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Lidocaine and its Analogues Inhibit IL-5-Mediated Survival and Activation of Human Eosinophils

Shinji Okada, John B. Hagan, Masahiko Kato, Jennifer L. Bankers-Fulbright, Loren W. Hunt, Gerald J. Gleich, and Hirohito Kita

Eosinophils and cytokines active on eosinophils, especially IL-5, are believed to be critically involved in chronic allergic diseases. IL-5 activates eosinophils and enhances their survival in vitro by delaying apoptosis. In this study, we found that lidocaine and six analogues blunt responses of eosinophils to IL-5. Lidocaine and its derivatives inhibit IL-5-mediated eosinophil survival in a concentration-dependent manner (IC50 \text{mM} = 110 \mu M for 30 pg/ml IL-5). At suboptimal lidocaine concentrations, the eosinophil survival response to IL-5 shifts and more IL-5 is required to maintain survival. The inhibitory effect requires at least 24-h exposure of eosinophils to lidocaine, and the protein kinase C activator, PMA, completely reverses the inhibition. A multiparameter flow-cytometric analysis shows that lidocaine hastens the apoptosis of eosinophils normally delayed by IL-5. Lidocaine does not affect IL-5R expression or IL-5-induced protein tyrosine phosphorylation. Lidocaine also inhibits eosinophil survival mediated by IL-3 or granulocyte-macrophage CSF, although less potently than that mediated by IL-5. Furthermore, lidocaine inhibits eosinophil superoxide production stimulated by IL-5, granulocyte-macrophage CSF, or IL-3, but not that stimulated by platelet-activating factor, immobilized IgG, or PMA. Lidocaine and its derivatives show novel immunomodulatory properties and are able to blunt eosinophil responses to cytokines in addition to their local anesthetic or antiarrhythmic properties. Thus, lidocaine and its derivatives may represent a new class of therapeutic agents to treat patients with allergic diseases. The Journal of Immunology, 1998, 160: 4010 – 4017.
activation of eosinophils. We report that lidocaine and its derivatives induce apoptosis of eosinophils cultured with IL-5. Furthermore, eosinophil superoxide production stimulated with cytokines was selectively inhibited by lidocaine. Thus, lidocaine and its derivatives may possess novel immunomodulatory effects, as shown by their ability to blunt the eosinophils’ responses to cytokines.

Materials and Methods

Reagents

Human rIL-5 was generously provided by Schering, Kenilworth, New Jersey. Human GM-CSF and IL-3 were purchased from Genzyme (Boston, MA). Lidocaine, other local anesthetics, dexamethasone, and PMA were purchased from Sigma Chemical (St. Louis, MO).

Eosinophil purification

Eosinophils were purified from peripheral blood obtained from normal volunteers, as described previously (20). Briefly, heparinized venous blood was layered onto 1.085 g/ml Percoll (made in PIPES buffer, pH 7.4, supplemented with 50 mM NaCl, 5 mM KCl, 25 mM NaOH, and 5.4 mM glucose) and centrifuged at 2000 rpm in Beckman CS-6KR (Beckman Instruments, Fullerton, CA) for 30 min with no brake. Plasma, mononuclear cells, and Percoll layers were removed, and erythrocytes were lysed by osmotic shock. The remaining eosinophil/neutrophil pellet was mixed with CD16-bound micromagnetic beads (Miltenyi Biotec, Sunnyvale, CA) and incubated for 1 h. The cells were then separated using a magnetic cell separation system (MACS; Miltenyi Biotec). The eluate was collected, and cell number and eosinophil purity were determined. Purity was always at least 95%. All procedures were performed at 4°C or on ice.

Eosinophil survival assay

The survival assay was performed as previously described (4). Briefly, 50-μl aliquots of cell suspension, containing 2 × 10⁶ cells/ml, were put into 96-well cell culture plates (Costar, Cambridge, MA) in Hybri-Care medium (American Type Culture Collection, Rockville, MD) supplemented with 50 μg/ml gentamicin, 0.1% human serum albumin (HSA), and 10% heat-inactivated defined calf serum (HyClone Laboratories, Logan, UT). Serial dilutions of lidocaine and various concentrations of IL-5, GM-CSF, or IL-3 in the Hybri-Care medium (50 μl) were added to the wells (total volume 100 μl). To examine the effects of the activation of protein kinase C (PKC) on eosinophil survival, three dilutions of PMA (0.05, 0.15, and 0.5 μM final concentration) were added. Cells were cultured for 4 days at 37°C, 5% CO₂ in a humidified incubator. After culture, 50 and 0.5 nM final concentration) were added. Cells were cultured for 4 days. At the end of the last incubation, cells were centrifuged at 3000 rpm for 5 min. Supernatants for PMA were removed carefully from each well, and 10 μl of fluorescein diacetate (0.2 mg/ml in PBS) was added. After a 30-min incubation on ice, 0.5 μl of propidium iodide (PI) (0.5 μg/ml in Hybri-Care medium) was added. After an additional 5 min, the numbers of live and dead cells were determined using a hemacytometer and a fluorescent microscope. Percentage of viability was calculated by dividing the number of live cells by total cell number.

Flow-cytometric analysis of apoptosis

Multiparameter flow-cytometric assay was used to discriminate and quantify viable, apoptotic, and necrotic cells stained with the DNA-binding fluorophores Hoechst 33342 and PI, as previously described (21, 22). Briefly, after various time periods, cultured eosinophils were collected into polystyrene test tubes. Hoechst 33342 (Molecular Probes, Eugene, OR) and PI diluted in PBS were added simultaneously to the cell suspension such that their final concentrations were 10 and 32 μM, respectively. Five minutes after staining, more than 5000 cells were analyzed using a Becton Dickinson FACSVantage flow cytometer (San Jose, CA) with UV laser excitation at 357 nm. Blue fluorescence (Hoechst 33342 bound to DNA, between 430 and 530 nm) and red fluorescence (PI bound to DNA, above 630 nm) were measured for each cell analyzed. Because Hoechst 33342 is permeable for intact cell membranes and PI is impermeable, two-dimension scattergram (blue vs red) showed three distinct populations of cells. Dead cells fluoresced red due to PI, whereas viable and apoptotic cells fluoresced blue due to Hoechst 33342. Apoptotic cells were distinguished from viable cells by their higher intensity of blue fluorescence (21, 22). Three populations were distinct and there was no overlap among them. The percentages of each population in total numbers of the cells were calculated by Lysis II software (Becton Dickinson). In some experiments, each population was sorted and was subjected to DNA electrophoresis using the method of Smith et al. (23) to confirm the designation of the population.

Analysis of IL-5-induced protein tyrosine phosphorylation

Eosinophils, 1 × 10⁶ cells in 100 μl of RPMI (supplemented with 25 mM HEPES and t-glutamine), were preincubated with various concentrations of IL-5 for 15 min at 37°C in an incubator. After the last incubation, cells were centrifuged at 3000 × g for 30 s. After removing supernatants, pellets were lysed with sample buffer (5% 2-ME, 2% SDS, 10% glycerol, and 1 mM orthovanadate in Tris-HCl, pH 6.8). The samples were boiled 15 min and loaded on an 8.75% SDS-PAGE. After electrophoresis, the proteins were transferred to a polyvinylidene difluoride membrane (Immobilon PDVF; Millipore, Bedford, MA) in a buffer containing 25 mM Tris and 192 mM glycine. The blots were blocked overnight with 2% BSA and 0.5% Tween-20 in Tris-buffered saline (TBS). After several washes with TBS containing 0.5% Tween-20, the blots were incubated 2 h at room temperature with 0.1 μg/ml of 4G10 Ab to phosphorylated tyrosine, followed by a 1-h incubation with peroxidase-conjugated rabbit anti-mouse IgG Ab (0.5 μg/ml) (DAKO, Carpinteria, CA) in TBS containing 2% BSA and 0.5% Tween-20. After three washes, blots were incubated 1 min with enhanced chemiluminescence solution (Amersham, Arlington Heights, IL) and exposed to x-ray film.

125I-labeled IL-5-binding assay

The binding of 125I-labeled IL-5 to eosinophils was measured according to the method described previously (24) with modifications. Suspensions with 2 × 10⁶ cells in 100 μl of binding medium (RPMI 1640, pH 7.4, supplemented with 25 mM HEPES, 1% BSA, and 0.1% NaN₃) were preincubated with various concentrations of 125I-labeled IL-5 (Amersham) in 50 μl binding medium, cell suspensions were incubated 15 min at 37°C with or without 1 mM of lidocaine. After the addition of various concentrations of 125I-labeled IL-5 (Amersham) in 50 μl binding medium, cell suspensions were incubated 16 h on a rotating table at 4°C. Cell suspensions were suspended, overlayed onto 200 μl of defined cell serum, and centrifuged at 3000 rpm for 5 min. Pellets in the separation tubes were rinsed on dry ice and cut away from the supernatant. Radioactivity in the pellets was measured in a gamma counter (Packard Instrument, Downers Grove, IL). Non-specific binding was determined by the incubation with 1 μM of unlabeled IL-5, and specific binding was calculated by subtracting non-specific binding from total binding. Non-specific binding was less than 0.2% of total binding.

Superoxide anion production assay

Generation of superoxide by eosinophils was measured by superoxide dismutase (SOD)-inhibitable reduction of cytochrome c, as described elsewhere (25), with slight modifications. Unless stated otherwise, 96-well flat-bottom tissue culture plates (Falcon 3072; Becton Dickinson) blocked with 2.5% HSA were utilized. For the experiments with immobilized IgG, 96-well flat-bottom tissue culture plates were first coated overnight with 50 μg/ml of human serum IgG (Organon Teknika Corp., Durham, NC) and then blocked with 2.5% HSA. Freshly isolated eosinophils were suspended in HBSS with 10 mM HEPES and mixed with 100 μM cytochrome c (Sigma Chemical) at 1 × 10⁶ cells/ml. After washing the plates, 100 μl of cell suspension was dispensed onto plates, and the reactions were initiated by adding 50 μl of serial dilutions (0.1, 0.3, and 1 mM final concentrations) of lidocaine, followed by 50 μl of stimuli (25 ng/ml and 1 μM final concentrations for cytokines and platelet-activating factor (PAF) diluted in HBSS or HBSS alone. Immediately after the addition of stimuli, the reaction wells were measured for absorbance at 550 nm in a microplate auto-reader (Thermomax; Molecular Devices, Menlo Park, CA), followed by repeated readings. Between absorbance measurements, the plate was placed at 37°C in an incubator. Each reaction was conducted in duplicate and against an identical control reaction that contained 20 μg/ml of SOD (Sigma Chemical). Superoxide anion generation was calculated with an extinction coefficient of 21.6 cm⁻¹ M⁻¹ for reduced cytochrome c at 550 nm and was expressed as nmol cytochrome c reduction/1 × 10⁵ cells.

Statistical analysis

All values are expressed as the mean ± SEM and, unless noted otherwise, represent three or more experiments performed in duplicate. Significance of differences in viability was determined using ANOVA with post hoc analysis of Tukey’s least significant difference or paired t test.

Results

Effect of lidocaine on eosinophil viability

As reported earlier (4, 26), in the absence of cytokines or other stimuli, eosinophils underwent apoptosis, and by PI staining, more than 90% of cells were dead after 96 h in culture (Fig. 1A).
In contrast, when 30 pg/ml of IL-5 was added to the culture, approximately 70% of cells survived at day 4 (Fig. 1A). Lidocaine inhibited this IL-5-mediated eosinophil survival in a time-dependent manner. That is, lidocaine showed no or a minimal inhibitory effect by 24 h in culture (day 1); the inhibitory effect was apparent by 72 or 96 h (Fig. 1A). After 96 h of culture, the concentration-response curve showed inhibition of survival at 0.01 mM of lidocaine; 1 mM of lidocaine completely inhibited eosinophil survival mediated by 30 pg/ml of IL-5 (Fig. 1B). The IC_{50} of lidocaine with 30 pg/ml of IL-5 was 0.11 mM.

The effects of lidocaine on IL-5-induced eosinophil survival were studied further by varying the concentration of IL-5 (Fig. 2). IL-5 in the absence of lidocaine enhanced eosinophil survival in a concentration-dependent manner. The enhancement of survival was seen with as low as 1 pg/ml IL-5 and reached a plateau at 300 pg/ml. Lidocaine at 0.01 and 0.1 mM shifted the IL-5 concentration-response curve to the right, increasing the concentrations of IL-5 needed to induce 50% survival of eosinophils. In the presence of 0, 0.01, or 0.1 mM of lidocaine, IL-5 EC_{50} values were 18, 60, and 90 pg/ml, respectively. Furthermore, the effects of 0.01 or 0.1 mM of lidocaine were largely overcome by 300 pg/ml or higher concentrations of IL-5 (approximately 10% of inhibition). In contrast, the effects of 1 mM of lidocaine on eosinophil survival were pronounced; the IL-5 concentration-response curve was flat, and IL-5, up to 1000 pg/ml, could not overcome the inhibitory effects of 1 mM of lidocaine.

GM-CSF and IL-3 share the β-chains of their receptors with those for IL-5; thus, these three cytokines possess a number of similar biologic effects on mature eosinophils (reviewed in Ref. 27). When we examined the effects of these cytokines on eosinophil survival, GM-CSF and IL-3 enhanced eosinophil survival in a concentration-dependent manner similar to IL-5 (Fig. 3). Eosinophil survival reached a plateau at more than 70% with 10 pg/ml GM-CSF or 100 pg/ml IL-3. Lidocaine also inhibited eosinophil survival induced by GM-CSF or IL-3 (Fig. 3); however, the effects were less pronounced compared with lidocaine’s effect on IL-5-mediated survival (Fig. 2). For instance, the shift to the right of the GM-CSF or IL-3 concentration-response curves by 0.01 or 0.1 mM of lidocaine was minimal. In fact, the IL-3 concentration-response curves in the presence of 0, 0.01, or 0.1 mM of lidocaine were almost superimposable. Furthermore, 1000 pg/ml of GM-CSF or IL-3 could partially overcome the effects of 1 mM of lidocaine, and maintained more than 50% of eosinophil survival. Thus, eosinophil survival mediated by IL-5 is preferentially inhibited by lidocaine compared with survival mediated by GM-CSF or IL-3.

**Effect of lidocaine and dexamethasone on eosinophil apoptosis**

According to a previous report, the death of cytokine-deprived eosinophils is most likely a multistep process (26). When eosinophils are cultured in the absence of cytokines, such as IL-5 and GM-CSF, they contract in size and have condensed, single, and intensely fluorescent nuclei, which are characteristic of apoptotic cells. At this stage, DNA fragmentation is seen by electrophoresis; however, eosinophil cell membranes are still intact and exclude membrane-impermeable dyes, such as PI and trypan blue (26). As cell death proceeds, the apoptotic cells eventually undergo necrosis and become completely dead cells. At this stage, cell membranes are not intact and become permeable to PI. Cytokines delay the rate of, but do not prevent, apoptosis and subsequent cell death of eosinophils (26). This delayed apoptosis is most likely the mechanism for eosinophil longevity when cultured with the cytokine (26). Furthermore, glucocorticoids, which inhibit eosinophil survival, have been known to induce apoptosis of eosinophils (28). Therefore, we examined whether lidocaine’s inhibition of eosinophil survival was due to the induction of apoptosis. To access cell apoptosis and cell death simultaneously and quantitatively, we stained cells with Hoechst 33342 and PI and analyzed them by multiparameter flow cytometry (21, 22). Eosinophils were cultured...
for 48 h in the absence or presence of IL-5. As shown in Figure 4, apoptotic cells, with condensed nuclei and with intact cell membranes (Hoechst 33342-strong, PI-negative, R2), are clearly discriminated from dead cells with disrupted cell membranes (PI-positive, R3) or viable cells (Hoechst 33342-weak, PI-negative, R1). Eosinophils treated with IL-5 for 48 h show few apoptotic cells (2%) compared with eosinophils cultured in the absence of cytokine (18%). Furthermore, as shown in Figure 5, when eosinophils were cultured in the absence of IL-5, apoptotic cells increased in a time-dependent manner, reached a peak at 72 h, and decreased slightly at 96 h. Consistent with the appearance of apoptotic cells, the number of dead cells (Fig. 5B) increased in a time-dependent manner when cells were cultured in the absence of IL-5. In contrast, in the presence of 10 pg/ml of IL-5, eosinophil apoptosis (Fig. 5A) and cell death (Fig. 5B) were delayed significantly (p < 0.05 at 48 and 72 h for apoptosis, and p < 0.05 at 48, 72, and 96 h for cell death, n = 3). When the effects of lidocaine were examined in this system, 1 mM of lidocaine hastened apoptosis of eosinophils cultured with IL-5. As shown in Figure 5C, significantly larger numbers of apoptotic cells were observed in the presence of lidocaine than in the absence of IL-5 at 48 h, as well as at 72 h (p < 0.05, n = 3). Dexamethasone, at 1 μM, also enhanced apoptosis of eosinophils at 72 h, consistent with a previous report (28). Interestingly, at earlier time points (48 h), significantly higher numbers of apoptotic cells were observed with lidocaine than with dexamethasone (p < 0.05, n = 3) (Fig. 5C). These findings suggest that lidocaine, like dexamethasone, induces apoptosis of eosinophils, although the kinetics of cell apoptosis differs between the two agents.

Mechanisms of eosinophil apoptosis induced by lidocaine

To examine the mechanisms of eosinophil apoptosis induced by lidocaine, a series of experiments were performed. It has been reported previously that eosinophils possess approximately 1000 high affinity IL-5 binding sites per cell (29), and binding of IL-5 to this receptor is critical for survival of eosinophils in vitro (30). Therefore, we first examined whether lidocaine affects binding of IL-5 to the receptors on eosinophils. As a result, in the absence of lidocaine, approximately 1500 high affinity IL-5 binding sites per cell were detected on eosinophils in the 125I-labeled IL-5-binding assay (1491 sites/cell, kDa = 49.8 pM). However, 1 mM of lidocaine did not affect the number or affinity of IL-5R (1422 sites/cell, kDa = 48.8 pM).

Eosinophils incubated with IL-5 show rapid activation of multiple tyrosine kinases, such as Lyn (31) and the janus kinase (JAK)2 (32, 33), leading to subsequent activation of various intracellular signaling molecules and cellular functions. Furthermore, increased tyrosine phosphorylation of proteins is implicated in prolonged survival of eosinophils (34). Therefore, we examined whether tyrosine phosphorylation of proteins induced by IL-5 is affected by lidocaine in eosinophils. Cells were preincubated with serial dilutions of lidocaine (3 to 0.1 mM), stimulated with IL-5, lysed, and analyzed by immunoblot using an anti-phosphotyrosine

FIGURE 3. Effect of lidocaine on GM-CSF-mediated (A) and IL-3-mediated (B) eosinophil viability. In A, eosinophils were cultured for 4 days with varying concentrations of GM-CSF and lidocaine. Data represent means ± SEM from five independent experiments. Significant inhibition in viabilities was observed with 1 mM of lidocaine (p < 0.001, n = 5). In B, eosinophils were cultured for 4 days with varying concentrations of IL-3 and lidocaine. Data represent means ± SEM from five independent experiments. Significant inhibition in viabilities was observed with 1 mM of lidocaine (p < 0.02, n = 5).

FIGURE 4. An example of the flow-cytometric analyses of eosinophil apoptosis and cell death. Eosinophils were incubated without (left panel) or with 100 pg/ml of IL-5 (right panel) for 48 h. Cells were harvested, stained with Hoechst 33342 and PI, and analyzed by multiparameter flow cytometry, as described in Materials and Methods.
mAb. IL-5 induced tyrosine phosphorylation of a number of proteins, most remarkably those with molecular masses of 140 kDa, 110 kDa, 60 to 75 kDa, and approximately 43 kDa (data not shown). However, lidocaine did not affect the phosphorylation of any of these proteins (data not shown).

Finally, in certain circumstances, activation of PKC can suppress apoptosis of leukocytes (35). In this study, we found that the activation of PKC by PMA enhances eosinophil survival in vitro (Fig. 6A); this enhancement was not affected by 1 mM lidocaine or 1 µM dexamethasone (Fig. 6A). Furthermore, as shown in Figure 6B, the inhibitory effect of 1 mM lidocaine on IL-5-mediated eosinophil survival was reversed completely by 0.15 nM PMA. Altogether, these findings suggest that lidocaine does not affect the receptor binding or early signal transduction of IL-5 in eosinophils. The activation of PKC most likely bypasses the target molecule(s) of the inhibitory effects of lidocaine on eosinophil survival.

Effects of lidocaine on superoxide production by eosinophils

In this study, we investigated whether other functions of eosinophils mediated by cytokines are modulated by lidocaine. It is commonly accepted that cytokines are not strong agonists for superoxide production. However, as shown previously, cytokines, such as IL-5, GM-CSF, and IL-3, do induce large amounts of superoxide production from eosinophils when eosinophils can adhere to substrate through β2 integrins (25, 36). As shown in Figure 7, this superoxide production was also inhibited by lidocaine in a concentration-dependent manner. A close examination of the kinetic curve shows that the inhibitory effects of 1 mM of lidocaine were not obvious until after 45 min of incubation; subsequently, the inhibition became more pronounced with longer incubation. Figure

**FIGURE 5.** Effects of IL-5 and lidocaine on eosinophil apoptosis. In A and B, eosinophils were incubated with or without 10 pg/ml of IL-5 for up to 96 h. In C, eosinophils were incubated with no drug, 1 µM dexamethasone, or 1 mM lidocaine in the presence of 10 pg/ml IL-5. The number of apoptotic and dead eosinophils was discriminated and quantitated by multiparameter flow cytometry, as described in Materials and Methods, and Figure 4. Apoptotic cells are defined as Hoechst 33342-strong positive (condensed nuclei) and PI-negative (intact cell membrane). Dead cells are defined as PI-positive (broken cell membrane). Data represent means ± SEM from three independent experiments. *Denotes significant difference (p < 0.05) compared with no drug at the same time point; † denotes significant difference (p < 0.05) compared with dexamethasone at the same time point.

**FIGURE 6.** Effects of lidocaine on PMA-induced eosinophil survival. In A, eosinophils were cultured for 4 days with varying concentrations of PMA in the absence (open circles) or presence of 1 mM lidocaine (closed circles) or 1 µM dexamethasone (closed triangles). Data represent means ± SEM from five independent experiments. In B, eosinophils were cultured with varying concentrations of lidocaine in the presence of 30 pg/ml of IL-5 and PMA (open circle, 0; closed triangle, 0.05 nM; closed square, 0.15 nM). Mean ± SEM from three experiments are shown. *; **Denote significant differences compared with 0 nM of PMA in the presence of the same concentrations of lidocaine (*p < 0.05, **p < 0.01).
IC50 values using 30 pg/ml of IL-5 (Table I) show that tetracaine varied considerably among them (data not shown). The calculated a concentration-dependent manner, although the potency of agents IL-5. All of the agents listed above inhibited eosinophil survival in were drawn for each agent using mean values from three independent experiments, derivatives for 4 days in the presence of 30 pg/ml IL-5. Concentration response curves of each agent is also shown as means from five independent experiments; *denotes significant differences (p < 0.05) compared with 0 mM of lidocaine.

Effects of lidocaine derivatives on eosinophil survival induced by IL-5

Finally, we examined whether derivatives of lidocaine, including tetracaine, benoxinate, dibucaine, proparacaine, procaine, and bupivacaine, also inhibit eosinophil survival induced by IL-5. Eosinophils were incubated with serial dilutions of lidocaine and its derivatives, from 10^{-2} to 10^{-7} M, in the presence of 30 pg/ml of IL-5. All of the agents listed above inhibited eosinophil survival in a concentration-dependent manner, although the potency of agents varied considerably among them (data not shown). The calculated IC_{50} values using 30 pg/ml of IL-5 (Table I) show that tetracaine was the most potent and bupivacaine was the least potent. Tetracaine, dibucaine, benoxinate, proparacaine, and procaine inhibited IL-5-mediated eosinophils’ survival by more than 50% at 0.1 mM concentration. Thus, not only lidocaine, but also other local anesthetics, can inhibit eosinophil survival.

Discussion

Lidocaine and its derivatives are blockers of voltage-gated Na^+ channels, and are used widely to suppress the activation of excitabile cells (37). Lidocaine may also affect the functions of nonexcitable cells, such as neutrophils and macrophages, although relatively high concentrations (e.g., >10 mM) of lidocaine were used for some of the previous studies (38, 39). In this study, we provide evidence for the induction of apoptosis, as well as the inhibition of cellular function by lidocaine in human eosinophils. Several lines of evidence suggest that these effects of lidocaine are not due to nonspecific cytotoxicity. 1) As examined by multiparameter flow cytometry, the inhibition of cell survival by lidocaine was due to apoptosis, which is the physiologic mechanism of cell death (23), but not due to necrosis (Fig. 5). 2) The eosinophil apoptosis and cell death were time dependent and required at least 24 h of exposure to lidocaine (Fig. 1). 3) Cell survival induced by PMA was not affected by lidocaine, and the inhibitory effects of lidocaine on IL-5-induced survival were reversed by the addition of PMA (Fig. 6). 4) Eosinophil superoxide production induced by cytokines was inhibited by lidocaine (Fig. 8). In contrast, superoxide production induced by PAF, immobilized IgG, or PMA was not inhibited by lidocaine, suggesting that lidocaine is not a general inhibitor of eosinophil function. 5) Finally, lidocaine did not affect the binding of IL-5 to eosinophils or IL-5-induced tyrosine phosphorylation of intracellular proteins in eosinophils. These findings suggest that lidocaine and its derivatives inhibit activation and survival of eosinophils in a noncytotoxic manner, and lidocaine may have preferential inhibitory effects on cytokine-mediated eosinophil survival and activation.

Interestingly, among several cytokines active on eosinophils, eosinophil survival induced by IL-5 was inhibited most profoundly by lidocaine compared with that induced by IL-3 or GM-CSF; the difference was most obvious between IL-5 (Fig. 2) and IL-3 (Fig. 3). It was claimed that, in mature eosinophils, IL-5, GM-CSF, and IL-3 share the β-chains of their receptors, which are needed for signal transduction, and this is most likely why these three cytokines possess a number of similar biologic effects on mature eosinophils (reviewed in Ref. 27). However, a careful examination of the literature suggests that this is not always the case. For example, Hansel et al. reported that IL-3 and GM-CSF, but not IL-5, induce the expression of HLA-DR in blood eosinophils (40). Scoggan et

Table I. Comparison of the effects of lidocaine and its derivatives on IL-5-mediated eosinophil survival*

<table>
<thead>
<tr>
<th>Agents</th>
<th>IC_{50} on 30 pg/ml IL-5 (μM)</th>
<th>Percent Inhibition of Viability at 100 μM of Each Agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracaine</td>
<td>37</td>
<td>86.1 ± 5.8</td>
</tr>
<tr>
<td>Dibucaine</td>
<td>46</td>
<td>79.3 ± 12.0</td>
</tr>
<tr>
<td>Benoxinate</td>
<td>75</td>
<td>63.8 ± 18.0</td>
</tr>
<tr>
<td>Propracaine</td>
<td>105</td>
<td>49.5 ± 16.1</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>110</td>
<td>51.3 ± 8.6</td>
</tr>
<tr>
<td>Procaine</td>
<td>180</td>
<td>21.8 ± 14.9</td>
</tr>
<tr>
<td>Bupivacaine</td>
<td>250</td>
<td>33.9 ± 14.6</td>
</tr>
</tbody>
</table>

* Eosinophils were cultured with various concentrations of lidocaine or its derivatives for 4 days in the presence of 30 pg/ml IL-5. Concentration response curves were drawn for each agent using mean values from three independent experiments, and IC_{50} values were calculated. Percent inhibition of eosinophil viability by 0.1 mM of each agent is also shown as means ± SEM from three experiments.
al. found that GM-CSF, but not IL-5, enhances leukotriene production by HL-60 cells, which express similar numbers of receptors to these cytokines with comparable affinities (41). Finally, binding of 125I-labeled IL-5 to human eosinophils was inhibited partially by GM-CSF or IL-3 (24). In contrast, the binding of 125I-labeled GM-CSF or 125I-labeled IL-3 to eosinophils was inhibited only minimally by IL-5. Thus, in contrast to common understandings, these findings point to potential qualitative and/or quantitative differences in eosinophils’ responses to IL-5, GM-CSF, and IL-3. Our findings with lidocaine are consistent with this latter speculation, and suggest that, as far as eosinophil survival is concerned, the eosinophils’ response to IL-5 is more sensitive to lidocaine than their responses to GM-CSF or IL-3.

Glucocorticoids are used extensively and successfully for the treatment of patients with allergic diseases. Although several mechanisms are responsible for the beneficial effects of glucocorticoids in allergic diseases, one of them is the induction of eosinophil apoptosis (11, 12). In this study, we found that lidocaine and glucocorticoids are similar in terms of their induction of cellular apoptosis in eosinophils. However, there are several differences between lidocaine and glucocorticoids. 1) The effect of cellular apoptosis was approximately 24 h earlier with lidocaine than with dexamethasone (Fig. 5). 2) The inhibitory effect of dexamethasone on eosinophil survival was similar among IL-5, IL-3, and GM-CSF, and was overcome by high concentrations of any of these cytokines (12). In contrast, we found that IL-5-induced survival was the most sensitive to lidocaine, and lidocaine’s inhibition of viability was not overcome by high concentrations of IL-5. 3) Glucocorticoids did not inhibit effector function of eosinophils unless cells were exposed to them for a prolonged period (reviewed in Ref. 8). In contrast, lidocaine inhibited eosinophil superoxide production when added simultaneously with stimuli. Thus, we may conclude that the effect of lidocaine on eosinophils is glucocorticoidic; however, the mechanisms of action are most likely different. This could be advantageous in the treatment of patients with allergic diseases because lidocaine could be used in combination with glucocorticoids or in the patients resistant to glucocorticoid treatment. In fact, nebulized lidocaine has been used safely and successfully to treat glucocorticoid-dependent patients with asthma, and it replaced or reduced the amounts of glucocorticoids needed for treatment (19). Moreover, concentrations of lidocaine, up to 10 mM, were found in BAL fluids from patients with asthma who safely underwent bronchoscopy (18), indicating that the concentrations of lidocaine used in our study are pharmacologically relevant. Thus, lidocaine shows promise as another class of immunomodulatory drug that can be substituted for glucocorticoids in the treatment of patients with asthma.

The question is, then, how does lidocaine inhibit cell survival and function in IL-5-stimulated eosinophils? In eosinophils, binding of IL-5 to its receptors induces rapid activation of multiple tyrosine kinases, such as Lyn (31) and JAK2 (31, 32), leading to subsequent activation of various intracellular signaling molecules and cellular functions. However, in our study, the numbers of binding sites for IL-5 and their affinities were not affected by 1 mM of lidocaine (Fig. 6). Surprisingly, IL-5-induced protein tyrosine phosphorylation was not affected by lidocaine, suggesting that events further downstream in IL-5 signaling are most likely the targets of lidocaine. This speculation agrees with our functional assay results, in which not only the eosinophil superoxide production induced by IL-5, but also that induced by IL-3 and GM-CSF, was inhibited by lidocaine. Perhaps the target molecule(s) for lidocaine in eosinophils is involved in multiple cellular events, including survival and functional activation. It is generally accepted that lidocaine selectively interacts with the voltage-dependent Na^+ channel and inhibits the Na^+ flow into excitable cells (37, 42). Although little is known regarding the presence of Na^+ channels in leukocytes, Rajotte et al. (43) reported that Na^+ influx was inhibited by amiloride in a myeloid cell line. Furthermore, they found that amiloride inhibits the abilities of GM-CSF and IL-3 to prevent apoptosis in this cell line (43). Our preliminary studies using the patch clamp technique also show that eosinophils mount inward currents, most likely due to influx of Na^+ ions through the channels (data not shown). Thus, the inhibition of Na^+ flow by lidocaine is one of the potential mechanisms of eosinophil apoptosis and decreased activation. Another potential mechanism is the modulation of intracellular pH (pH_i) by lidocaine. Lidocaine inhibited realignment of the cytosol in neutrophils (39), suggesting that lidocaine may also inhibit the Na^+ /H^+ antiporter. Elevation of pH_i due to activation of the Na^+ /H^+ antiporter is a common response to many growth factors (reviewed in Ref. 44). Adhesion of lymphocytes to extracellular matrix proteins also triggers the activation of the Na^+ /H^+ antiporter and elevation of pH_i (45); as described previously (25, 36), cellular adhesion is a critical step for triggering effector functions of cytokine-stimulated eosinophils. It is unknown whether any one of these mechanisms is predominant or whether they work synergistically to inhibit survival and activation of eosinophils. It is noteworthy that lidocaine preferentially inhibits the effects of cytokines on eosinophils, as shown by its inhibitory effects on survival and superoxide production induced by cytokines, but not on those induced by IgG, PAF, or PMA (Figs. 2, 3, and 8). Furthermore, because the binding of IL-5 and IL-5-mediated protein tyrosine phosphorylation was not affected by lidocaine, the inhibitory effects of lidocaine are not likely due to the inhibition of upstream signaling of eosinophils stimulated with IL-5. Thus, lidocaine may directly affect intracellular event(s) critically involved in cell survival and activation of eosinophils. Interestingly, a recent study shows that in vivo blockade of the voltage-activated K^+ channel in pigs reduced thymic development of T cell subsets (46), suggesting that potential roles of ion channels in immunity may be more diverse than expected and may involve activation, proliferation, development, and survival of immune cells. Therefore, further studies of lidocaine’s mechanism of action on eosinophils may lead to new therapeutic strategies for patients with allergic diseases. In addition, lidocaine may be useful to dissect the regulatory mechanisms of cell activation and survival induced by cytokines and may provide new insights into the roles of ion channels in regulating nonexcitable cells.

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References


