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Xanthine oxidase generation of toxic oxygen metabolites in acute uveitis

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Summary

Lodoxamide, a xanthine oxidase inhibitor, has antiphlogistic effects in the treatment of acute uveitis. The role of xanthine oxidase generated free radicals is discussed.

Introduction

Toxic oxygen metabolites generated by stimulated neutrophils are an important mechanism of tissue damage in acute uveitis [1]. The vascular endothelium is an additional source of free radicals that is also important in acute inflammation [2].

Superoxide radicals are generated by the plasmalemma NADPH-oxidase calalysed reduction of molecular oxygen that accompanies the 'oxygen burst' of stimulated neutrophils [3]. Xanthine oxidase (XO) catalysed superoxide generation appears to be the major mechanism involved in free radical generation from vascular endothelial cells [4]. There is a complex interrelationship between the neutrophil and vascular endothelium in acute inflammation that is only partly understood.

These recent observations have directed attention to the importance of xanthine oxidase generated free radicals in acute inflammation. First it has been demonstrated that products of inflammation such as C_{5a} or tumor necrosis factor promote the conversion of xanthine dehydrogenase (XD) into xanthine oxidase [4]. Second is the observation that histamine enhances xanthine oxidase activity [5].

Lodoxamide (LO), an analog of disodium chromoglycate, is a highly effective inhibitor of XO [6]. This study is an evaluation of the effect of LO in the treatment of experimental phacoanophylactic endophthalmitis (EPE).

Methods

Because of space limitations, details of methodology are referenced. The production of EPE [7], tissue processing and morphometry have been extensively described [8]. XO and XD levels were measured by uric acid production as described by Friedl et al. [5]. Histamine levels were measured by the Amak Inc (Westbrook Maine) radio-immuniossay kit. Hydroxyl radical scavenging was determined by deoxyribose degradation described by Hallowell and Gutteridge [9]. Iron binding was assayed by the bleomoycine dependent degradation of DNA described by Gutteridge et al. [10]. Complement was determined by total hemolytic complement activity [11] and the crossed immunoelectrophoresis technique of Chapman and Ward [12].

Results

LO was an effective antiphlogistic agent in treating EPE producing a 43% reduction in inflammation. Comparing the effects of LO to DMSO demonstrated that the antiphlogistic effect cannot be attributed to free radical scavenging.

LO did not bind iron so that the antiphlogistic effect could not be attributed to interference with the Haber Weiss reaction. LO had no effect on serum complement levels.

Discussion

LO is an effective antiphlogistic agent in EPE. The antiphlogistic effect cannot be attributed to conventional mechanisms of antioxidant activity such as hydroxyl radical scavenging or iron chelation. Complement depletion is known to inhibit the development of EPE [13] but LO had no effect on complement levels.

Lodoxamide is a membrane stabilizing agent which inhibits mast cell degranulation and histamine and leukotrine release [14]. Leukotrines may be involved in immune complex disease [15]. Preliminary observations suggest that mast cells are important in pathogenesis of experimental allergic uveitis [16]. Although mast cell products have not been directly implicated in EPE, these observations and the effect of histamine on XO activity suggests that modulating mast cell activity is one of the antiphlogistic mechanisms of LO in EPE.

LO like other membrane stabilizers also has calcium channel blocking activity [17]. Calcium channel blockers may interfere with superoxide production in stimulated acute inflammatory cells by disrupting intracellular signal transduction [18]. Although this is effective in vitro our preliminary studies have not observed antiphlogistic effects of the calcium channel blocker verapamil in experimental uveitis.

LO is a potent XO inhibitor. XO has been demonstrated to be an important generator of toxic oxygen metabolites in acute inflammation [15]. XO generating systems induce severe ocular inflammation [19]. There is considerable XO activity in the normal rats eye. It is reasonable to hypothesize that one of the important antiphlogistic mechanisms of LO is the inhibiting effect on XO activity. Studies on the effects of LO on ocular histamine and XO activity are in progress.

Table. Antiphlogistic effect vs. scavenging.

Agent	Reduction of inflammation	Hydroxyl radical scavenging (ED_{50})
Lodoxamide tromethamine (5 mg/kg)	43%	482 ± 109 (μM)
Dimethyl sulfoxide	39%	213 ± 42 (μM)

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