Amygdalin inhibits angiogenesis in the cultured endothelial cells of diabetic rats

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Abstract

Background: Angiogenesis contributes to different physiological and pathological conditions. The aim of this study was to investigate for the first time the antiangiogenic effects of amygdalin on the cultured endothelial cells of diabetic rats.

Materials and Methods: A total of 20 streptozotocin-induced diabetic rats were divided into two equal groups of control and amygdalin-treated animals. Eight weeks after the induction of diabetes, amygdalin was injected intraperitoneally (3 mg/kg) to the rats of the treatment group. One day later, rats were sacrificed; the aortic arteries were excised and cut as 2 mm rings. Each aortic ring was incubated in a cell-culture well for 7 days. The process of angiogenesis was monitored by counting the number of microvessels and primary microtubules in each well.

Results: Optic microscopy showed proliferation and migration of new endothelial cells to the fibrin gels. The endothelial cells produced primary microtubules which gradually made several branches and finally made a vascular matrix. The number of microvessels and primary microtubules in each well was significantly lower in the amygdalin-treated vs. control group (P < 0.01). Conclusion: Therefore, amygdalin exerts inhibitory effects on angiogenesis in aortic rings of diabetic rats and may pave a new way for treatment of unfavorable angiogenic conditions.

Keywords: Amygdalin, angiogenesis, diabetes, endothelial cells, rat's aortic rings

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Introduction

The growth of blood vessels, namely angiogenesis, is responsible for growth and repair of tissues, and contributes to several malignant, ischemic, inflammatory, immune and infectious disorders. Therefore, substances with inhibitory effects on angiogenesis and endothelial proliferation may be beneficial in treatment of pathological angiogenic conditions and can probably change the face of medicine in the next decades. Aortic rings model is commonly used for understanding the vascular structural changes that take place during angiogenesis. Different stages of growth and development of endothelial cells and formation of new vascular channels can be studied with aortic rings. The
endothelial cells can be stimulated by vascular endothelial growth factor (VEGF) to initiate the angiogenic processes. [2,13,14] This model can be used for evaluation of the pro- and antiangiogenic effects of different substances.

Amygdalin is a cyanogenic glycoside compound which is found in the plants of the rosaceous family and in pits of several fruits and raw nuts. [15,16] It is composed of a benzaldehyde group, which is known as an analgesic compound, [17] and a hydrocyanic acid, which is suggested to have antineoplastic effects. [18] The current study for the first time evaluates the antiangiogenic properties of amygdalin on the cultured endothelial cells derived from the aortic rings of diabetic rats.

Materials and Methods

Adult male Sprague-Dawley rats (age, 6 weeks; body weight, 230-250 g) were used in this study. Rats were housed in a temperature controlled vivarium (a temperature of 23 ± 3°C and a relative humidity of 50 ± 10%) with a 12:12 h light-dark cycle and had free access to rat chow and water ad libitum. Diabetes was induced in animals by intravenous injection of streptozotocin (60 mg/kg body weight in Na-citrate buffer, pH 4.5). Blood glucose levels were checked every week using an Accu-check blood glucose meter (Roche Diagnostics, Basel, Switzerland) and rats with blood glucose levels ≥ 200 mg/dl for 2 consecutive weeks were considered diabetic. A total of 20 streptozotocin-induced diabetic rats were divided into two equal groups of control and amygdalin-treated animals. Eight weeks after the initial administration of streptozotocin, amygdalin (Sigma, USA) was injected intraperitoneally (3 mg/kg in 2 ml H_2O) to the rats of the treatment group. In the control group water (2 ml) was injected intraperitoneally. One day later, rats were sacrificed and their aortic arteries were surgically excised and cut as 2mm rings.

The rings were washed by phosphate buffer serum (PBS, Sigma, USA; Ph 7.4) and each ring was embedded in a solution of 50 ml PBS (pH 7.4) with gentamycin (1.6 g/l) and maintained at 4°C. A Dulbecco's modified Eagle's medium (DMEM, Ph 7.4, Sigma); as well as a culture media of Hams F12 (Ph 7.4, Sigma) were used. These media were filtered (0.22 μm filters, Millipore) under laminar-flow hood and combined together with ratio of 50% to 50%. Then, 20% fetal calf serum (FCS, Sigma) along with 2.5 mg/ml VEGF (Sigma) were added to the combined media.

We used 24-well culture plates (Greiner Bio-One, Germany) in this study. Each well finally contained 1 ml of the mixture media, an aortic ring, and two layers of fibrin gel filled up above and underneath the ring. To produce a fibrin gel layer, 2.5-μl thrombin (Sigma; concentration: 500 ku/l) and 250 μl fibrinogen (Sigma; concentration: 8 g/l) were mixed together in each well under laminar-flow hood and then the plate was placed in incubator (37°C and CO_2 5%) for 1 hour. The aortic ring was placed in each well and 1 ml of the mixture of media was added. A mixture of thrombin and fibrinogen (5-μl thrombin and 250-μl fibrinogen) was then filled above the mixture and the plate was incubated at 37°C and CO_2 5% for 7 days. The process of angiogenesis was monitored in each well by optic microscope. The angiogenic response of aortic cultures was measured by counting the number of microvessels, according to the published criteria. The number of the primary microtubules in each well (at the 7th day of incubation) was determined using a hemocytometer device. The groups were compared using Mann-Whitney U test and P<0.05 was considered statistically significant.

Results

The new endothelial cells with nucleus, clear borders and distinct cellular membranes proliferated within the cultures media and migrated out of the rings to the fibrin gels. The endothelial cells produced primary microtubules. These microtubules gradually extended in the fibrin gel and made several branches. The branches gradually attached together and made a vascular matrix which finally occupied the space around the aortic rings. We observed that the process of angiogenesis in the aortic rings of all the 10 amygdalin-treated rats was diminished in comparison to the control rings (Figure 1). The number of microvessels in the aortic rings of amygdalin-treated rats tended to be lower than the control rings (mean ± SD: 23.1 ± 7.8 vs. 72.4 ± 11.3, P < 0.01). The average number of primary microtubules in each well at the 7th day of incubation was significantly lower in the group treated with amygdalin compared with the control group (mean ± SD: 4.2 ± 1.0 × 10^4 vs. 6.5 ± 1.1 × 10^4, P < 0.001).

Discussion

We found that amygdalin inhibits angiogenesis reflected by reduced number of developed microvessels in aortic rings of diabetic rats. Further, since microtubules are primarily involved in angiogenesis, [19] inhibition of angiogenesis may be at least in part due to decreased number of microtubules following amygdalin treatment.

Angiogenesis, the sprouting of new capillary blood vessels from preexisting microvasculature, is necessary for the development and maintenance of tissues and organs and is an important component in a number of pathological settings, including diabetic retinopathy, rheumatoid arthritis, and tumor growth. [20] In vitro investigation is the main assay for studying angiogenesis. [21] No previous study has evaluated the antiangiogenic activity of amygdalin; however, there are some studies which have shown the anti-angiogenic properties of other molecules with cyanogroups. Lee et al. showed that coumarin molecules that contain cyano groups exert antiangiogenic activity and can be utilized as lead compounds to develop potential nontoxic angiogenesis inhibitors. [22] Likewise, there are also some other reports to support the antiangiogenic effects of molecules with cyanogroups. [23,24,25] In our study, for the first time, we observed that amygdalin inhibits angiogenesis in aortic rings of diabetic rats. It is suggested that amygdalin may inhibit angiogenesis in a similar way to other cyanogroup-containing molecules.

Amygdalin had been suggested as an unconventional treatment of cancer; however, now the effectiveness of such a treatment is disputed and there are not sufficient studies available to prove this function. [26,27] Angiogenesis is a vital process for growth of cancer cells. [28] The antiangiogenic effect of amygdalin observed in the current study might play a
Amygdalin inhibits angiogenesis in the cultured endothelial cells of diabetic rats. This observation may be a consequence of reduced number of microtubules following amygdalin administration. However, further investigations are needed to elucidate the molecular mechanisms involved in this effect of amygdalin. Our findings may have therapeutic relevance and may pave a new way for treatment of unfavorable angiogenic conditions.

In conclusion, to the best of our knowledge the current study for the first time shows inhibition of angiogenesis by amygdalin in a rat model of diabetes mellitus. This observation may be a consequence of reduced number of microtubules following amygdalin administration. However, further investigations are needed to elucidate the molecular mechanisms involved in this effect of amygdalin. Our findings may have therapeutic relevance and may pave a new way for treatment of unfavorable angiogenic conditions.

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