Antioxidative Effects of Anthocyanins on Rotator Cuff Tenofibroblasts
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ABSTRACT INTRODUCTION:

The unavoidable, age-related degeneration of rotator cuff tendons can progress eventually to tendon rupture and glenohumeral joint osteoarthritis. The exact cause of rotator cuff tear is still unknown. Apoptosis of the tenofibroblasts is a possible cause of rotator cuff tendon tear. The purpose of the current study is to evaluate whether anthocyanins, well-known powerful antioxidants, are able to prevent apoptotic cell death in rotator cuff tendon origin tenofibroblasts exposed to oxidative stress.

METHODS:

Cells and Anthocyanins Used in Experiment: Cryopreserved third-passage cells, originally isolated from the supraspinatus tendons of male Sprague-Dawley rats, were used for all experiments in the current study. The cells were cultured in a 30% FBS/DMEM medium. Then, FACS and RT-PCR for the Scleraxis gene confirmed that these cultured cells were tenofibroblasts (Fig. 1).

The anthocyanins used in this study were extracted from the black soybean (Glycine max (L.) Merr) seed coat. These anthocyanins, a mixture of cyanidin-3-glucoside, delphinidin-3-glucoside, and petunidin-3-glucoside, showed, in concentrations of up to 200µg/ml, no cytotoxicity for the cultured tenofibroblasts (p>0.05) (Fig. 2).

Assessment of Cell Viability, Apoptosis Rate, and Intracellular Reactive Oxygen Species (ROS) Production: The tenofibroblasts were divided into three main study groups: a control group, an H2O2-treated group, and an anthocyanin-H2O2-treated group. The oxidative stressor, to which each of the two groups of tenofibroblasts was exposed for 12 hours, was 0.5mM H2O2. The rates of cell viability were evaluated using the MTT assay. The rate of apoptosis, and of intracellular ROS production for each study group were evaluated using the MTT assay, the rate of apoptosis, and of intracellular ROS production in the H2O2-treated group were statistically significant differences among the control group, the H2O2-treated group, and the anthocyanin-H2O2-treated groups (p<0.0001) (Fig. 3).

Analysis for Intracellular ROS Production: The level of intracellular ROS production in the study groups was as follows: 1.67 in the H2O2-treated group, and 0.60 in the anthocyanin-H2O2-treated groups (Fig. 5). The level of intracellular ROS production in the H2O2-treated group was significantly higher than the levels in the control group and in the anthocyanin-treated group (p<0.01). The levels of intracellular ROS production in the 100µg/ml anthocyanin-H2O2-treated groups were significantly lower than those in the H2O2-treated group, the control group (p<0.01).

RESULTS:

Analysis of the Rates of Cell Viability: Cell viability was assumed to be 100% in the control group. Cell viability in each study group yielded the following measures: 23.41% in the H2O2-treated group, 54.00% in the 10µg/ml anthocyanin-H2O2-treated group, 88.05% in the 50µg/ml anthocyanin-H2O2-treated group, 89.62% in the 100µg/ml anthocyanin-H2O2-treated group, and 90.89% in the 200µg/ml anthocyanin-H2O2-treated group (Fig. 3). There were statistically significant differences among the control group, the H2O2-treated group, and the anthocyanin-H2O2-treated groups (p<0.01). The rates of cell viability in the anthocyanin-H2O2-treated groups were significantly higher than the rate in the H2O2-treated group (p<0.0001). Analysis of the Rates of Cell Apoptosis: The rates of apoptosis were 0.34% in the control group, 52.20% in the H2O2-treated group, and as follows in the anthocyanin-H2O2-treated subgroups: 42.30% in 10µg/ml, 10.20% in 50µg/ml, 9.15% in 100µg/ml, and 7.66% in 200µg/ml. The rates of apoptosis in each anthocyanin-H2O2-treated group was significantly lower than in the H2O2-treated group (p<0.0001) (Fig. 4). These results demonstrated that anthocyanins have a concentration-dependent antiapoptotic effect on H2O2-mediated apoptosis.

DISCUSSION:

This study demonstrated that anthocyanins, through antiapoptosis and the suppression of intracellular ROS production, have a concentration-dependent cytoprotective effect on rotator cuff tenofibroblasts exposed to the oxidative stress of H2O2. This finding supports the possibility that anthocyanins can play an important antiapoptotic role in the prevention of rotator cuff degeneration.

The current study suggests several avenues of further research into the possibility of preventive care for rotator cuff tendon degeneration. The molecular mechanisms of the antiapoptotic effect of anthocyanins must be determined. Because the involvement of H2O2...