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Inhibitory effects of rosmarinic acid on pterygium epithelial cells via redox imbalance and induction extrinsic and intrinsic apoptosis

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Pterygium is a common tumor-like ocular disease, which may be related to exposure of chronic ultraviolet (UV) radiation. Though the standard treatment for pterygium is surgical intervention, the recurrence of pterygium is high when no effective inhibitory drug is used after surgery. Rosmarinic Acid (RA) is a polyphenol antioxidant, which has many biological activities including anti-UV and anti-tumor. This study was to examine the inhibiting effects of RA on pterygium epithelial cells (PECs). The inhibitory effect of RA on the cytotoxicity of the PECs was determined by the MTT reduction assay. Intracellular ROS (reactive oxygen species) levels were measured using a fluorescent probe, DCFH-DA (2',7'-dichlorofluorescin diacetate). The levels of Superoxide Dismutase (SOD) and Catalase (CAT) were measured as indexes of antioxidant activities. Western blot analysis was used to determine the protein expression of Nrf2, HO-1, NQO1 and apoptosis associated proteins. RA significantly reduced the cell viability of the PECs. Treatment with RA could remarkably increase the Nrf2 protein expression levels in nucleus, HO-1 and NQO1 protein expression levels, and activities of SOD and CAT. As the result, intracellular ROS levels in PECs were decreased. In addition, the induction of extrinsic apoptosis on PECs by RA was also associated with the increasing of Fas, FADD, TNF-α and caspase 8 protein expression levels. Moreover, the induction of PECs intrinsic apoptosis cell death through up-regulation of cytochrome c, Bax and caspase 9, down-regulation of Bcl-2 and inactivation of pro-caspase 3. Our study demonstrated that RA could inhibit the viability of the PECs via regulation the extrinsic and intrinsic apoptosis pathway. Therefore, RA may have potential for pterygium therapeutic medication.

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