a specific •OH radical scavenger. In brief, tissues were processed in cold for MSA which was allowed to react with Fast blue BB salt to yield a yellow product. This was measured spectrophotometrically at 425 nm using benzene sulfinic acid as the standard. The values obtained were expressed as nm of •OH / g tissue.

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