MRI study the neuroprotection of Melatonin in the COX-1 gene wild-type mice

G. Li[1,2], L-Y. Zou[3], C. R. Jack Jr. [4], E. S. Yang[2]

[1]Faculty of Medicine, The University of Hong Kong, HK, Hong Kong, [2]The Jockey Club MRI Centre, The University of Hong Kong, HK, Hong Kong, [3]Department of Medicine, The University of Hong Kong, HK, Hong Kong, [4]Radiology, Mayo Clinic and Foundation, Rochester, Minnesota, United States

Introduction:

Up regulation of cyclooxygenase 1 and 2 (COX-1, COX-2) mRNA, protein, and activity promotes inflammation. Melatonin is a potent antioxidant and free radical scavenger that readily diffuses through cell membranes. It exerts a protective effect against experimental ischemic damage[1]. Expression of COX -2 in carrageenan-treated rats is completely inhibited by melatonin[2]. To our knowledge however, a possible neuroprotective effect of melatonin, via alteration of COX -1 expression, in ischemia has not been investigated. This study aimed to explore whether melatonin had neuroprotective effects in a mouse photothrombotic stroke model, and whether this neuroprotective effect was mediated by COX-1.

Materials and Methods:

Heterozygous (COX-1-gene +/-) knockout mice (129/C57BL) were established at the National Institute of Environmental Health Science, NC[3]. Focal cerebral ischemia was induced with a photothrombolic technique. A laser Doppler flowmeter was used to determine the cerebral blood flow (CBF) in ischemicpenumbra[4]. Melatonin (15 mg/kg) or its vehicle (i.e. used as placebo) was given via an intraperitoneal injection at 0.5 h before stroke, 24 h and 48 h after photothrombosis. The brains were removed and embedded in gel for MRI scanning which was performed at 3T. Parameters for 3D FSE axial brain images were TR = 2000 ms, TE = 90 ms, BW = 15.6KHz, FOV = 8 × 4cm, matrix size = 384 × 256, NEX = 1, slice thickness = 1 mm without slice gap. These were followed by 3D SPGR with the following imaging parameters: TR = 25 ms, TE = 5 ms, BW = 15.6 KHz, FOV = 8 × 6 cm, matrix 512 × 192, NEX = 10, slice thickness 1 mm without slice gap. Infarct volume (mm^3) was calculated by manually delineating hyperintense areas from 3D SPGR and FSE images. Results were expressed as lesion volume percentage relative to the volume of the contralateral hemisphere[5]. Cerebral edema index was expressed as ipsilateral hemisphere area percentage relative to the contralateral hemisphere area[4]. Numerical results ,were expressed as mean ±□ SE. Data were analyzed with one-way ANOVA test. The level of significance was set at $P<0.05$.

Results:

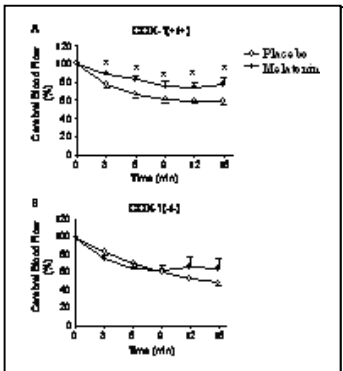


Fig. 1 Normalized regional cerebral blood flow during illumination in the penumbra (CBF, in percent) in the COX-1 gene wild-type (+/+) and homozygous (-/-) knockout mice with injection of melatonin or placebo (n=6). A, CBF in the COX-1 gene wild-type (+/+) mice group. B, CBF in the COX-1 gene homozygous (-/-) knockout mice group. *P<0.05. as compared to the placebo group respectively.

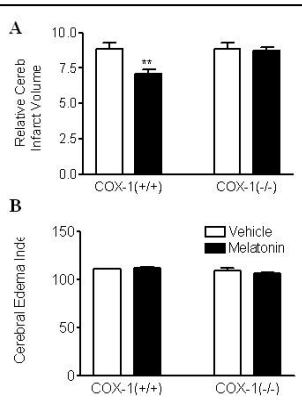
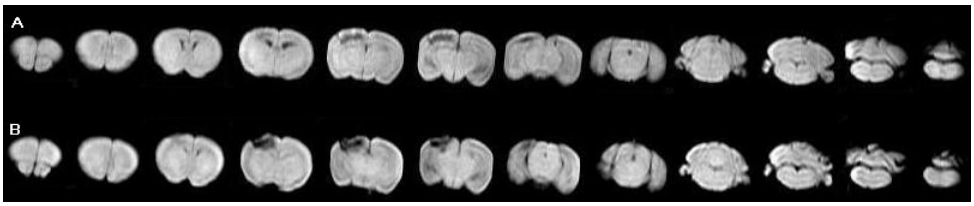


Fig. 2. A, quantification of infarct volumes of the COX-1 gene wild-type (+/+) and homozygous (-/-) knockout mice with injection of melatonin or placebo. Data are expressed as lesion volume percentage of the contralateral hemisphere volume. B, cerebral edema index of the COX-1 gene wild-type (+/+) and homozygous (-/-) knockout mice with injection of melatonin or placebo data are expressed as ischemic hemisphere volume percentage of the contralateral hemisphere volume **P<0.01 as compared to the vehicle group respectively.

Fig. 3 Infarct volume produced by photothrombosis at 72 h after stroke (n=6). A, cerebral infarct determined by T2-weighted MRI in the COX1gene wild-type (+/+) with melatonin treatment. B, cerebral infarct determined by T2-weighted MRI in the COX1 gene wild-type group without melatonin treatment.



Discussion and Conclusion:

The main findings of this study are: (1) multiple doses of melatonin at 15 mg/kg/day started 0.5 h before onset of ischemia significantly reduces infarct volume at 72 h after photothrombotic stroke in the COX-1 gene wild-type (+/+) mice, whereas, there is no neuroprotective effect of melatonin found in the COX-1 gene homozygous (-/-) knockout mice. (2) single dose of melatonin at 15 mg/kg/day started 0.5 h before onset of ischemia significantly improves the CBF in penumbra in the COX-1 gene wild-type (+/+) mice, this neuroprotective effect is not found in the COX-1 gene homozygous (-/-) knockout mice. (3) neither melatonin administration nor COX-1 gene significantly influences the cerebral edema index. (4) the neuroprotective effects of melatonin are mediated or partly mediated by inhibition of COX-1. In conclusion, our results indicate melatonin does exhibit neuroprotective effects in a mouse photothrombotic stroke model, and this neuroprotective effect is mediated by COX-1.

References: 1. Reiter RJ. *Prog Neurobiol* 1998; 56(3):359-384. 2. Cuzzocrea S et al., *J Pineal Res* 1999; 27(1):9-14.
3.Shenawy SM et al., *Pharmacol Res* 2002; 46(3):235-243. 4. Cheung RT et al., Neurosci Lett 2002; 330(1):57-60.
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