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Original article

Inhibitory effect of curcumin on angiogenesis in a streptozotocin-induced diabetic rat model: An aortic ring assay

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ABSTRACT

Background: Curcumin (diferuloylmethane) has been associated with the inhibition of angiogenesis, as well as the prevention of cancers and inflammatory processes. The aim of this study was to assess the efficacy of curcumin in suppressing angiogenesis in the cultured endothelial cells of rat aortic rings.
Methods: Eight-week-old male Wistar rats were randomized into five groups each with a different treatment and cell culturing paradigm: controls cultured in the absence of VEGF (vascular endothelial growth factor) (C), controls cultured in the presence of VEGF (C-V), controls treated with curcumin and then cultured in media lacking VEGF (C-TC), diabetics cultured in media supplemented with VEGF (D-V) and diabetics treated with curcumin and then cultured in media supplemented with VEGF (D-V-TC). Each group consisted of 8 animals. Diabetes was induced in by streptozotocin (STZ; 60 mg/kg body weight, IV). After 8 weeks, animals were sacrificed and their aortas were excised. Ring-shaped explants were embedded in a 96-well culture plate. Angiogenesis response was measured by counting the number of primary microtubules in each well.
Results: Optic microscopy revealed that the D-V group had the highest number of microvessels, while angiogenesis was not observed in the C or C-TC groups. The number of primary microtubules was significantly lower in the D-V-TC group compared to the D-V group ($P < 0.05$). The D-V-TC group had a significantly higher number of microvessels compared to the C-TC group ($P < 0.05$).
Conclusion: Curcumin attenuates angiogenesis response in stertozotocin-induced diabetic rats.

1. Introduction

Curcumin (Diferuloylmethane) is the principal component and active ingredient of turmeric (Curcuma longa), an organic extract of Curcuma, a rhizomatous herbaceous perennial plant.[1] This phytochemical has long been used as a food additive and coloring agent.[2] Accumulating evidence suggests that curcumin exhibits various biofunctions.[3] Emerging data have revealed that curcumin possesses remarkable anti-oxidant, anti-inflammatory, anti-angiogenesis, and anti-carcinogenic properties.[4–9]

Diabetes mellitus (DM) is a major worldwide concern and its long-term complications involve various organ systems.[10] Hyperglycemia plays a definite role in the clinical manifestation of DM.[11] However, excessive angiogenesis, the formation of new blood vessels from pre-existing vasculatures,[12] may also be an important

Abbreviations: C, controls in the absence of VEGF; C-V, controls in the presence of VEGF; C-TC, controls treated with curcumin cultured in the absence of VEGF; D-V, diabetics in a culture containing VEGF; D-V-TC, diabetics treated with curcumin in a culture containing VEGF; DM, diabetes mellitus; VEGF, vascular endothelial growth factor; DMSO, dimethyl sulfoxide; CPCSEA, Committee for the Purpose of Control and Supervision of Experiments on Animals; PBS, phosphate buffer saline; MMP, matrix metalloproteinases; UPA, urokinase plasminogen activator; NF-kB, nuclear factor kappa; AP-1, activator protein 1.

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factor in some long-term pathological conditions, including diabetic nephropathy and retinopathy. In vitro angiogenesis assays contribute to the investigation of both angiogenic inducer and inhibitor agents.[13] The rat aortic ring model is a well-established in vitro assay for assessing angiogenesis,[13] and many consider it the best system for simulating physiological conditions found *in vivo*.[14] Furthermore, it has the advantage of promoting all of the key steps of the angiogenesis process.[15] Vascular endothelial growth factor (VEGF) is a prominent proangiogenic mediator[16] and therefore can be used as a local inducer of angiogenesis with the intent of monitoring the potency of inhibitory factors.[14]

In the study presented here, we explored the angiogenic effects of curcumin on cultured endothelial cells derived from the streptozotocin (STZ)-induced diabetic rat aortic rings.

2. Materials and methods

2.1. Experimental design

Eight-week-old adult male Wistar rats with an average body weight of 240 ± 20 g (obtained from Pasteur Institute of Iran) were randomly allotted into five groups: controls cultured in the absence of VEGF (C) (Fig. 1), controls cultured in the presence of VEGF (C-V) (Fig. 2), controls treated with curcumin and then cultured in a medium lacking VEGF (C-TC) (Fig. 3), diabetics cultured in media supplemented with VEGF (D-V) (Fig. 4), and diabetics treated with curcumin and then cultured in media supplemented with VEGF (D-V-TC) (Fig. 5). Each group contained eight animals. All rats were provided standard laboratory rodent chow and water *ad libitum*. After 2 weeks, the experimental rats (groups D-V and D-V-TC) received a single intravenous injection of 60 mg/kg of body weight of STZ,[17] whereas the control group received 60 mg/kg of vehicle. STZ was prepared fresh by dissolving in Na-citrate buffer (Sigma-Aldrich), pH = 4.5. In order to verify the diabetic condition, 96 h after the STZ injection, blood glucose levels of the experimental groups were measured using a portable glucose analyzer, Accu-check Blood Glucose Meter (Roche Diagnostics, Basel, Switzerland). Rats with blood glucose levels <15 mmol/L (270 mg/dl) received another injection of STZ. Following the second injection, animals with blood glucose levels higher than 15 mmol/L for 2 weeks were considered to be diabetic.

Rats in the curcumin-treated groups (groups C-TC and D-V-TC) were given a solution of 100 mg/kg of curcumin per day (dissolved in 2 ml DMSO) by intragastric administration. Treatment with curcumin began 3 days prior to STZ administration and was continued for 8 weeks.

2.2. Animal care

Rats were housed in individual and separate cages in a temperature (23 ± 3 °C) and humidity (50 ± 10%) controlled vivarium with a 12 h light/dark cycle. Body weight was recorded weekly and food intake was monitored daily. All animals had free access to water and chow throughout the study. All animal protocols used were in accordance with the guidelines for care and use of laboratory animals provided by the CPCSEA and was approved by the animal ethics review committee of our institution.

2.3. Rat aortic ring bioassay

At the end of 8 weeks, animals were sacrificed by inhalation of anesthetics under a fume hood. The thoracic aorta was surgically excised. After deliberate removal of periaortic fibro-adipose tissue, the aorta was sectioned into 2 mm ring-like segments. The ring-shaped explants were rinsed in PBS (Sigma-Aldrich, USA; pH = 7.4) and were embedded in a solution containing 1.6 g/L of gentamicin dissolved in 50 ml PBS (pH = 7.4). Containers were maintained at 4 °C. The culture medium was prepared in a laminar-flow cabinet by mixing a culture media of Hams F12 (pH = 7.4, Sigma-Aldrich) and Dulbecco's modified Eagle's medium (DMEM, pH = 7.4, Sigma-Aldrich) in a ratio of 50:50. Both media were filtered through a 0.22 μm hydrophilic cartilage membrane (Durapore, EMD Millipore). Immediately, after aortic ring embedding, 2.5 mg/L VEGF (Sigma-Aldrich) and 20% fetal calf serum (FCS, Sigma-Aldrich) were added to the culture medium of groups C-V, D-V and D-V-TC.

To prepare the culture media, a 96-well culture plate (Greiner Bio-One, Germany) was covered with fibrin gel and was allowed to set-up for 1 h at 37 °C, 5% CO_2. Fibrin gel was obtained by combining 2.5 μl thrombin (Sigma-Aldrich; concentration: 500 ku/L) and 250 μl fibrinogen (Sigma-Aldrich; concentration: 8 g/L), under the laminar-flow cabinet. Then, the aortic explant was embedded in each fibrin-coated well and 1 mL of culture media was added to the medium and was covered with an additional layer of fibrin gel. The rings were incubated at 37 °C, 5% CO_2 for 7 days. All assays were performed in duplicates.

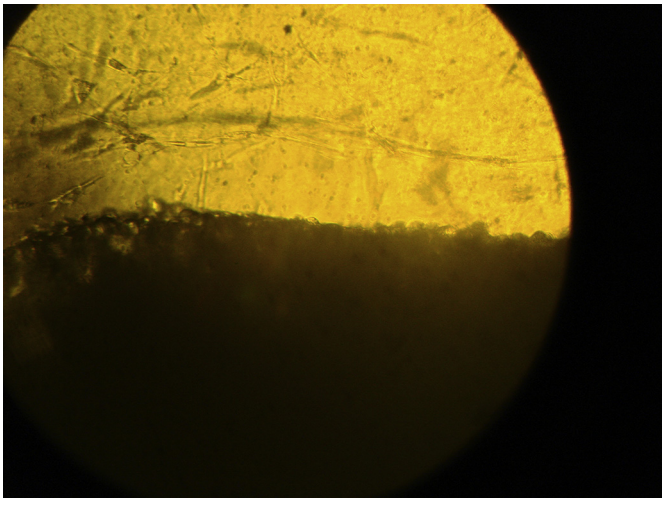

Fig. 1. Optic microscopic view of endothelial cells of aortic ring in Group C.

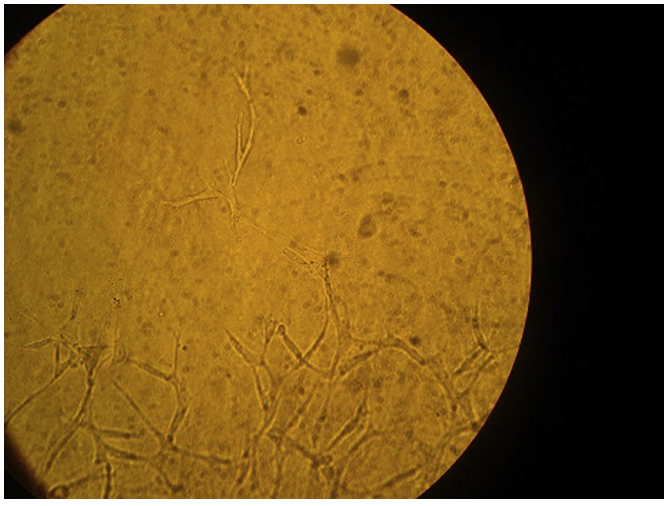

Fig. 2. Optic microscopic view of endothelial cells of aortic ring in Group C-V.