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Effects of Gold Nanoparticles on Vascular Endothelial Growth Factor and its Receptor in an Animal Model of Uveitis in Rats [Version 1, 2 Approved with Reservations]

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Abstract

Endotoxin-induced uveitis (EIU) is characterized by leukocyte infiltration, breakdown of the blood-ocular barrier, and retinal cell death. Vascular endothelial growth factor (VEGF) contributes to the retinal vascular hyperpermeability. Gold nanoparticles (GNP) exerted antiangiogenic activities and reduced macrophage infiltration and inflammation in animal models of arthritis and retinopathy of prematurity. Thus we hypothesized that GNP treatment can decrease retinal levels of VEGF in an animal model of lipopolysaccharide induced uveitis (LPS). EIU was induced by the administration of LPS in male Wistar rats. Two hours after LPS administration, saline, prednisolone acetate 1%, aurochloric acid (GS) 2 mM solution or GNP (40 mg/ml) were topically applied to both eyes of rats and repeated every 6 hours for 24 hours. After 24 hours the aqueous humor was sampled bilaterally and the retinas were excised. VEFG was measured using ELISA assay and VEGF receptor 2 (VEGFR2) by western blot. There were a significantly increase in the levels of VEGF, but not VEGFR2 in the retina of animals exposed to LPS, and this was related to an increase in the levels of aqueous humor MPO activity (r=0. 87, p<0. 001). None of the treatments were able to decrease the levels of VEGF in the retina.

Keywords

Gold Nanoparticles; Nanoparticles; Biological Activity; Uveitis; Vascular Endothelial Growth Factor; Anti-Inflammatory Activity.

Introduction

Endotoxin-induced uveitis (EIU) is an acute intraocular inflammatory condition that mimics human disease and is induced in an animal model by the systemic injection of sublethal doses of lipopolysaccharide (LPS). The inflammatory response of EIU peaks at 24 h and is characterized by leukocyte infiltration, breakdown of the blood-ocular barrier, and retinal cell death [1]. In this context, vascular endothelial growth factor (VEGF) has been implicated in the pathophysiology of the disease and directly contributes to the retinal vascular hyperpermeability, angiogenesis and inflammation that are clinically observed [2]. In addition, macular oedema contributes to the pathophysiology of a number of retinal diseases, including diabetic retinopathy, ischaemic retinopathies and uveitis, and VEGF contributes to retinal macular oedema, particularly in diabetic and ischaemic retinopathies [3]. However, rats developing autoimmune uveitis have high levels of VEGF in the retina, but no neovascularization [4].

Intraarticular administration of nanogold ameliorates the clinical course of arthritis in rats [5]. Gold nanoparticles (GNP) exerted antiangiogenic activities and subsequently reduced macrophage infiltration and inflammation, which resulted in attenuation of arthritis [5]. In addition, GNP could be a potent inhibitor to retinal neovascularization without retinal toxicity retinopathy of prematurity [6].

In view of these properties of GNP and its effects upon VEGF we hypothesized that GNP treatment can decrease retinal levels of VEGF in an animal model of LPS-induced uveitis and this can be related to its ability to decrease eye inflammation.

Materials and Methods

GNPs Preparation and Characterization

GNP were prepared as we previously described and characterized employing UV-vis spectroscopy, XRD diffractometry and Transmition Electron Microscopy [7]. In brief, aurochloric acid solution was warmed up to 90°C and sodium citrate was added. After vigorous stirring and refluxing, the solution was cooled at room temperature (20 + 2°C) and the GNP were purified by serial centrifugations. The resulting powder was re-suspended in saline solution (NaCl 0. 9%) and stored at 7 ºC until use.

Animals

Male Wistar rats weighing 300–350 g obtained from Universidade do Extremo Sul Catarinense breeding colony were housed individually under standard conditions. All studies were approved by the Ethics Committee from Universidade do Extremo Sul Catarinense. Experimental animals were first randomly divided into five groups (6 animals per group): control, lipopolysaccharide (LPS), LPS + prednisolone, LPS + GS and LPS + GNP.

EIU and Treatments

EIU was induced by the administration of LPS (*Escherichia coli*, serotype 055: B5; Sigma-Aldrich, St. Louis, MO) 100 µg/100 µL pyrogen-free 0. 9% sodium chloride into subcutaneous tissue [8]. Two hours after LPS administration, saline, prednisolone acetate 1% (Latinofarma Pharmaceutical Industries LTDA), GS (aurochloric acid 2 mM) or GNP (40mg/ml) were topically applied to both eyes of rats and repeated every 6 hours for 24 hours. Both GS and GNP were prepared under sterile conditions as isotonic solutions. After 24 hours, rats had been anesthetized with ketamine chlorohydrate (50 mg/kg) and xylazine 2% (20 mg/kg). The retinas were excised and stored at -80°C to posterior analyses.

Retinal Inflammatory Response

As an index of neutrophil infiltration it was measured myeloperoxidase activity as previously described [9]. Briefly, aqueous humor was homogenized (50 mg/mL) in 0. 5% hexadecyltrimethylammonium bromide and was centrifuged at 15, 000*g* for 40 minutes. An aliquot of supernatant was mixed with a solution of 1. 6 mM tetramethylbenzidine and 1 mM H_2O_2. Activity was measured spectrophotometrically as the change in absorbance at 650 nm at 37°C.

Enzyme-Linked Immunosorbent Assay for VEGF

To measure VEGF, we used 6 rats (12 retinas) for each group. VEGF were estimated with enzyme-linked immunosorbent assay (ELISA) kits (Prepotech, Ribeirao Preto, Brazil) according to the manufacturer's instructions. The tissue sample concentration was calculated from a standard curve and corrected for protein concentration.

Western Blot Analyses for VEGF Receptor 2 in Retinas

Retinal samples were homogenized in Laemmli-sample buffer (62. 5mM Tris-HCl, pH 6. 8, 1% (w/v) SDS, 10% (v/v) glycerol) and equal amounts of cell protein (30 µg/well) were fractionated by SDS-PAGE and electro-blotted onto nitrocellulose membranes. Protein loading and electro-blotting efficiency were verified through Ponceau S staining, and the membrane was blocked in Tween-Tris buffered saline (TTBS: 100mM Tris-HCl, pH 7. 5, containing 0. 9% NaCl and 0. 1% Tween-20) containing 5% albumin. Membranes were incubated overnight at 4ºC with primary antibody diluted at 1:1000 in TTBS (1:1000) (anti-VEFGR2, was purchased from Millipore, USA) and then washed with TTBS. Anti-IgG linked to a peroxidase was then incubated with the membrane for additional 2 h at room temperature (1:5000 dilution range), the membrane was washed again and the immunoreactivity was detected by enhanced chemiluminescence using Pierce WestPico Chemiluminescence kit. Densitometric analysis of the films was performed with ImageQuant software. Blots were developed to be linear in the range used for densitometry.

Statistical Analysis

To evaluate the differences across various experimental conditions, one-way analysis of variance was performed and SNK test served to evaluate differences between individual pairs of experimental conditions. The correlation between VEGF and VEGFR2 and MPO activity was performed by Pearson test. In all analyses, $p < 0.05$ was considered statistically significant.

Results

As shown in Figure 1, there were a significantly increase in the levels of VEGF in the retina of animals exposed to LPS, and this was related to an increase in the levels of aqueous humor MPO activity ($r=0.87$, $p<0.001$). Differently from our primary hypothesis none of the treatments were able to decrease the levels of VEGF in the retina, actually, GS could even increase VEGF levels (Figure 1).

Since the majority of the effects of VEGF are mediated by its binding to VEGFR2 we determined the content of VEGFR2 in these animals, but we could not find any significant difference in its content when compared normal and LPS-injected animals, and none significant effect of any treatment (data not shown).

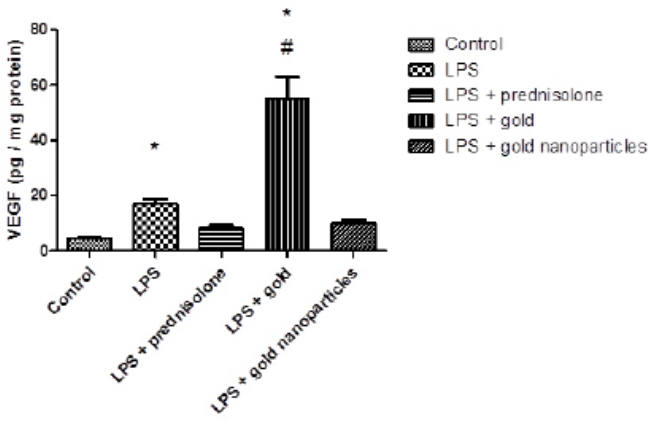

Figure 1: Effects of GNP or prednisolone or GS on VEGF levels in the retina 24 hours after the administration of LPS. Data are expressed as mean ± SD. *Different from control. # Different from LPS.

Discussion

In the present study the administration of GNP 2 hours after LPS injection did not decrease VEGF and VEGFR2 levels in the rat retina. This result is differently from our primary hypothesis that is supported by some data that demonstrate a decrease in VEGF levels after GNP treatment in different animal models, such as arthritis and retinopathy of prematurity [5, 6]. Since gold binds strongly to thiols and amines [10], nanogold inhibits VEGF-induced endothelial cell proliferation by interacting with the sulfur/amines present in its heparin-binding domain, and thereby inhibits VEGF-induced signaling [11].

The ability of GNP to bind VEGF seems to be related to its size and charge [11]. When compared 5 to 20 nm diameter particles it was shown that larger particles showed maximum effect (complete inhibition), while 5 nm GNP exhibited a modest (25%) inhibition of the in vitro activity of VEGF. Other studies have demonstrated that after intravenous injection of gold nanoparticles, 100 nm nanoparticles were not detected in the retina whereas 20 nm nanoparticles passed through the blood-retinal barrier and were distributed in all retinal layers [12]. We here used GNP around 29 nm and we could not find any evidence in the literature on the binding of this size GNP to VEGF, thus one can postulate that our particles were too large to bind it. In addition, it was shown that surface modification of GNPs with various charged ligands prevents their ability to bind VEGF emphasizing the role of the naked NP surface. Studies have demonstrated that the anti-angiogenic property of bare gold nanoparticles is lost when the particles are covered with nonfunctional charged ligands [11], how GNPs used in it study are electrically charged and present potential Zetta of -30 mV may be effective role in angiogenesis are lost.

The studies published to date are no consensus as to biological effect of GNPs as for particle size or functionality of charges in compound, but also regarding for ocular structure which is composed to three layers cornea, which may hamper penetration of active ingredient [13, 14].

In addition to eye anatomical features, the administration route also is essential for compound penetration into tissue and its bioavailability and effect, as described in previous studies demonstrated that topically has low absorption intraocular, approximately 5% dose [15, 16]. Furthermore, drainage of tear film to tearing and blinking reflex upon instill the eye drop and the limited volume, allowing compound elimination quickly [13]. Some studies demonstrated nanoparticles show effect in more internal ocular tissues such as the retina after subconjunctival administration [17] and not topically as used in the study, which may at least in part to confirm absence of GNP effect in angiogenesis mediated by VEGF. In addition, we did not measure gold concentration in the retina, then we can not ascertain that GNP can really reach retina to promote a decrease in VEGF concentrations.

We find a significant relation between retinal VEGF and MPO activity, suggesting that some of the aqueous humor inflammatory cells can cross the retinal-blood barrier [18]. However, since we had previously demonstrated that GNP could decrease MPO activity and it did not interfere in VEGF levels this is not probable. Most probable is that inflammatory cells came from the iris and ciliary region [19] and promote retinal inflammation that is followed by increase in the retinal levels of VEGF.

In summary, the present study does not support a role for GNP in decreasing retinal VEGF levels in an animal model of LPS-induced uveitis.

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