Effects of an Antagonist of the Gastrin-Releasing Peptide Receptor in an Animal Model of Uveitis

David Valter Pereira,1 Amanda Valnir Steckert,1 Franciele Mina,1 Fabricia Petronilho,1,2 Rafael Roesler,3,4 Gilberto Schwartzmann,4 Cristiane Ritter,1 and Felipe Dal-Pizzol1,2

PURPOSE. Some studies have shown the role of gastrin-releasing peptide (GRP) on the production and release of cytokines both in animal models and in humans with inflammatory diseases, but there are no reports on the effects of GRP in ocular inflammatory disease, mainly uveitis. The authors report on the effects of the GRP receptor (GRPR) antagonist RC-3095 in a well-established model for uveitis induced by the administration of lipopolysaccharide (LPS), comparing its effects with those of glucocorticoids.

METHODS. Adult male Wistar rats (weight range, 250–300 g; n = 6 per group) were randomly divided into four groups: saline, LPS + saline, LPS + dexamethasone, LPS + RC-3095. Two hours after LPS administration, RC-3095 (0.3 mg/kg, single dose, subcutaneously) or dexamethasone (1 mg/kg, each 6 hours, subcutaneously) was administered. After 24 and 48 hours, rats were anesthetized, aqueous humor was sampled, and the irides were removed. Aqueous humor tumor necrosis factor-α, monocyte chemoattractant protein-1 concentration, myeloperoxidase activity were determined. In addition, oxidative damage to the irides was determined by the measure of thiobarbituric acid reactive substances and protein carbonyl content.

RESULTS. The acute administration of RC-3095 exhibited anti-inflammatory actions, characterized by a reduction of myeloperoxidase activity and a decrease in tumor necrosis factor-α and monocyte chemoattractant protein-1 levels, to a greater extent than dexamethasone. In addition, RC-3095 elicits important action against irides oxidative damage.

CONCLUSIONS. These findings suggest that GRP participates in the inflammatory response in an animal model of uveitis, making GRPR a target for new therapeutic options in the treatment of uveitis. (Invest Ophthalmol Vis Sci. 2009;50:5300–5303) DOI: 10.1167/iovs.09-3525

Uveitis is an important cause of blindness worldwide. In the United States its incidence is reported to be 17 per 100,000 to 24 per 100,000 inhabitants per year, and its prevalence is reported to be 38 per 100,000 to 204 per 100,000 inhabitants per year.1

The proinflammatory cytokines interleukin (IL)-1β, -2, -6, interferon-γ, and tumor necrosis factor (TNF)-α have all been detected within the ocular fluids or tissues in the inflamed eye, together with others, such as IL-4, -5, -10, and transforming growth factor-β. The chemokines IL-8, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein (MIP)-1α, MIP-1β, and fractalkine are also thought to be involved in the associated inflammatory response.2–7

The evidence for the involvement of neuropeptides in inflammatory diseases has focused predominantly on a proinflammatory role for substance P (SP)8–10 and an immunomodulatory role for neuropeptide Y, the proinflammatory action of neuropeptide U, and an anti-inflammatory role for vasoactive intestinal peptide.11–13 The mechanism of action of these neuropeptides in modulating inflammatory diseases is, at least in part, due to their ability to affect cytokine production. For example, SP increases the production of the proinflammatory cytokines TNF-α, IL-6, and IL-1β whereas vasoactive intestinal peptide inhibits the production of the proinflammatory cytokines TNF-α, IL-6, and IL-12 and stimulates the production of the anti-inflammatory cytokines IL-10 and IL-1Ra.14

In this context, gastrin-releasing peptide (GRP) and its receptor are widely distributed in mammalian peripheral tissues and in the central nervous system. Recently, effects of these peptides on the production and release of cytokines were described both in animal models and in humans with inflammatory diseases.15

There is the description of GRP in the normal retina, but there is no description in the literature of a role for GRP in pathologic conditions in the eye. Thus, using a pharmacologic tool, we have demonstrated a role for GRP in the development of the inflammatory response in an animal model of uveitis.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 250 to 300 g each were used. The animals were caged at 22°C, with a 12-hour light/12-hour dark cycle and free access to food and water until the time of the experiments. All experimental procedures involving animals were performed in accordance with the National Institutes of Health Guide for the Care and Use of Animals and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and with the approval of our institutional ethics committee. Experimental animals were first randomly divided into four groups: saline, lipopolysaccharide (LPS) + saline, LPS + dexamethasone, and LPS + RC-3095. Uveitis 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. Two hours after LPS administration, GRP receptor antagonist

From the 1Laboratório de Fisiopatologia Experimental, Programa de Pós-Graduação em Ciências da Saúde, Unidade Acadêmica de Ciências da Saúde, Universidade do Extremo Sul Catarinense, Criciúma, SC, Brazil; 2Programa de Pós Graduação em Ciências Biológicas—Bioquímica, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil; 3Grupo de Pesquisa em Neurofarmacologia Celular e Molecular, Departamento de Farmacologia, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil; 4Laboratório de Pesquisas em Câncer, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil; and 5Laboratório de Pesquisas em Câncer, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.

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Corresponding author: Felipe Dal-Pizzol, Laboratório de Fisiopatologia Experimental, Programa de Pós-Graduação em Ciências da Saúde, Unidade Acadêmica de Ciências da Saúde, Universidade do Extremo Sul Catarinense, 88806–000 Criciúma, SC, Brazil; pizz@unesc.net.

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Iridies Oxidative Damage

The inflammatory response to LPS induced oxidative damage in the irides observed as an increase in TBARS and protein carbonyl activity both at 24 hours and 48 hours after LPS administration (Figs. 2, 3).

**RESULTS**

**Aqueous Humor Inflammatory Response**

LPS induced, as expected, inflammatory alterations in aqueous humor, as determined by increases in TNF-α and MCP-1 levels (Table 1) and in myeloperoxidase activity (Figs. 1A, 1B) 24 hours and 48 hours after its administration. The blockade of GRPR by RC-3095 was able to decrease TNF-α levels both at 24 hours and 48 hours after LPS (Table 1), and this effect was more pronounced when compared to dexamethasone (Table 1). In addition, RC-3095, but not dexamethasone, decreased MCP-1 levels in the aqueous humor (Table 1). These effects were followed by a decrease on myeloperoxidase activity in RC-3095-treated animals (Figs. 1A, 1B). Interestingly, dexamethasone induced an increase in myeloperoxidase activity both at 24 hours and 48 hours after LPS administration (Figs. 1A, 1B).

**Irides Oxidative Damage**

The inflammatory response to LPS induced oxidative damage in the irides observed as an increase in TBARS and protein carbonyl activity both at 24 hours and 48 hours after LPS administration (Figs. 2, 3).

**DISCUSSION**

In the present study, the administration of RC-3095 2 hours after LPS injection significantly reduced inflammation in the anterior chamber, even when administered as a single dose. RC-3095 effects seemed to be superior to the continuous administration of dexamethasone, suggesting that the anti-inflammatory effects of inhibiting GRPR was higher than of glucocorticoids in the model used.

Several new therapeutic agents have been tested in the LPS model of uveitis. In general, these agents are effective to decrease inflammation in this model when administered before followed by Tukey test. All statistical analyses were performed using a statistical package (SPSS 12.0 for Windows; SPSS, Inc., Chicago, IL).

**Table 1.** Effects of RC-3095 or Dexamethasone on TNF-α and MCP-1 Levels in Aqueous Humor 24 and 48 Hours after Administration of Lipopolysaccharide

<table>
<thead>
<tr>
<th></th>
<th>TNF-α (ng/mL)</th>
<th>MCP-1 (ng/mL)</th>
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<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td>Saline</td>
<td>20 ± 3</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>LPS + saline</td>
<td>150 ± 14†</td>
<td>85 ± 15*</td>
</tr>
<tr>
<td>LPS + dexamethasone</td>
<td>60 ± 7†</td>
<td>63 ± 10*</td>
</tr>
<tr>
<td>LPS + RC-3095</td>
<td>50 ± 8†</td>
<td>35 ± 8†</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD. *Different from saline. † Different from LPS + saline. ‡ Different from LPS + dexamethasone.
or immediately after LPS. In our model, the administration of a single dose of RC-3095 was superior to multiple dexamethasone doses, even when administered 2 hours after LPS. The blockade of GRPR was able to decrease TNF-α and myeloperoxidase in the aqueous humor until 48 hours after LPS administration, suggesting that GRPR is involved in long-term inflammation in the uveal tract. It was unexpected that dexamethasone seemed to increase some of these inflammatory parameters when administered after LPS. In this context, Elgebaly et al. demonstrated that dexamethasone was not able to decrease neutrophil infiltration in hydrogen peroxide-injured corneas. In a mouse model of an acute exacerbation of chronic asthma, elevated expression of mRNA for TNF-α, granulocyte-macrophage colony-stimulating factor, and IL-8 were not suppressed by dexamethasone. In addition, ocular lymphocytes isolated from patients with uveitis who had been treated with topical glucocorticoids expressed highly elevated levels of CXCR4. Given that the influx of neutrophils to the anterior chamber is dependent on local production of chemokines and that we have here demonstrated that, under these conditions, dexamethasone did not decrease MCP-1, we suggest that RC-3095 could have presented a more important anti-inflammatory effect in this model.

Several reports have demonstrated that the production of reactive species, such as superoxide anion radical (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), hydroxyl radical (OH$^-$), and peroxinitrite occurs at the site of inflammation and contributes to tissue damage. Reactive oxygen species (ROS) could be mediators of inflammation induced by cytokines and chemokines, which in turn induce intracellular ROS generation by mitochondrial respiratory chain reaction, arachidonic metabolic reaction, and membrane-bound superoxide-generating enzyme NADPH oxidase. Further, ROS activate redox-sensitive transcription factors such as NF-κB and AP-1, which play a central and crucial role in uveal tract inflammation. Zhang et al. showed that NF-κB translocation and expression of E-selectin, TNF-α, and IL-6 are involved in the pathogenesis of LPS-induced uveitis. In addition, all the GRP receptors characterized to date are guanine nucleotide binding (G)-protein-coupled, have seven transmembrane domains, and activate phospholipase C to increase intracellular concentrations of inositol phosphates, diacyl glycerol, and calcium. Among the multiple intracellular signaling pathways that mediate the effects of GPCRs, a family of related serine-threonine kinases, collectively known as ERKs or MAPKs, appears to play a central role. The induction of AP-1 by GRP is primarily mediated by the JNK and p38 MAPK cascades. In addition, Levine et al. showed that bombesin stimulates NF-κB activation and expression of proangiogenic factors in prostate cancer cells. Therefore, the decrease of inflammatory response and oxidative damage in LPS-induced uveitis observed in RC-3095–treated animals can be related to the regulation of NF-κB activation.

The present study included some limitations. First, animal models of endotoxin-induced uveitis depend on systemic immune cell activation, and RC-3095 could have blunted this response. To avoid this, we administered RC-3095 after LPS injection, when eye inflammation was installed. At this time, changes in the blood aqueous barrier occur; thus, we expected that the observed decrease in eye inflammation would have depended on a direct effect of RC-3095 in the eye. Even in experimental autoimmune uveoretinitis, systemic activation of T cells is of major importance, and this limitation was also present. Despite these limitations, our model was useful in elucidating a variety of mediators that are activated in the eye and in testing a wide variety of potential pharmacologic inhibitors.

Second, the study focused on parameters of oxidative damage and inflammatory response, but not on data concerning clinical and histologic analyses. Thus, we cannot ascertain whether the observed alterations in inflammatory response contributed to a decrease in parameters with more clinical significance, but we do believe that the observed alterations probably induced similar improvements in clinical signs of uveitis in these animals.

In conclusion, the present results support the view that in an LPS-induced uveitis model, the GRPR antagonist RC-3095 has anti-inflammatory properties that can be related to the reduction of oxidative damage.

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References


