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Comparative study of Curcumin and Lucentis on retinal neovascularization

Running Title: Curcumin on retinal neovascularization in mice

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Abstract
Introduction To investigate the effect of Curcumin on retinal neovascularization in mice with oxygen-induced lesions.
Methods 7-day-old (P7) C57/BL6J mice were randomly divided into control group, OIR group, DMSO group, 100 mg/kg, 50 mg/kg and 25 mg/kg curcumin group and Lucentis group (15 mice per group). Mice in the experimental group were fed for 5 days in high oxygen partial pressure environment, and then in normal oxygen air environment for another 5 days. Corresponding interventions were given at 12-16 days of age (P12-16). At 17 days of age (P17), the eyeball was removed and the retina was paved with Isolectin GS-IB4 fluorescence staining. Real-time PCR was used to detect VEGF mRNA levels in tissues and cells. The protein expression level of VEGF was detected by Western blot.
Results Immunofluorescence showed that curcumin injection could significantly reduce the formation of retinal neovascularization and astrocyte injury in OIR, and 100 mg/kg curcumin group had the best effect. Compared with the control group, mRNA and protein expression of VEGF in retina of mice in OIR and DMSO groups were significantly up-regulated ($P<0.05$); Compared with OIR group, curcumin group and Lucentis group were down-regulated ($P<0.05$). The protein expression and mRNA level of VEGF in HRCECs of curcumin group decreased with the increase of curcumin concentration, and the effect of curcumin group at 80μmol/L was similar to that of Lucentis group. In the HRCECs cultured with the same concentration of curcumin, the protein expression and mRNA level of VEGF decreased with the prolongation of drug intervention time.
Conclusion Curcumin can down-regulate the expression of VEGF in retinal tissues and cells, thereby inhibiting retinal neovascularization and HRCECs cell proliferation.
Keywords: Curcumin; Lucentis; Oxygen-induced retinopathy; Retinal neovascularization; VEGF

Introduction

The formation and proliferation of retinal neovascularization (RNV) is the basis for the development of many fundus vascular diseases [1,2]. This pathological process is often accompanied by exudative, hemorrhagic, proliferative and other changes. According to the clinical characteristics of the disease, it can be divided into proliferative diabetic retinopathy (PDR), retinopathy of prematurity (ROP) and so on [3,4]. A mice model of oxygen-induced retinopathy (OIR) with reproducible and quantifiable proliferative retinal neointima formation is suitable for examining the pathogenesis of retinal neointima formation and therapeutic interventions in ROP and other vascular lesions[5]. This model has been widely used in studies related to ROP, PDR and to assess the efficacy of anti-angiogenic compounds[6-7].
Overexpression of vascular endothelial growth factor (VEGF) is the basis for RNV formation [8]. The anti-VEGF drug Lucentis is currently one of the effective treatments for RNV [9-10], but because of its high price and the need for multiple intraocular injections, there is a need to find a more cost-effective alternative treatment.
Curcumin is the main active ingredient extracted from turmeric, which has anti-inflammatory, anti-tumor and antioxidant pharmacological effects [11-12]. Studies have confirmed that curcumin can inhibit neovascularization by downregulating VEGF expression [13-14], but the therapeutic mechanism of curcumin in RNV diseases is not clear. In this experiment, we investigated the effects of curcumin and Lucentis on RNV formation and VEGF expression in oxygen-induced retinopathy (OIR) at animal level and cellular level.

1 Materials and Methods

1.1. Main materials, reagents and instruments

Meterials and reagents: Curcumin (458-37-7, Alfa , USA); Lucentis (Novartis , Switzerland); Isolectin (GS-IB4, 1214130, Invitrogin, USA); GFAP (GA5 , 3665, cell signaling technology, USA); polyclonal murine anti-VEGF antibody (ab46154, Abcam, USA); Trizol reagent (15596018, Invitrogen , USA); Western blot-related reagents (Roche Biologicals, Germany); Real-time PCR-related reagents and primer kits (Takara, Japan).
Apparatus: homemade laboratory animal chambers; oxygen concentration detector (MSA primax, USA) ; confocal fluorescence microscope (LSM 510 META, Zeiss, Germany); Real-time PCR instrument (ABI 7000, Bio-rad, USA), western blot protein electrophoresis instrument (Bio-rad, USA).

1.2 Experimental animals and cells grouping

A total of 105 SPF-grade healthy 7-day-old (P7) C57BL/6J mice, half male and half female, weighing (4.71 ± 0.63) g, were purchased from the Experimental Animal Center of Shanxi Medical University, and all mice were examined in

both eyes before modeling to exclude ocular lesions. The 105 mice were randomly divided into control group, OIR group, DMSO group, 100 mg/kg curcumin group, 50 mg/kg curcumin group, 25 mg/kg curcumin group and Lucentis group (5 mg/kg prepared and 0.1 ml injected intraperitoneally) using the random number table method, with 15 mice in each group. 30 eyes were taken from each group of mice for immunofluorescence staining experiments, Real-time PCR experiments and Western blot experiments, 10 eyes for each experiment.
Primary human retinal capillary endothelial cells were used for the cell experiments, and the cells were extracted and routinely cultured for one week for subsequent treatment. The groups were as follows: TN group: normal glucose group (glucose concentration of 1.0 mmol / L); TH group: high glucose group (glucose concentration of 5.5 mmol / L); TL group: Lucentis group; T1 group: high glucose + 5 μmol/L curcumin group; T2 group: high glucose + 10 μmol/L curcumin group; T3 group: high glucose + 20 μmol/L curcumin group; T4 group: high glucose + 40 μmol/L curcumin group.

1.3 Establishment of animal models
Control mice were housed in normoxic air at (23 ± 2)°C until 17 days of age (P17). A total of 90 mice from each experimental group and their nursing mothers (3 mice from each group, 18 mice in total) were placed in an experimental animal chamber connected to an oxygen concentration detector at P7, with a constant O_2 volume fraction of (75 ± 2)%, temperature (23 ± 2)°C and humidity (55 ± 2)%. Normal fluorescent lighting was given, and the light was turned off for 12 h/12 h on a day/night cycle, with regular observation twice daily. Bedding was changed, food added and water changed every 2 days. Mice were kept under high oxygen partial pressure for 5 days, then removed from the oxygen chamber at 12 days of age (P12) and placed in normoxic air for another 5 days.
At P17, the mice in each group were executed, the samples were taken, and subsequent experiments were performed.
1.4 Drug intervention and sampling
At P12-P16, the corresponding drug interventions were given to each experimental group. The single-day injection dose for each experimental group was as follows: DMSO group: 1‰ DMSO solution of 0.1 ml/each; Lucentis group: Lucentis injection of 0.1 ml/each; Curcumin groups: concentrations of 100, 50, 25 mg/kg curcumin (dissolved in 1‰ DMSO), all 0.1 ml/each, respectively. The administration method was intraperitoneal injection for 5 consecutive days.
At P17, the mice in each group were executed by ether anesthesia, and the eyes were removed for retinal stretched preparation (10 eyes per group), Real-time PCR (10 eyes per group) and Western blot assay (10 eyes per group).
1.5 Culture and identification of HRCECs
Culture of HRCECs: Human post-transplant donor eyes were obtained by aseptic surgery, the eyes were cut open parallel along the equator under a microscope, and the retinal nerve layer was bluntly peeled using microscopic instruments, and HRCECs were obtained after digestion, clipping, homogenization, and filtration. DMEM medium containing 10% fetal bovine serum, 100 u/mL penicillin, and 50 u/mL gentamicin was added to the HRCECs, and placed in 5% CO_2 , 37℃ incubator for routine culture, and the growth of retinal microvascular endothelial cells was closely observed. The culture medium was changed every 1-2 days, and the primary cells were passaged when the wall area was greater than 80%.
Identification of HRCECs: The growth characteristics and morphological features of HRCECs were observed using an inverted phase contrast microscope, and cells in good growth condition were inoculated at a density of 4. 5×10^4 cells in a 24-well plate with sterile coverslips and incubated in an incubator. After the cells crawled all over the coverslip, the plate was rinsed twice with 1x PBS, the PBS was discarded and samples were fixed with 4% paraformaldehyde for 10 min. After three times rinsing with 1x PBS, the PBS was discarded and anti-VIII primary antibody was added and incubated overnight at 4 ℃. The above rinsing steps were repeated, and the cells were incubated with fluorescent secondary antibody dilution (1:1000) for 1 h at room temperature, rinsed and blocked, and the intracellular expression of factor VIII was observed and images were collected using fluorescence microscopy.
1.6 Relative area ratio of retinal neovascularization and central nonperfused areas
At P17, the eyes of each group of mice were removed for stretched preparation of retina, and Isolectin GS-IB4 immunofluorescence staining was performed according to the above immunofluorescence staining procedure, and Isolectin GS-IB4 expression was observed using fluorescence microscopy. Fluorescence images were collected and the relative areas of retinal neovascularization (absolute area of RNV/total retinal area) and central non-perfused area of

the retina (absolute area of non-perfused area/total retinal area) were analyzed using ImageJ software, and the experiments were repeated three times to obtain the average value.

1.7 MTT method to detect proliferation of HRCECs

HRCECs in regular culture were digested, centrifuged and resuspended, and inoculated in 96-well plates (no cells were added to the edge wells, only culture medium was added as a blank control for zeroing and evaporation prevention) with 5×10^3 cells per well and 200 μL of culture medium, and 5 replicate wells were set up for each group. After the cells adhered, the culture medium was discarded and drugs were added according to the experimental groups: 200 μL normal glucose culture medium was added to each well in the TN group; 200 μL high glucose culture medium was added to each well in the TH group; 200 μL Lucentis (1 mg/mL) was added to each well in the TL group; for T1-T4 group, curcumin diluted in high glucose culture medium was added to each well in the 96-well plate in concentrations of 5, 20, 40, and 80 μmol/L, respectively, 200 μL per well. The cells were incubated for 24 h, 48 h and 72 h, respectively, and then 20 μL of MTT powder solution was added to each well and incubated for 4 h. The medium was discarded and the cells were washed with PBS three times, and then 20 μL of DMSO solution was added to each well and shaken for 10 min until the blue-purple crystals dissolved. The 96-well plate was placed into the ELISA monitor and the wavelength was selected as 490 nm. The results were recorded and calculated according to the following formula: cell survival rate (%) = average OD value of experimental group (T1-T4 group) / average OD value of control group (TN, TH group) × 100%; cell inhibition rate (%) = (1 - OD value of experimental group / OD value of control group) × 100%. The experiment was repeated three times to take the average value for calculation.

1.8 Real-time PCR for mRNA expression of VEGF in mice retinal tissues

All operations were performed on an ultra-clean table. Total RNA was extracted from the retinal tissues of each group of mice according to the kit instructions, and the RNA was reverse transcribed into cDNA. The reaction program was set to incubate at 42℃ for 50 min and heat at 95℃ for 5 min, followed by PCR amplification, and the experiment was repeated three times.

The primer sequences used for PCR were as follows:

Genes	5' primers	3' primers
VEGF	TTGCTGCTCTCTACCTCCACCAT	TGATTCTGCCCTCCTCCTTCT
β-actin	TGGCACCCAGCACAATGAA	CTAAGTCATAGTCCGCCTAGCA

1.9 Western blot assay to detect the protein expression level of VEGF in the retinal tissue of each group of mice

The total protein of each group of mice retina was extracted, and the protein supernatant was separated for quantitative determination of protein concentration. 10% SDS-PAGE gels were prepared, and the samples were loaded according to the measured protein concentration. After 1.5 h of electrophoresis at 120 V and 1.5 h of membrane transfer at 250 mA, the membranes were blocked with 5% skim milk powder for 1 h. mice anti-TGF-β1 monoclonal antibody (1:200) and rabbit anti-PN monoclonal antibody (1:1000) were added and incubated overnight at 4℃. On alternate days, the membranes were washed 3 times using PBS, and goat anti-mice and goat anti-rabbit secondary antibodies (1:5000) were added, respectively, and incubated at room temperature for 1 h. They were washed 3 to 4 times using PBST. Protein strips were exposed using an electrochemical exposure meter and grayscale values were analyzed using Image J software. The experiment was repeated three times to take the average value for calculation.

1.10 Statistical analysis

This study used SPSS 25.0 analysis software to statistically analyze the data, and all data were expressed as mean ± standard deviation (X ± S). Means between groups were statistically analyzed using the LSD method, and comparisons between multiple groups were statistically analyzed using one-way ANOVA. The difference was statistically significant at $P<0.05$.

2 Results

2.1 Growth of neovascularization in retinal stretched preparation of each group of mice

The immunofluorescence results of P17 retinal stretched preparation in all groups of mice showed that curcumin intervention reduced the generation, clustering and accumulation of RNV in the OIR model and effectively prevented the damage of astrocytes. In the control group, there was no retinal neovascularization, and astrocytes showed normal stellate and dendritic shape without scarring. Compared with the control group, the OIR group and DMSO group had retinal vascular varicosities in the form of beaded clusters, with a large central non-perfused area and a large number of highly fluorescent stained neovascular clusters in the periphery, and astrocyte heterotypy and scarring were obvious. Compared with the OIR and DMSO groups, the area of retinal neovascularization and non-perfused area was reduced in the curcumin and Lucentis groups, and astrocyte heterogeneity and scarring were reduced. Moreover, the effect of curcumin was concentration-dependent, with the best effect in the 100 mg/kg group, and the difference between the 100 mg group and the Lucentis group was not significant, so 100 mg/kg was the best concentration for injection (Figure 1; Table 1).

2.2 Effect of different concentrations of curcumin on the proliferation of HRCECs under high glucose environment

The number of HRCECs cells cultured with high glucose was significantly increased compared with the normal glucose group, and the difference was statistically significant ($P<0.05$). After high glucose + different concentrations of curcumin (5, 20, 40, and 80 μmol/L) culture, the inhibition rate of HRCECs cells gradually increased with the increase of curcumin concentration ($P<0.05$). After the same concentration of curcumin intervention, the cell inhibition rate of HRCECs increased with the increase of drug intervention time, and the difference was statistically significant ($P<0.05$) (Table 2).

2.3 Real-time PCR to detect the mRNA expression of VEGF in the mice retina of each group

Compared with the control group, the mRNA levels of retinal VEGF were upregulated by 103.83% in the OIR group and 101.25% in the DMSO group, with statistically significant differences ($P<0.05$). There was no statistically significant difference between the two groups in the OIR and DMSO groups ($P>0.05$). Compared with the OIR group, the mRNA levels of retinal VEGF was downregulated (10.78% in the 25 mg/kg group, 30.46% in the 50 mg/kg group, and 43.51% in the 100 mg/kg group) in the curcumin group and was downregulated by 44.18% in the Lucentis group, all with statistically significant differences ($P<0.05$), but there was no statistically significant difference between the 100 mg/kg curcumin group and the Lucentis group ($P > 0.05$) (Figure 2).

2.4 Western blot detection of the protein expression of VEGF in the mice retina of each group

The expression of VEGF protein in the P17 mice retina in the OIR and DMSO groups was significantly upregulated compared with the normal control group ($P<0.05$), but there was no statistical difference between the two groups ($P>0.05$). Compared with the OIR group, the expression was decreased in the curcumin group and Lucentis group ($P<0.05$), but there was no statistical difference between the 100 mg/kg curcumin group and Lucentis group ($P>0.05$) (Figure 3).

2.5 Effect of different concentrations of curcumin on VEGF protein expression in HRCECs cells under high glucose environment

The protein expression of VEGF in HRCECs of different groups in this experiment was detected by Western blot. The results showed that the protein expression of VEGF in HRCECs of high glucose group was higher than that of normal glucose group, and the gray value analysis results of its protein bands were statistically different ($P < 0.05$); at the same culture time, the protein expression of VEGF in HRCECs decreased with the increase of curcumin concentration compared with Lucentis group and high glucose + different concentrations of curcumin group (5, 20, 40, 80 μmol/L) ($P < 0.05$). The protein expression of VEGF decreased with increasing duration of drug intervention in HRCECs with the same concentration of curcumin intervention ($P < 0.05$) (Figure 4).

2.6 Effect of different concentrations of curcumin on VEGF mRNA levels in HRCECs cells under high glucose environment

Real-time PCR was performed to detect the mRNA levels of VEGF in HRCECs of different groups: under the same incubation time, the mRNA levels of VEGF in HRCECs of the high glucose group were higher than those in the normal

glucose group, and the results were statistically different ($P < 0.05$); under the same incubation time, the mRNA levels of VEGF in HRCECs decreased with the increase of curcumin concentration compared with the Lucentis group, and the effect of 80 μmol/L curcumin group was similar to that of the Lucentis group, with statistical differences ($P < 0.05$). In HRCECs cultured with the same concentration of curcumin intervention, the mRNA levels of VEGF decreased with increasing time of drug intervention, with a statistical difference ($P < 0.05$) (Figure 5).

3 Discussion

RNV tends to trigger increased vessel wall permeability leading to leakage, vessel wall breakage and bleeding, which then leads to pathological connective tissue proliferation. RNV disease is retinal neovascularization, formation of vitreous mechanization and retinal proliferative membrane, resulting in reduced visual function and, in severe cases, blindness or even ocular atrophy [15-16]. Previous studies on RNV proliferative disease have focused on the angiogenic damage of RNV, but the role of abnormally proliferating pathological RNV on retinal neovascularization and astrocytes has not explained in detail. In this study, it was showed that scarring of high oxygen concentration-induced retinal astrocytes was evident, and most of them lost their normal stellate and dendritic morphology.

In the early 1950s, it was suggested that ROP might be associated with the toxic side effects of clinical treatment with high concentrations of oxygen [17-18] and a corresponding animal model of OIR was established. The retinal vascular development of C57BL/6J neonatal mice is similar to that of preterm infants, so the OIR model maximally simulates the whole process of pathophysiological changes of RNV and has become a mature model for the study of retinal neovascular diseases. In this study, we used OIR mice and primary HRCECs to investigate and explain the drug therapeutic effects and application prospects against OIR from in vitro and in vivo levels, respectively. After curcumin intervention during hypoxic phase, it could morphologically inhibit RNV production in OIR mice while causing astrocyte damage reduction and more similar morphology to normal stellate and dendritic morphology.

Studies have confirmed that RNV production mainly results from abnormal expression of VEGF, so anti-VEGF therapy is particularly important. Lucentis is a commonly used anti-VEGF drug in clinical practice, and Lucentis was used as a positive control in this study, but it only aims at single-target therapeutic study and is expensive, and there is a lack of studies on multi-target and multi-pathway neovascularization inhibition in clinical practice. Studies have shown that curcumin has a wide range of pharmacological effects and low clinical side effects. He et al. found that Ets-1 expression levels were downregulated and retinopathy was reduced after curcumin treatment in diabetic rats, suggesting that curcumin may alleviate the symptoms of diabetic retinopathy by inhibiting Ets-1 expression [19]. It was confirmed that curcumin alleviated retinal nerve cell damage in rabbits with acute hypertension by upregulating Thy-1 expression in retinal nerve cells, subsequently suggesting that curcumin may have a protective effect on the retina [20]. This study found that intervention of curcumin during hypoxia significantly reduced RNV formation in OIR mice, thus confirming that curcumin can inhibit RNV production effectively in a dose-dependent manner, further supporting the results demonstrated by Yang F et al. in 2018 that curcumin can alleviate symptoms such as photoreceptor cell membrane disorder and retinal thinning by ameliorating diabetes-induced capillary basement membrane thickening, ganglion cell and inner nuclear layer cell apoptosis [21].

Compared with the control group, VEGF mRNA levels were elevated in the OIR group and decreased significantly after curcumin and Lucentis intervention during the hypoxic phase. The changes in protein levels of VEGF were basically consistent with mRNA levels in all groups, suggesting that the effects of curcumin were similar to Lucentis and were dose-related. In vitro experiments demonstrated that the protein expression and mRNA levels of VEGF in HRCECs decreased with the increase of curcumin concentration in Lucentis group and high glucose + different concentrations of curcumin (5, 20, 40, 80 μmol/L) groups, and the effect of curcumin at 80 μmol/L was similar to that of Lucentis group. In HRCECs cultured with the same concentration of curcumin, the protein expression and mRNA levels of VEGF decreased with the increase of drug intervention time, and these data further supported the conclusions obtained from in vivo experiments. Further related studies will be conducted subsequently to clarify the principle and mechanistic pathway of curcumin action on RNV. The exact mechanism by which curcumin regulates VEGF expression is unknown, and further experiments are needed to understand how curcumin regulates VEGF.

In summary, curcumin morphologically protected astrocytes while inhibiting pathological neovascularization in the retina of OIR mice. By downregulating VEGF expression, curcumin inhibited oxygen-induced retinal neovascularization and suppressed the proliferation of HRCECs cells with effects similar to those of Lucentis, and curcumin is expected to be an effective treatment for RNV disease with promising clinical applications.

Statements

Statement of Ethics

This study was conducted in accordance with the declaration of Helsinki.This study protocol was reviewed and approved by he Ethics Committee of Shanxi Aier Eye Hospital, approval number [No.20210717]. Written informed consent was obtained from all participants.

Conflict of Interest Statement

All of the authors had no any personal, financial, commercial, or academic conflicts of interest separately.

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Author Contributions

(I) Conception and design: Lu Yang
(II) Administrative support:Ximei Zhang, Dongping Li, and Guohong Zhou
(III) Provision of study materials or patients: Jiewei Liu and Yan Gao
(IV) Collection and assembly of data: Shufang Du and Yanyun Shi
(V) Data analysis and interpretation: Yong Li and Na Di
(VI) Manuscript writing: All authors
(VII) Final approval of manuscript: All authors

Data Availability Statement

All data generated or analyzed during this study are included in this article.Further enquiries can be directed to the corresponding author.

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Figure Legend:

Figure 1 A: Morphology showing inhibition of neovascular proliferation while protecting astrocytes after curcumin injection at different concentrations.

IB4 (red, neovascular staining); GFAP (green, astrocyte staining). Scale bar: 100 μm.

B: Statistical plot of retinal neovascularization area after curcumin intervention with different injection concentrations;

C: Statistical plot of non-perfused area after curcumin intervention with different injection concentrations. Data statistics are mean ± standard error.

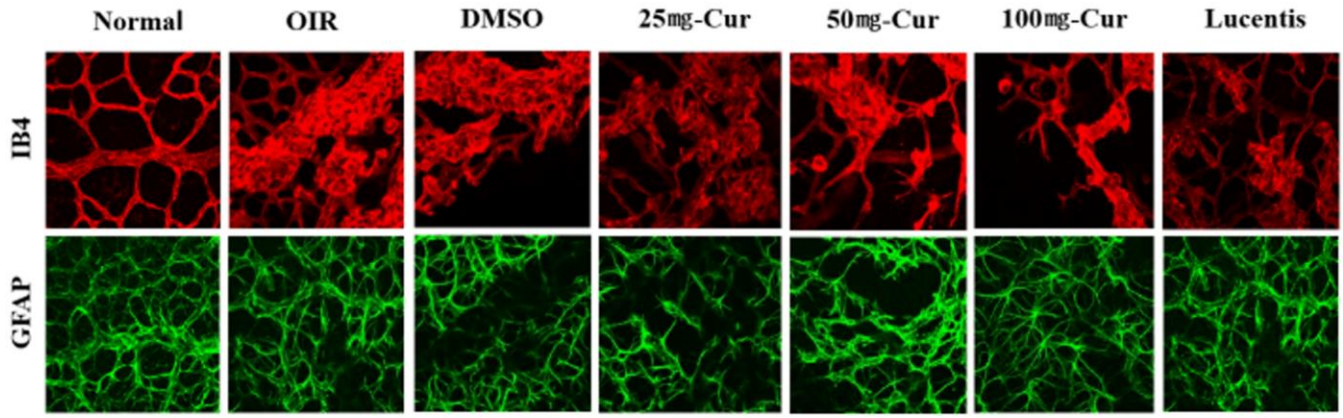
Figure 2 Real time-PCR on the expression level of VEGF mRNA in the mice retina of each group

Note: # Compared with the normal group（P＜0.05）；* Compared with the OIR group（P＜0.05）.

Figure 3 VEGF protein strips in the mice retina of each group and Fluorescence density analysis of VEGF strips

Figure 4 VEGF protein expression levels in HRCECs of different groups

Figure 5 The mRNA expression levels of VEGF in different groups of HRCECs



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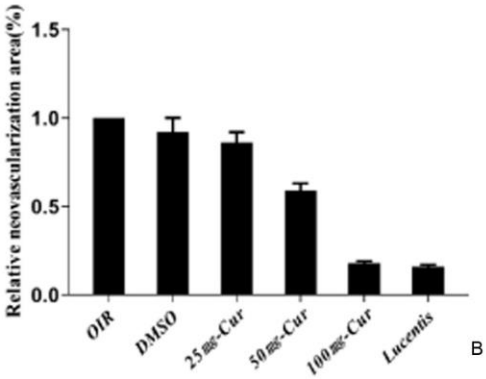
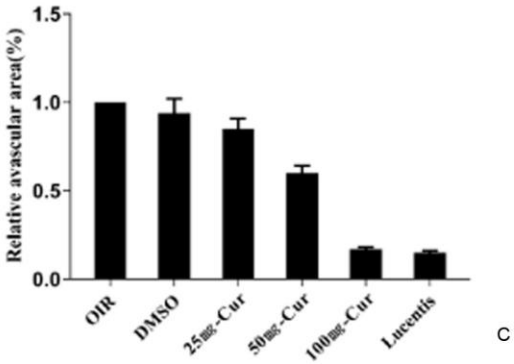


B



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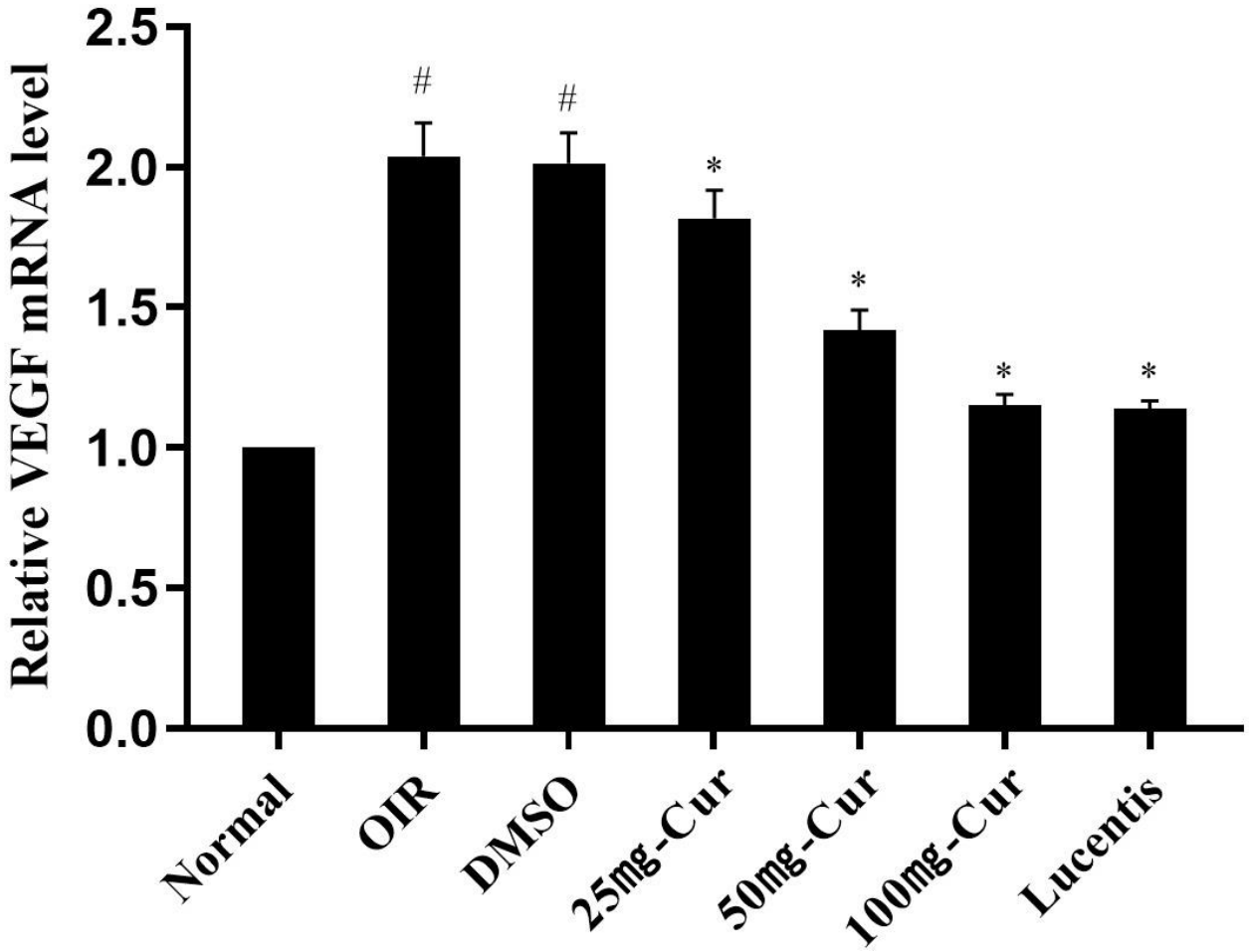
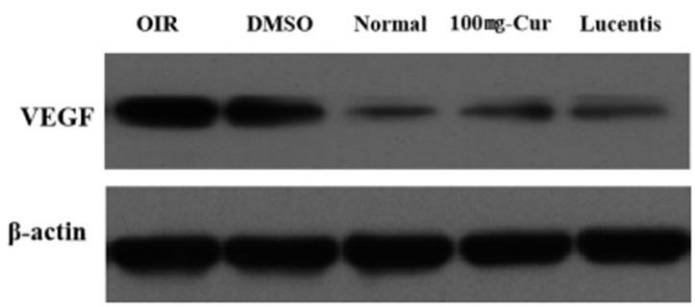
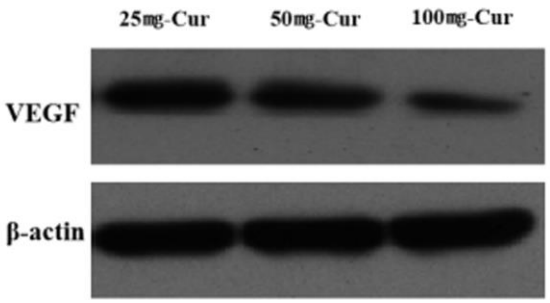
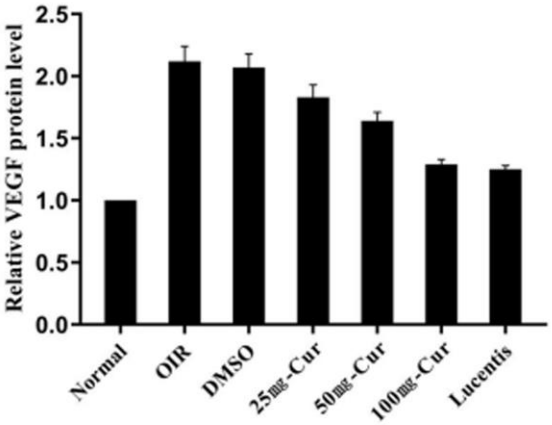
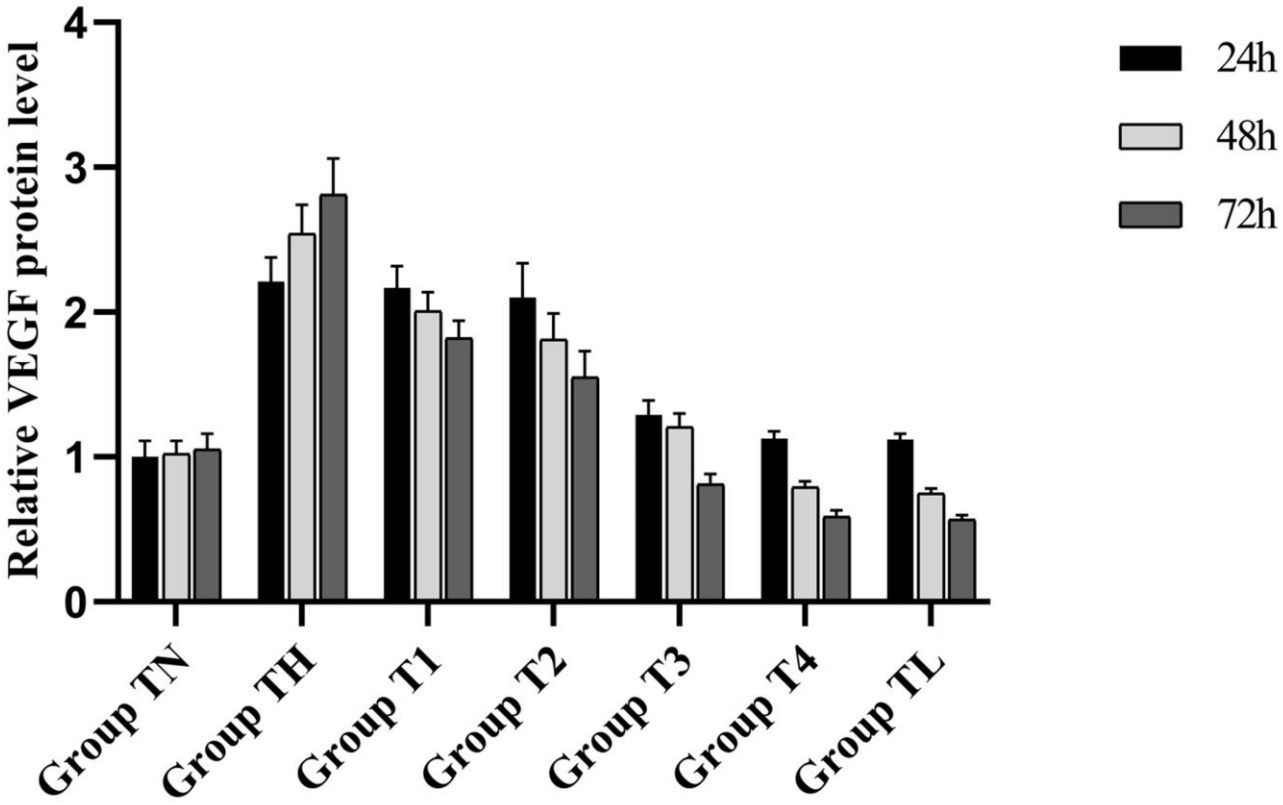

Relative VEGF mRNA level
2.5
2.0
1.5
1.0
0.5
0.0
#
#
*
*
*
*
Normal
OIR
DMSO
25mg-Cur
50mg-Cur
100mg-Cur
Lucentis


OIR
DMSO
Normal
100mg-Cur
Lucentis
VEGF
β-actin


25mg-Cur
50mg-Cur
100mg-Cur
VEGF
β-actin


Relative VEGF protein level
2.5
2.0
1.5
1.0
0.5
0.0
Normal
OIR
DMSO
25mg-Cur
50mg-Cur
100mg-Cur
Lucentis


Relative VEGF protein level
4
3
2
1
0
24h
48h
72h
Group TN
Group TH
Group T1
Group T2
Group T3
Group T4
Group TL

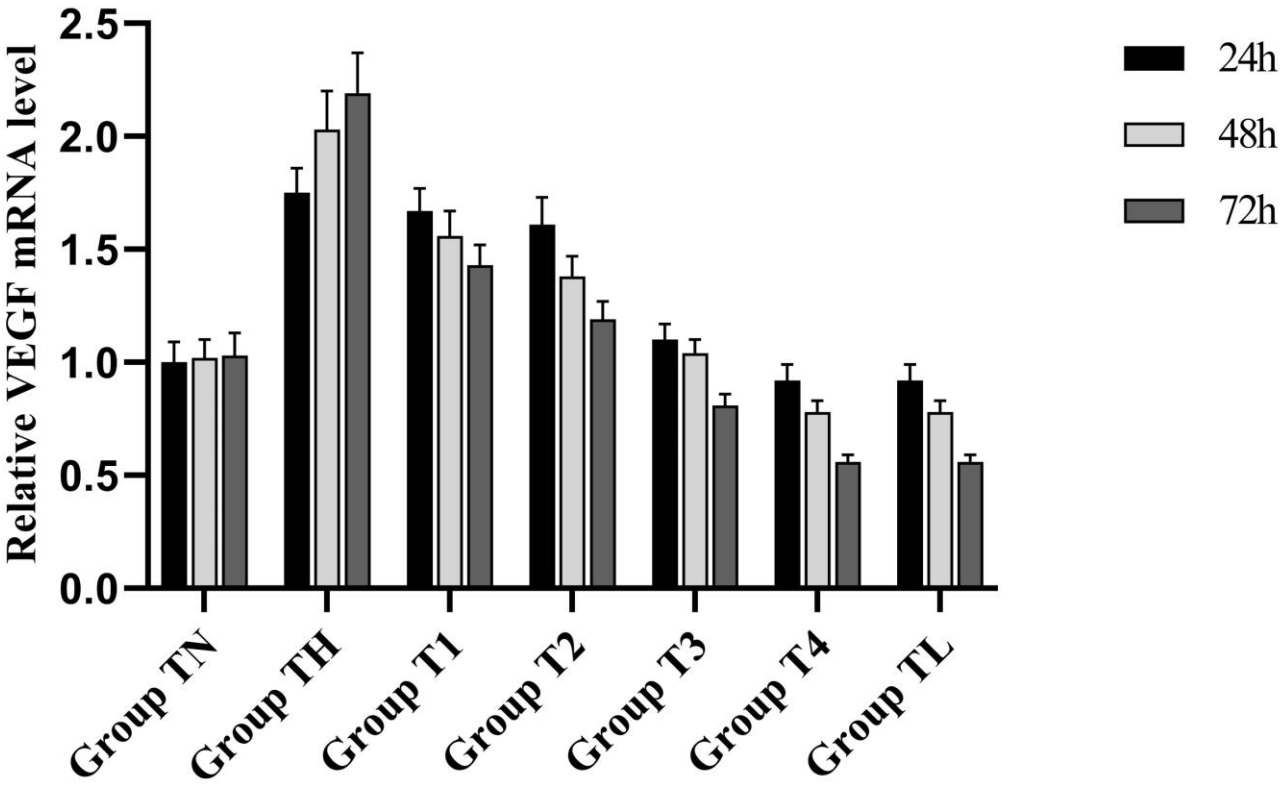

Relative VEGF mRNA level
2.5
2.0
1.5
1.0
0.5
0.0
Group TN
Group TH
Group T1
Group T2
Group T3
Group T4
Group TL
24h
48h
72h

Table 1: Reduction of retinal neovascularization and non-perfused area in OIR mice after curcumin intervention at different injection concentrations

(All units are in μm2)	OIR group	DMSO group	25 mg curcumin group	50 mg curcumin group	100 mg curcumin group	Lucentis Group
Relative neovascularization area ratio	3194879/ 7269798= 43.94%	3195743/ 7271964= 43.92%	2471458/ 7094368= 34.85%	1623673/ 7412365= 21.91%	581583/ 7157832= 8.14%	578556/ 7314239= 7.91%
Relative avascular area ratio	2698727/ 7269798= 32.98%	2697645/ 7271964= 32.96%	1953473/ 7094368= 27.44%	1428324/ 7412365= 19.26%	392245/ 7157832= 5.49%	397163/ 7314239= 5.42%
Area ratio of reduction of neovascularization compared to OIR group	1	1	20.73%	50.15%	81.48%	81.78%
Area ratio of reduction in the non-perfused area compared to the OIR group	1	1	16.82%	41.62%	83.36%	83.54%

Table 2 Effect of different concentrations of curcumin and Lucentis on the proliferation of HRCECs cells (n = 5)

Group	A490 Value		
	24 h	48 h	72 h
TN group	0. 4013±0. 128	0. 4248±0. 0113	0. 4567±0. 0110
TH Group	0. 6279±0. 008*	0. 6591±0. 007*	0. 6953±0. 005*
TL Group	0. 3957±0. 007#	0. 3653±0. 005#	0. 3165±0. 005#
T1 group	0. 5664±0. 006#	0. 5438±0. 005#	0. 5221±0. 007#
T2 group	0. 4732±0. 009#	0. 4382±0. 004#	0. 4047±0. 008#
T3 group	0. 4269±0. 013#	0. 4023±0. 008#	0. 3667±0. 007#
T4 group	0. 3974±0. 008#	0. 3665±0. 006#	0. 3172±0. 008#

Compared with TN group,* P<0. 05; compared with TH group,# P<0. 05