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Inhibition of LTB_4 binding to human neutrophils by nordihydroguaiaretic acid

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Abstract

Nordihydroguaiaretic acid (NDGA) was investigated for its ability to interact with leukotriene B_4 receptors on human polymorphonuclear leukocytes (hPMNs). ^{3}H-LTB_4 binding to specific receptors was reduced in a dose-dependent manner with maximal reduction at 100 μM NDGA and an IC_{50} of about 50 μM. Binding of another inflammatory stimulus, N-formyl-norleucyl-leucyl-phenylalanine (FNLP) was not affected by similar treatment. Chemotaxis and enzyme release stimulated by LTB_4 and oligopeptide were inhibited by NDGA. In addition, LTB_4-triggered inflammation *in vivo* in mice was inhibited by systemic administration of NDGA. These data suggest that LTB_4 receptor antagonism may contribute to inhibition of inflammation by NDGA.

Introduction

Recently, several laboratories have reported that specific receptors are present on hPMNs for LTB_4 [1–4], and that receptor affinity correlates with biologic potency [1]. This suggests that receptor antagonists to LTB_4 may provide a novel pharmacotherapeutic mechanism for new antiinflammatory compounds. The findings in this study suggest that nordihydroguaiaretic acid (NDGA) is an LTB_4 receptor antagonist, and that this activity may contribute to its antiinflammatory activity both *in vitro* and *in vivo*.

Materials and methods

^{3}H-FNLP (specific activity = 50 Ci/mmol) and ^{3}H-LTB_4 (specific activity < 100 Ci/mmol) were purchased from New England Nuclear (Boston, MA); native LTB_4 was purchased from Biomol, Inc. (Philadelphia, PA). Formyl-methionyl-leucyl-phenylalanine (FMLP) and NDGA were obtained from Sigma Chemical Company (St. Louis, MO). Silicone oil (Versilube F50) was purchased from General Electric Company (Waterford, NY).

Human polymorphonuclear leukocytes were prepared from blood drawn from 2–3 donors by venipuncture into heparinized tubes. hPMNs were purified (> 95% homogeneity) by standard dextran sedimentation, centrifugation on Hypaque/Ficoll, and hypotonic lysis of erythrocytes. Cells were resuspended in modified Hanks' balanced salt solution.

LTB_4 binding studies were run in polypropylene microfuge tubes containing 2×10^6 cells and varying concentrations of ^{3}H-LTB_4 (final concentration 5–100 nM). Parallel incubations with native LTB_4, final concentration 10 μM, were performed to determine non-specific binding. The binding assay was run for 45 min at 4 °C, and terminated

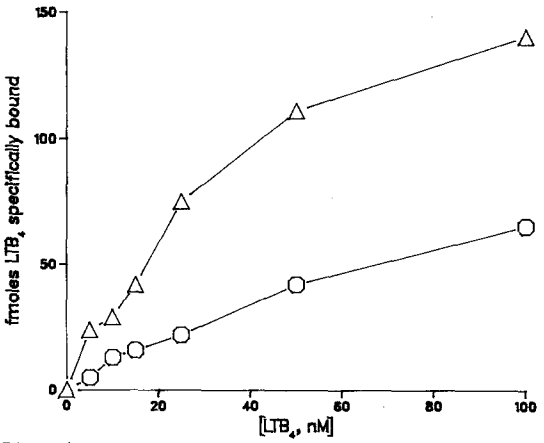

Figure 1
Specific binding of LTB_4 (5–100 n*M*) to hPMNs in the absence (△) or presence (○) of 100 μ*M* NDGA. Cells were incubated for a total of 60 min with NDGA, including at 15 min pre-incubation at 37 °C. Curves were extrapolated to the zero point. Each point was determined in triplicate and the data expressed as mean values of 3 experiments.

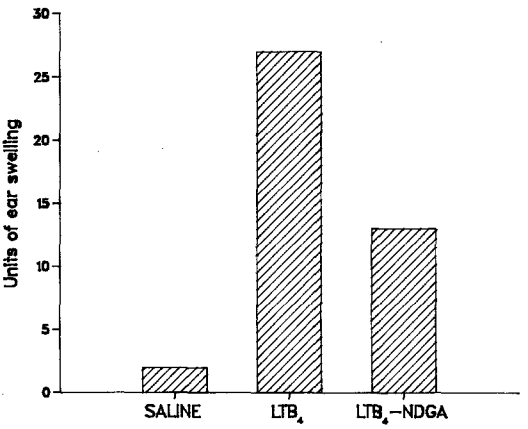

Figure 2
Inhibition of LTB_4-induced inflammation *in vivo* by 100 mg/kg NDGA. NDGA was administered i.p. 30 min prior to LTB_4 challenge. The bar on the left represents saline controls for both the i.p. and LTB_4 injections, the center bar represents saline injection i.p. prior to LTB_4 injection, and the right bar represents the NDGA-treated LTB_4-challenged condition. Data are expressed as units of ear swelling (10^{-4} inches/unit) as the mean of 6 animals per condition, averaged from 3 experiments.

by centrifugation through oil. ^{3}H-FNLP binding was estimated under similar conditions.

Chemotaxis was determined using Boyden chambers with filter paper separating cells (2×10^5 cells/assay) from chemoattractant. The standard medium was supplemented with 1.4 m*M* $MgCl_2$ and 1% BSA. Cells were incubated for 1 hour at 37 °C with FMLP or LTB_4 (1.5×10^{-6} *M*) in the presence or absence of NDGA. Filters were removed, stained and fixed, and the migration determined by measuring the distance from the primary plane of cells to the leading front (2–3 cells in focus in the microscope field).

β-glucuronidase release was measured by using aliquots of cells (5×10^6 cells/tube) pre-incubated for 10 min at 37 °C with cytochalasin B (5 μg/ml) and NDGA (100 μm). FMLP or LTB_4 (2×10^{-6} *M*) was added for 5 min at 37 °C, after which tubes were placed in an ice bath, centrifuged, and supernatants sampled. The aliquots were incubated with phenolpthalein glucuronide overnight at 37 °C, and β-glucuronidase release was quantitated by an ELISA procedure.

A new model of LTB_4-dependent inflammation was developed which involved injection of LTB_4 (1 μg in 20 μl PBS) into left ears of mice. A control condition using PBS injection only was performed in parallel, and data expressed as the difference in ear thickness between uninjected (right) ears and PBS or LTB_4 injected (left) ears at 2 hours. NDGA (100 mg/kg, i.p.) was administered 30 min before LTB_4. Data are expressed as units of ear swelling; 1 unit equals 10^{-4} inches.

Results

The specific binding of LTB_4 to hPMNs was inhibited by NDGA (Fig. 1). This effect was dose-dependent, with an IC_{50} of about 50 μ*M*. Length of incubation with the antioxidant, either during cell isolation or just 15 min prior to addition of LTB_4, did not affect the magnitude of the reduction of LTB_4 binding (maximal inhibition ~60%). Binding of another proinflammatory receptor-mediated stimulus, ^{3}H-formyl-norleucyl-leucyl-phenylalanine, (FNLP), was not altered under equivalent conditions.

Bioresponses in hPMNs to LTB_4, chemotaxis and enzyme release, were also decreased in the presence of NDGA, with IC_{50}'s similar to that observed for binding inhibition. In contrast to the inability of NDGA to influence FNLP binding,

FMLP-stimulated bioresponses were inhibited by NDGA (100 μM).
Ear swelling produced by LTB_4-injection in the ears of female Balb/C mice was mediated by PMN influx, as shown by histologic samples and marker enzyme analysis. Administration of 100 mg/kg NDGA (i.p.) to these mice 30 min prior to LTB_4 markedly reduced the resultant inflammation (Fig. 2).

Discussion

Lin et al. [5] have previously reported that isolation of hPMNs in the presence of 30 μM NDGA significantly increased LTB_4 binding (by approximately 50%), whereas NDGA addition to the binding assay only produced no effect. Data were not available in this report for concurrent LTB_4-stimulated bioresponses using cells prepared in the absence or presence of NDGA. In contrast, we find that incubation with NDGA, either during cell isolation or only during the binding assay, specifically inhibited LTB_4 binding to hPMNs. This molecular binding effect was manifested in reduced cellular and systemic biological response to LTB_4. Inhibition of ligand binding to hPMNs by NDGA is not a generalized effect, since FNLP binding to hPMNs was not affected, however, NDGA also inhibited oligopeptide-stimulated responses. Taken together, these results suggest that antioxidants such as NDGA may provide important leads to the development of antiinflammatory compounds with multiple sites of action.

References

[1] D. W. Goldman and E. J. Goetzl, *Specific binding of leukotriene B_4 to receptors on human polymorphonuclear leukocytes.* J. Immunol. *129,* 1600–1604 (1982).
[2] R. A. Kreisle and C. W. Parker, *Specific binding of leukotriene B_4* to a receptor on human polymorphonuclear leukocytes. J. Exp. Med. *157,* 628–641 (1983).
[3] D. W. Goldman and E. J. Goetzl, *Heterogeneity of human polymorphonuclear leukocyte receptors for leukotriene B_4-identification of a subset of high affinity receptors that transduce the chemotactic response.* J. Exp. Med. *159,* 1027–1041 (1984).
[4] R. M. Clancy, C. A. Dahinden and T. E. Hugli, *Oxidation of leukotrienes at the ω end: demonstration of a receptor for the 20-hydroxy derivative of leukotriene B_4 on human neutrophils and implications for the leukotriene receptors.* Proc. Natl. Acad. Sci. USA *81,* 5729–5733 (1984).
[5] A. H. Lin, P. L. Ruppel and R. R. Gorman, *Leukotriene B_4 binding to human neutrophils.* Prostaglandins *28,* 837–849 (1984).