Inhibition of C5a-induced basophil degranulation by disodium cromoglycate

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Abstract

Injection of purified porcine C5a into 24-hr basophil-rich cutaneous basophil hypersensitivity sites in the dose range $10^{-12}$--$10^{-10}$ moles/site produced cutaneous basophil anaphylaxis (CBA). The H$_2$ antihistamine antagonist mepyramine, given orally (3.0--30 mg/kg), inhibited the vasopermeability, but not the basophil degranulation, characteristic of CBA. The antiallergy agent disodium cromoglycate (DSCG), administered intravenously (3.0--30 mg/kg), inhibited vasopermeability and basophil degranulation. DSCG inhibition of mast cell degranulation was not important in the inhibition of CBA, since intact mast cells were found to be depleted at basophil-rich sites and absent at C5a-induced CBA sites from animals treated with DSCG.

C5a at $10^{-11}$ moles/site also induced vasopermeability and mast cell degranulation in normal guinea pig skin. Vasopermeability, but not mast cell degranulation, was inhibited by mepyramine at 30 mg/kg p.o. However, DSCG at 10 mg/kg i.v. failed to inhibit either the vasopermeability or the mast cell degranulation of this reaction. These results indicate that C5a induces the degranulation of both basophils and mast cells in the guinea pig, and that C5a-induced degranulation of basophils, but not mast cells, is inhibited by DSCG.

Introduction

C5a is known to induce histamine release from both rat and guinea pig mast cells and human basophils [1]. In addition, C5a is known to induce in vivo cutaneous mast cell degranulation in both the guinea pig and man [2]. However, the effects of C5a on basophil degranulation in vivo have not been described.

The antiallergy agent disodium cromoglycate (DSCG) has been shown to inhibit mast cell-dependent, IgE-mediated anaphylactic reactions in the rat, but has been either inactive or inconsistent in blocking mast cell-dependent anaphylactic reactions in the guinea pig [3]. Moreover, while DSCG is known to be inactive against antigen-induced human basophil histamine release in vitro [4], it has been found to inhibit the in vivo antigen-induced degranulation of both bone marrow [5] and cutaneous [6] basophils in the guinea pig. Intradermal challenge of appropriately sensitized guinea pigs produces basophil infiltration into a delayed skin reaction known as cutaneous basophil hypersensitivity (CBH) [7]. Rechallenge of these basophil-rich sites with additional antigen has been shown to produce a local anaphylactic response characterized by histamine release and basophil degranulation termed cutaneous basophil anaphylaxis (CBA) [8]. Basophil degranulation in the CBA reaction may be mediated by
antibody-dependent, as well as T-cell-dependent mechanisms [9]. Using the CBA reaction, we have initially studied whether C5a induces basophil degranulation in vivo in the guinea pig. We have then examined the effects of DSCG on in vivo C5a-induced basophil degranulation in this model compared to its effects on in vivo C5a-induced mast cell degranulation in normal skin.

Materials and methods
Preparation and purification of C5a
C5a was prepared as previously described [6]. After recalcification, complement activation in the presence of epsilon-amino-caproic acid, and acidification with HCl, C5a was purified from citrated porcine serum.

Cutaneous mast cell anaphylaxis
Reactions were produced by i.d. injection of normal guinea pigs with 1.4 $\times$ 10^{-11} moles porcine C5a in 0.1 ml PBS. To aid visualization and grading of the reactions, 5 mg Evans blue dye in 1.0 ml PBS were given i.v. before C5a injection. DSCG was given i.v. after dye injection and immediately before C5a injection; mepyramine was given p.o., 20 min prior to dye and C5a injections. Reactions were measured and graded 15 min following C5a injection. Extravasation was quantitated as the product of reaction diameter and intensity score. Reaction sites were then excised, fixed and processed into paraffin-embedded, Giemsa-stained 5 μ histologic tissue sections. Evaluation of mast cell degranulation was made by light microscopic counts at 750× of the number of intact mast cells per square millimeter remaining after C5a injection. Results were expressed as the percentage of inhibition of extravasation and the percentage of inhibition of mast cell degranulation.

Results
Effect of DSCG on the vasopermeability of C5a-induced CBA
Injection of 0.1 ml PBS into the center of twenty-four hour basophil-rich sites produced a small, insignificant increase in vasopermeability (Figure 1). Injection of C5a in the dose range 10^{-12}–10^{-10} moles/site, however, produced a concentration-dependent increase in vasopermeability at these sites. Vasopermeability reactions induced by 1.4 $\times$ 10^{-11} moles of C5a typically measured 12 mm in diameter and were graded 4+ in intensity, producing an extravasation index of 48 (Figure 1). Mepyramine significantly inhibited the extravasation of C5a-induced CBA in the dose range 3.0–30 mg/kg p.o. Mepyramine at 30 mg/kg p.o. typically inhibited C5a-induced CBA by 77% (Figure 1). DSCG also significantly inhibited C5a-induced CBA in the dose range 3.0–30 mg/kg i.v. DSCG at 10 mg/kg i.v. typically inhibited C5a-induced CBA by 64% (Figure 1).

Effect of DSCG on the basophil degranulation of C5a-induced CBA
A typical 750× field within the papillary dermis of a basophil-rich site 3 mm from reaction center was found to contain a mean of 35 basophils per field (Table 1). Light microscopic cell counts at typical reaction sites following C5a injection revealed a 77% reduction in the number of intact

![Figure 1](image-url)

Figure 1
Effect of DSCG on the vasopermeability of C5a-induced CBA. Control (■) represents extravasation produced by 0.1 ml PBS. C5a (□) injected at 1.4 $\times$ 10^{-11} moles/site. DSCG (●) given immediately before C5a injection. Mepyramine (●) given 20 min before C5a injection. Indices of extravasation represent mean ± SE of three determinations.