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Lidocaine-induced apoptosis and necrosis in U937 cells depending on its dosage

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ABSTRACT

Local anesthetics are known to affect a variety of cellular responses other than the action of anesthetics through the Na^+ channel blockade. In this study, we examined the effect of a common local anesthetic lidocaine on the cellular activity and viability of human histiocytic lymphoma U937 cells. The cellular activity and viability were assessed by WST-1 reduction activity and trypan blue exclusion test, respectively. Induction of apoptosis was monitored by DNA ladder formation, reduction of mitochondrial transmembrane potential ($\Delta\Psi m$), caspase-3 activity and nuclear morphology. Lidocaine at concentrations below 12 mM induced apoptosis characterized by DNA fragmentation and chromatin condensation dose- and time-dependently. A pan-caspase inhibitor and a caspase-3 inhibitor blocked DNA ladder formation followed by the reduction of cell death. However, the caspase inhibitors did not affect the $\Delta\Psi m$, but cyclosporin A inhibited the collapse of $\Delta\Psi m$ followed by a reduction of cell death. Lidocaine-induced apoptosis was mitochondria- and caspase-dependent, but the collapse of $\Delta\Psi m$ was independent of caspase activation. At concentrations above 15 mM, lidocaine induced necrosis with early disruption of membrane integrity. These results indicate that lidocaine induced apoptosis and necrosis in U937 cells depending on its dosage.

Local anesthetics are known for their ability to block voltage-gated Na^+ currents. However, it is also known that they have other beneficial or adverse effects on a variety of cellular activities such as wound-healing, thrombosis, inflammatory responses, and cellular toxicity (4, 5, 16). One of the cellular toxicities of local anesthetics, an adverse effect, is an induction of necrosis or apoptosis in non-neuronal as well as neuronal cells *in vivo* and *in vitro* (4, 10, 12, 22, 27). It has been reported that administration of local anesthetics *in vivo* induced cell death in muscle cells (10) and neuronal cells (12). Necrosis results from catastrophic failure of cell integrity produced by toxic insults. Apoptosis is a well-controlled form of cell death, characterized by typical morphological changes and biochemical characteristics including shrinkage of the cytoplasm, blebbing of the plasma membrane, externalization of membrane phosphatidyl serine molecules, chromatin condensation, and DNA fragmentation (2, 21). These characteristics result from a series of different biochemical events, in which the mitochondrial transmembrane potential ($\Delta\Psi m$) and caspases, a family of cysteine proteases, play central roles in the execution of apoptosis (15). The $\Delta\Psi m$ results from the asymmetric distribution of protons and other ions on both sides of the inner mitochondrial membrane, and it maintains the mitochondrial functions (32). The mitochondrial permeability transition (MPT) is the regulatable opening of the MPT pore and involved in the maintenance of $\Delta\Psi m$. The disruption

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of MPT collapses the ΔΨm resulting in the release of proapoptotic components such as cytochrome c, SMAC/DIABLO, endonuclease G and caspases from the intermembrane collapses the ΔΨm resulting in the release of proapoptotic components such as cytochrome c, SMAC/DIABLO, endonuclease G and caspases from the intermembrane space (9, 15, 23).

Monocoyte/macrophage system is an important role in pulpal defense reaction particularly against physical or chemical stimuli (26, 29). In this study, we determined whether lidocaine exerts toxicity in human histiocytic lymphoma U937 cells which display many monocytic characteristics and are able to differentiate to macrophages, and investigated the possible involvement of the ΔΨm and caspases in cellular toxicity.

MATERIALS AND METHODS

Cell culture. The human histiocytic lymphoma cell line, U937, was obtained from the Japanese Cancer Research Resource Bank (Tokyo, Japan). The cells were cultured in RPMI 1640 medium (GibcoBRL, Rockville, MD) supplemented with 10% heat-inactivated fetal bovine serum, 10 mM HEPES, 50 mM 2-mercaptoethanol, 100 U/mL penicillin and 100 μg/mL streptomycin in humidified air with 5% CO_2 at 37°C in the presence or absence of lidocaine at various concentrations.

Cellular viability. Cellular viability was determined by the ability of the cells to exclude trypan blue. It was also measured by flowcytometric analysis using a FACSCalibur (Becton Dickinson, San Jose, CA) after staining the DNA with 100 μg/mL propidium iodide (PI).

Nuclear morphology. Apoptotic nuclear morphology was evaluated by fluorescence microscopy after staining with Hoechst 33258 (Wako Pure Chemical Co., Osaka, Japan). After incubation, the cells were collected, washed once with phosphate-buffered saline (PBS) and stained with 5 μM Hoechst 33258 for 5 min. Immediately after staining, the cells were observed with a fluorescence microscope (Carl Zeiss, Jena, Germany), equipped with an × 40 objective (350 nm excitation and 460 nm emission).

Cellular activity. Cellular activity was estimated by 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2, 4-disulfophenyl)-2-2H-tetrazolium, sodium salt (WST-1) (Dojindo Laboratories, Kumamoto, Japan) reduction activity (18). Exponentially growing cells were plated in 96-well culture plates at 5×10^5 cells/well. After incubation, the cells were fed with exposure medium containing 4% WST-1 reagent, incubated for 2 h, and the absorbance at 450 nm was measured with a Micro-plate Reader Model 450 (BIO-RAD, Hercules, CA).

DNA ladder formation. The cells were lysed in 10 mM Tris-HCl buffer, pH 7.4, containing 10 mM EDTA, and 0.5% Triton X-100 with 400 μg/mL of RNase A at 37°C for 1 h, and then with 400 μg/mL of proteinase K at 37°C for 1 h. The DNA was precipitated with an equal volume of 2-propanol. The extracted DNAs were electrophoresed on a 2% agarose gel, visualized by ethidium bromide staining under ultraviolet illumination and photographed using Digital Image Stocker (TOYOBO, Osaka, Japan).

ΔΨm assay. After incubation, the cells were collected, washed once with PBS, and further incubated at a cell density of 5×10^5 cells/mL in PBS containing 40 nM 3,3'-dihexyloxacarbocyanine iodide ($DiOC_6(3)$) at 37°C for 15 min. The cells were then washed once with PBS and resuspended in PBS, followed by analysis on FACSCalibur.

Assay for Caspase-3 activity. After incubation with lidocaine or etoposide, the cells were lysed in 50 mM Tris-HCl buffer, pH 7.5, containing 0.03% NP-40, 1.0 mM dithiothreitol, and the caspase-3 activity was determined by measuring the cleavage of acetyl-Asp-Glu-Val-Asp-*p*-nitroanilide (Ac-DEVD-pNA) using a Caspase-3 Colorimetric Assay Kit (Calbiochem, San Diego, CA) according to the manufacturer's instructions.

Western Blotting of poly(ADP-ribose)polymerase (PARP). The cells were dissolved in SDS-sample buffer containing 62.5 mM Tris-HCl, pH 7.4, 2% SDS, 5% 2-mercaptoethanol, 10% glycerol and 0.001% bromophenol blue, and boiled at 100°C for 5 min. The samples were subjected to SDS-polyacrylamide gel electrophoresis for separation. After transfer of the proteins in the gels to an Immobilon P filter (Millipore, Bedford, MA) by semi-dry blotting, the filter was incubated with primary antibody against PARP (1 : 1000 dilution, Santa Cruz Biotechnology, Santa Cruz, CA) and then with horseradish peroxidase-linked secondary antibody (1 : 5000). The immunoblots were analyzed using an ECL plus system (Amersham Pharmacia Biotech, Buckinghamshire, England) according to the manufacturer's instructions.

RESULTS

Lidocaine reduced WST-1 reduction activity and viability of U937 cells.

To examine the effect of lidocaine on U937 cells, we treated the cells with lidocaine at various concentrations, and determined the WST-1 reduction activity and cellular viability. The cellular viability assessed by the trypan blue exclusion test was almost similar to that determined by flowcytometric analysis using PI staining. Lidocaine reduced the WST-1 reduction activity and cellular viability in dose- and time-dependent manners (Fig. 1). Lidocaine at 3 mM had little effect on both the WST-1 reduction activity and cellular viability, but at concentrations above 9 mM, it inhibited the WST-1 reduction activity and reduced the cellular viability in a time-dependent manner. Lidocaine at concentrations above 15 mM decreased the WST-1 reduction activity revealing about 50% of the initial level at 12 h, and the cellular viability was reduced to about 60% and 35% at 12 and 24 h, respectively. The WST-1 reduction activity at 12 mM after 24-h incubation was at a similar level as the initial activity, but the viability decreased by about 65%, suggesting that lidocaine induced cell death as well as the inhibition of cellular activity and growth.

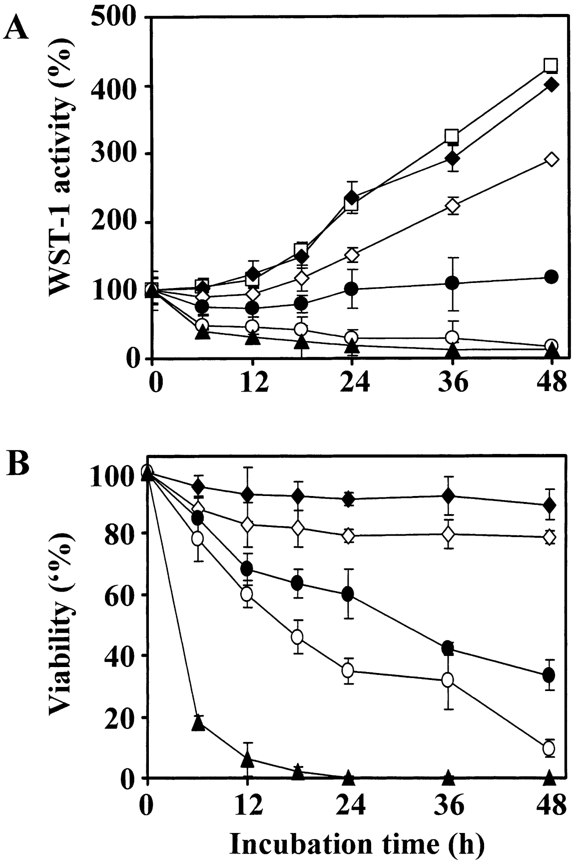

Fig. 1 Effects of lidocaine on WST-1 reduction activity and viability of U937 cells. Cells were incubated with lidocaine at concentrations of 0 mM (□), 3 mM (◆), 9 mM (◇), 12 mM (●), 15 mM (○), and 18 mM (▲) for the indicated times. WST-1 reduction activity (A) and cellular viability assessed by trypan blue exclusion test (B) were expressed as percent of the control. Data are means ± SD of three independent experiments.

Lidocaine at concentrations below 12 mM induced apoptosis.

To examine the mode of cell death induced by lidocaine, DNA ladder formation and nuclear morphology were assessed by agarose gel electrophoresis and Hoechst 33258 staining, respectively. At 24-h incubation, DNA fragmentation was observed in the cells treated with lidocaine at 9 and 12 mM (Fig. 2A), although it was not detected at concentrations above 15 mM. DNA fragmentation at 12 mM lidocaine became detectable at 18 h and predominant after 24 h (Fig. 2B). Marginal chromatin condensation was observed in the cells treated with 12 mM of lidocaine at 24 h, and fragmented chromatin, a typical nuclear morphology of apoptosis, was detected at 48 h (Fig. 2C). These results of DNA fragmentation and nuclear morphology suggested that lidocaine at concentrations below 12 mM induced apoptosis in U937 cells time-dependently.

Lidocaine-induced apoptosis was caspase-dependent.

To assess the involvement of caspases in lidocaine-induced apoptosis, the effects of caspase inhibitors on DNA fragmentation and cell death were examined using a pan-caspase inhibitor benzyloxycarbonyl-Val-Ala-Asp-fluoromethyl ketone (Z-VAD-fmk, Calbiochem, La Jolla, CA), and a caspase-3 inhibitor benzyloxycarbonyl-Asp-Glu-Val-Asp-fluoromethyl ketone (Z-DEVD-fmk, Calbiochem, La Jolla, CA). Since etoposide, an antitumor topoisomerase inhibitor, is known to induce apoptosis in U937 cells depending on caspase activation and the reduction of $\Delta\Psi m$ (7, 30), we compared the effects of caspase inhibitors on lidocaine-treated cells to those on etoposide-treated cells. We also measured the activity of caspase-3 in the treated cells.

Preincubation of cells with either Z-VAD-fmk or Z-DEVD-fmk inhibited the DNA ladder formation

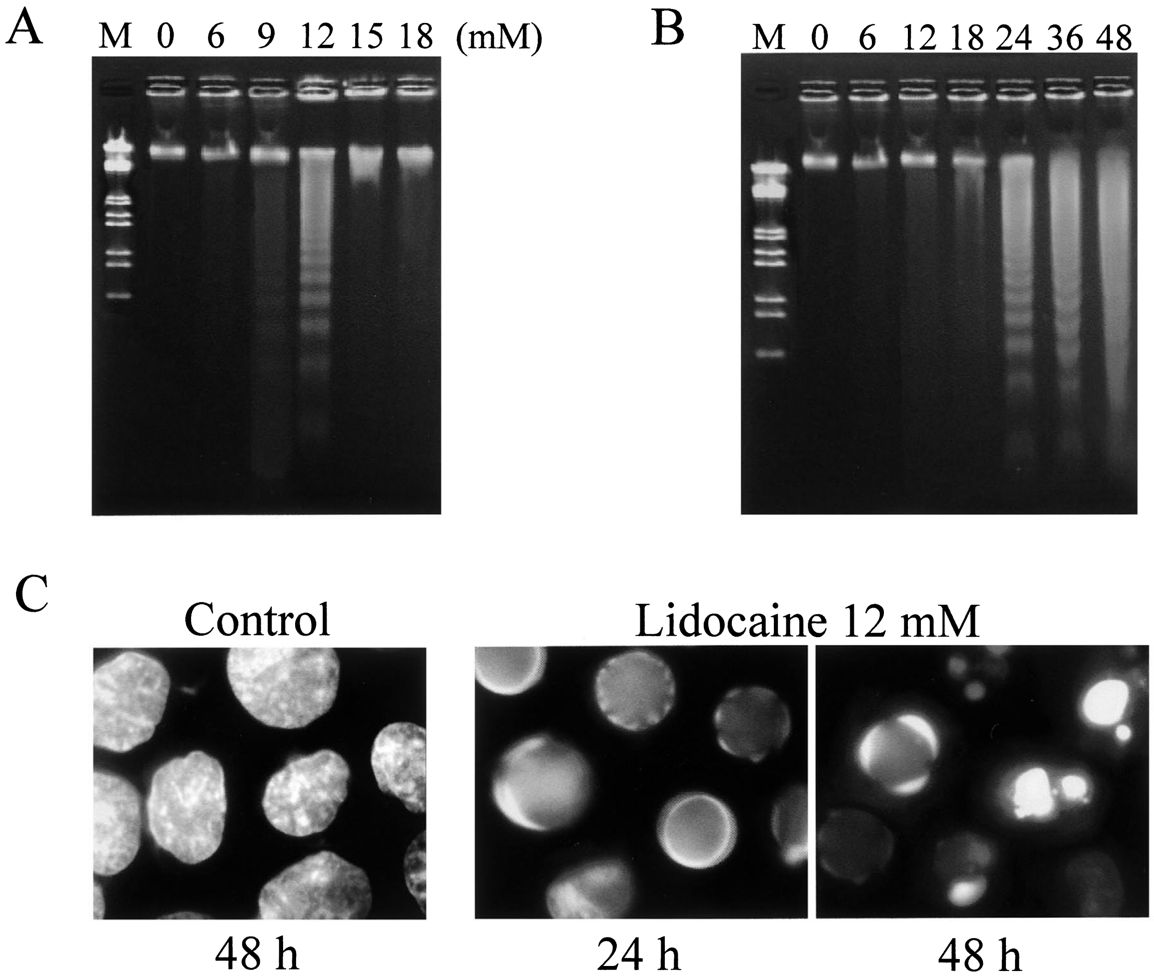

Fig. 2 Effects of lidocaine on DNA fragmentation and nuclear morphology. (A) Dose-dependent DNA fragmentation induced by lidocaine. U937 cells were incubated with lidocaine at concentrations indicated in the Figure for 24 h and DNA fragmentation was analyzed by agarose gel electrophoresis. M; marker. (B) Time course of DNA fragmentation induced by lidocaine. Cells were incubated with 12 mM lidocaine and DNA fragmentation was analyzed at the indicated times. M; marker. (C) Nuclear morphology of the control cells at 48 h, and of the cells treated with 12 mM lidocaine for 24 h and 48 h.

(Fig. 3A). Lidocaine induced cell death as assessed by PI staining revealing about 50% of PI positive cells, which was reduced to about 18% in the presence of either Z-VAD-fmk or Z-DEVD-fmk (Fig. 3B). Etoposide-induced DNA fragmentation was detected at 3 h and cell death was observed at 6 h (Fig. 4), both of which were also inhibited by the inhibitors (Fig. 4). Etoposide-treatment induced a progressive increase in the caspase-3 activity starting at 2 h and reaching about 25-fold above the control level at 6 h, while an increase in the caspase-3 activity in lidocaine-treated cells was observable at 12 h and reached about 12-fold at 24 h (Fig. 5A). Caspase-3 activity in the control cells was undetectable even after 24 h. Activation of caspase-3 was confirmed by the cleavage of PARP, a substrate of caspase-3, in both etoposide- and lidocaine-treated cells (Fig. 5B). The caspase inhibitors also inhibited the cleavage of PARP in both lidocaine- and etoposide-treated cells (Fig. 5B). These results indicate that the lidocaine-induced apoptosis depended on caspase-3 activation as in etoposide-treated cells, although the caspase-3 activation by lidocaine was slower than that in the etoposide-treated cells.

Lidocaine-induced apoptosis was dependent on the $\Delta\Psi m$ reduction, which was caspase-independent.

The mitochondrial involvement in lidocaine-induced apoptosis was examined by the flowcytometric analysis of the $\Delta\Psi$m using DiOC$_6$(3). Lidocaine-induced cell death was associated with a reduction of $\Delta\Psi$m (Fig. 6). To confirm whether the reduction of $\Delta\Psi$m

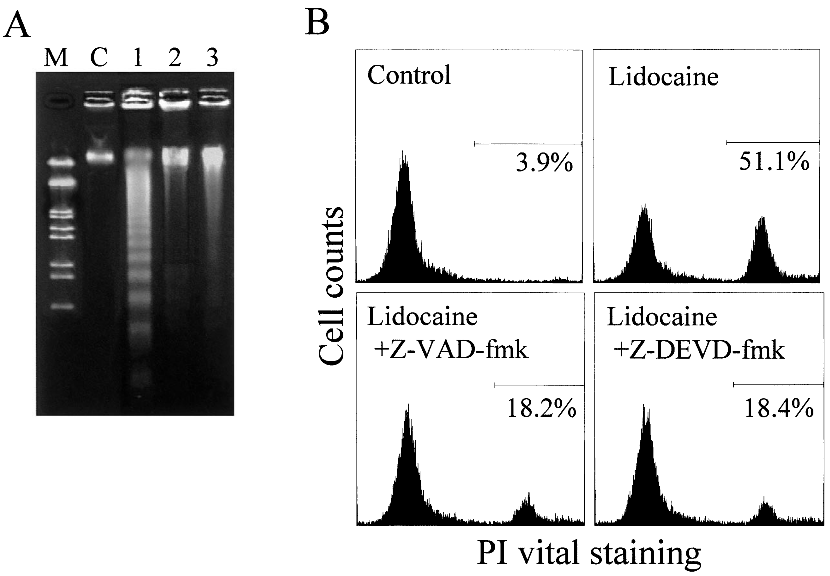

Fig. 3 Inhibition of lidocaine-induced DNA fragmentation and cell death by caspase inhibitors. (A) DNA fragmentation induced by lidocaine at 12 mM in the absence (lane 1) or presence of 100 µM Z-VAD-fmk (lane 2) or 100 µM Z-DEVD-fmk (lane 3). DNA fragmentation was analyzed at 24 h. M; marker, C; control. (B) Flowcytometric analysis of PI positive cells at 24 h in the presence or absence of caspase inhibitors. Data show a representative of three independent experiments.

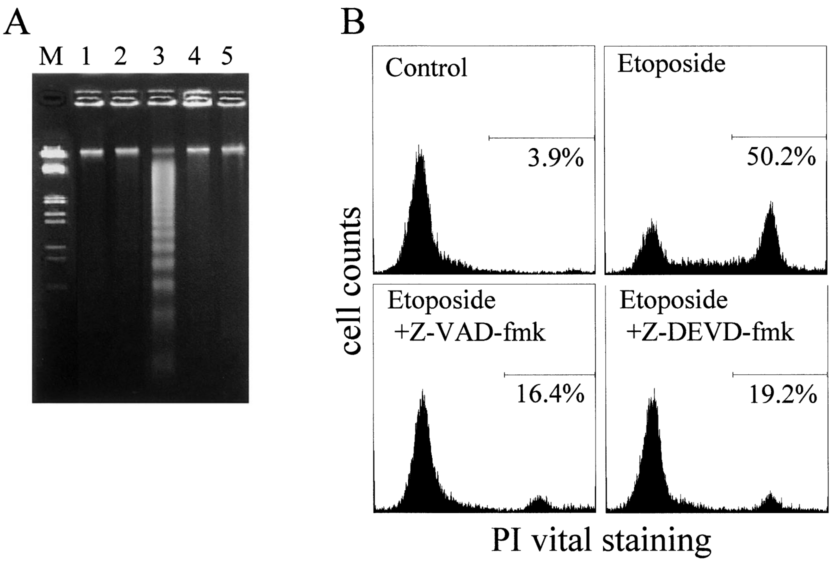

Fig. 4 Etoposide-induced DNA fragmentation and PI positive cells. (A) DNA fragmentation induced by 10 µM etoposide in the absence of caspase inhibitors at 0 h (lane 1), 3 h (lane 2), and 6 h (lane 3), or in the presence of 100 µM Z-VAD-fmk at 6 h (lane 4) or 100 µM Z-DEVD-fmk at 6 h (lane 5). M; marker. (B) Cells were pretreated with 100 µM Z-VAD-fmk or 100 µM Z-DEVD-fmk for 30 min and then incubated with 10 µM etoposide for an additional 6 h, and PI positive cells were analyzed by flowcytometry. Data show a representative of three independent experiments.

is involved in lidocaine-induced cell death, the effect of cyclosporin A, an inhibitor of the collapse of ΔΨm, on DNA fragmentation and cell death was determined. When the cells were pretreated with cyclosporin A for 5 h, lidocaine-induced collapse of the ΔΨm was inhibited followed by a reduction of cell death (Fig. 6). DNA fragmentation induced by lidocaine was also inhibited by cyclosporin A-pretreatment (data not shown). Cyclosporin A alone had no effect on DNA fragmentation and cell death. These results indicate that the collapse of the ΔΨm is involved in the induction of cell death.

Etoposide-induced apoptosis was associated with a reduction of ΔΨm and was also inhibited by preincubation with cyclosporin A (Fig. 7). The pan-caspase inhibitor Z-VAD-fmk did not inhibit the reduction of ΔΨm induced by lidocaine (Fig. 6), while the etoposide-induced ΔΨm reduction was inhibited by Z-VAD-fmk (Fig. 7), suggesting a different mechanism of the reduction of ΔΨm in lidocaine-treated cells from that in etoposide-treated cells.

All of these results indicate that the cell death induced by lidocaine at 12 mM is apoptosis through the mitochondria- and caspase-dependent pathway.

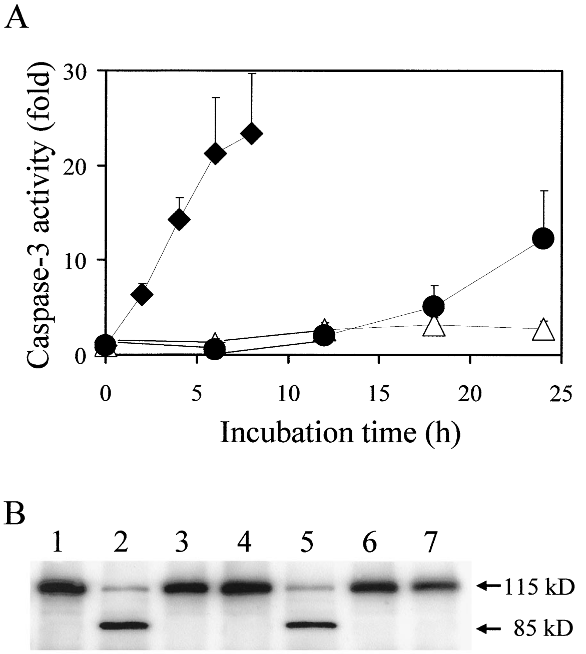

Fig. 5 Caspase-3 activity and poly(ADP-ribose)polymerase cleavage in lidocaine- and etoposide-treated cells. (A) Caspase-3 activities in the cells treated with 10 µM etoposide (◆), 12 mM lidocaine (●) or 15 mM lidocaine (△). Activity was expressed as fold of the control cells. Data are means ± SD of triplicate experiments. (B) Western blotting analysis of poly(ADP-ribose) polymerase. Control cells were incubated without lidocaine for 6 h (lane 1). Cells were treated with 12 mM lidocaine (lanes 2–4) for 24 h or 10 µM etoposide (lanes 5–7) for 6 h in the absence (lanes 2 and 5) or presence of 100 µM Z-VAD-fmk (lanes 3 and 6) or 100 µM Z-DEVD-fmk (lanes 4 and 7).

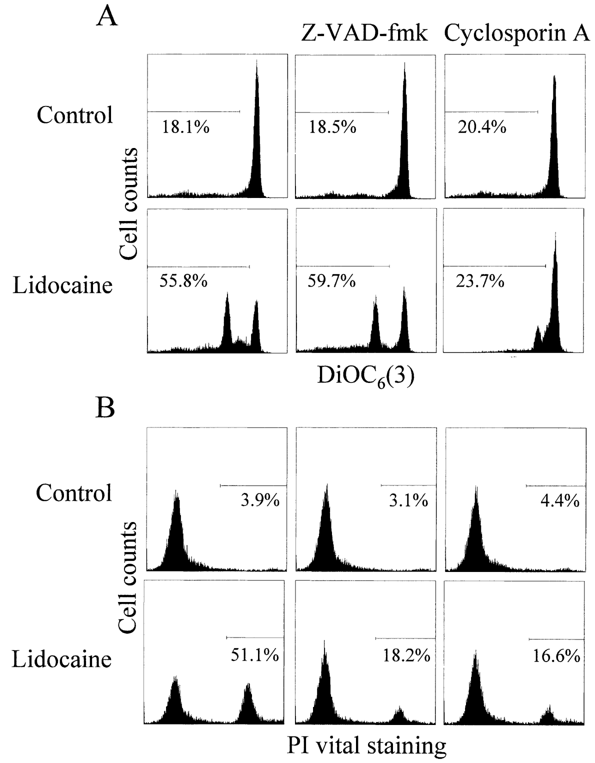

Fig. 6 Flowcytometric analysis of mitochondrial transmembrane potential ($\Delta\Psi$m) and PI positive cells. Cells were pretreated with 100 µM Z-VAD-fmk for 30 min or with 1 µM cyclosporin A for 5 h, then incubated with 12 mM lidocaine for an additional 24 h, and the $\Delta\Psi$m (A) and PI positive cells (B) were analyzed by flowcytometry. Data show a representative of three independent experiments.

Lidocaine at concentrations above 15 mM induced necrosis rapidly.

DNA ladder formation was not observed in the cells treated with lidocaine at concentrations above 15 mM (Fig. 2). Chromatin condensation was also not observed (data not shown). Cell death assessed by PI staining at 18 mM lidocaine was detected at 1 h and increased with incubation time, but a reduction of $\Delta\Psi$m was not observed (Fig. 8). Z-VAD-fmk affects neither the collapse of $\Delta\Psi$m nor the cell death. Indeed, the activation of caspase-3 was quite low in the cells treated with lidocaine at concentrations above 15 mM (Fig. 5A). These results suggest that lidocaine at higher concentrations induces cell death independent of caspases and the $\Delta\Psi$m reduction. Hoechst 33342 staining at 6 h showed irregular shape of nuclei with pyknotic chromatin, and no fragmented nuclei was observed. The early disruption of membrane integrity assessed by trypan blue exclusion and PI staining suggested that the cell death induced by lidocaine at higher concentrations was necrosis.

DISCUSSION

Local anesthetics are clinically useful compounds that exert a pharmacological effect by blocking nerve impulse propagation, but they have also adverse effects on a variety of cells. The present study showed that lidocaine inhibited the growth of U937 cells with a decrease in the WST-1 reduction activity and induced cell death without inducing the arrest of the cell cycle (Fig. 1). Lidocaine at concentrations below 12 mM induced chromatin condensation and internucleosomal DNA fragmentation (Fig. 2), which are typical characteristics of apoptosis, indicating the induction of apoptosis in U937 cells in a dose- and time-dependent manner. We also observed that prilocaine at 10 mM induced apoptosis with chromatin condensation and DNA fragmentation in U937 cells to the same extent as lidocaine (un-

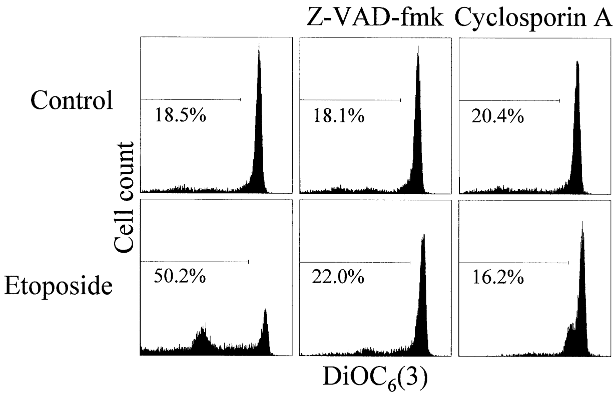

Fig. 7 Flowcytometric analysis of mitochondrial transmembrane potential (ΔΨm) of the cells treated with 10 μM etoposide. Cells were pretreated with 100 μM Z-VAD-fmk for 30 min or 1 μM cyclosporin A for 5 h, and then incubated with etoposide for 6 h, and the ΔΨm was analyzed by flowcytometry. Data are representatives of three independent experiments, which gave similar results.

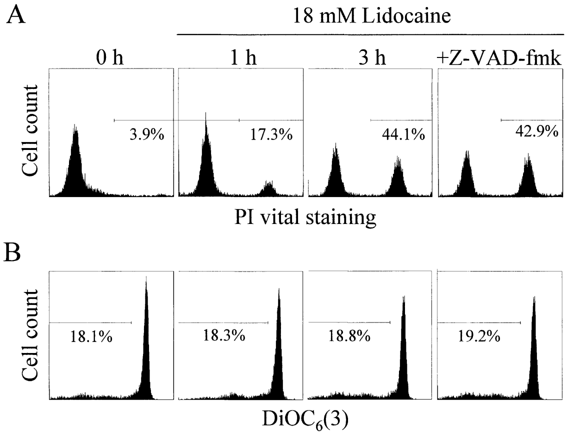

Fig. 8 Flowcytometric analysis of cell viability assessed by PI staining (A) and mitochondrial transmembrane potential (ΔΨm) (B) of the cells treated with 18 mM lidocaine for 1 and 3 h in the presence or absence of 100 μM Z-VAD-fmk. Data are displayed as one representative out of three experiments, which gave similar results.

published data). It has been reported that local anesthetic prilocaine induced apoptosis in cultured osteoblastic cells (4), and as did bupivacaine in immature muscle cells *in vitro* (12). However, the mechanism of apoptosis by local anesthetics in these cells has not been elucidated.

The caspases are a novel class of at least 12 cysteine proteases, and are known to be involved in the general mechanism in the induction of apoptosis (8). Particularly, caspase-3 plays a crucial role in the execution of apoptosis and its activation induces cleavage of PARP, DNA fragmentation and chromatin condensation (8). Lidocaine-induced apoptosis in U937 cells was caspase-dependent, since it was inhibited by Z-VAD-fmk, a pan-caspase inhibitor, and Z-DEVD-fmk, a caspase-3 inhibitor, resulting in the prevention of PARP cleavage and DNA fragmentation (Figs. 3 and 5B). Caspase-3 activation is either mitochondria-dependent or -independent (8, 15). In the mitochondria-dependent activation, the collapse of ΔΨm induces activation of caspase-9 followed by the activation of caspase-3 (8, 15). In the mitochondria-independent activation, caspase-3 is activated by initiator caspases such as caspase-8, which is activated by the formation of death-inducing signaling complexes with death receptors and adaptor proteins (8, 15).

The collapse of ΔΨm was independent of caspase activation in lidocaine-treated cells (Fig. 3). The MPT involved in the maintenance of ΔΨm consists of the adenine nucleotide transporter (ANT) located in the inner membrane and voltage-dependent anion channel (VDAC) in the outer membrane (24, 32). ANT is associated with cyclophilin D located in the matrix (32). Cyclosporin A binds to matrix cyclophilin D and prevents the ΔΨm reduction through the inhibition of MPT (24, 32). The reduction of ΔΨm induced by lidocaine was prevented by cyclosporin A (Fig. 6), indicating that lidocaine induced the MPT pore opening. The opening of the MPT pore induces the release of various proapoptotic components of the mitochondrial intermembrane space, resulting in the activation of caspase-9 and then caspase-3. Thus, the inhibition of the collapse of ΔΨm by cyclosporin A was followed by the inhibition of cell death with DNA fragmentation (Fig. 6), indicating that lidocaine-induced apoptosis is in fact mitochondria-dependent.

The collapse of ΔΨm is induced by a variety of apoptotic stimuli through different mechanisms (15, 24). Many anticancer drugs are known to induce Fas and/or FasL expressions (11, 17). Fas ligand (FasL) ligation to Fas induces the activation of caspase-8, which cleaves Bid, a proapoptotic Bcl-2 family protein. Cleaved Bid, truncated Bid (tBid), translocates to the mitochondrial membrane and induces the collapse of the ΔΨm followed by the progression of apoptosis (6, 25). However, we did not detect the expression of FasL in lidocaine-treated cells at 18 or 24 h by RT-PCR method (unpublished data). Moreover, the pan-caspase inhibitor Z-VAD-fmk did not affect the ΔΨm reduction in lidocaine-treated cells (Fig. 6A) and a caspase-8 inhibitor, benzyloxycarbonyl-Ile-Glu-Thr-Asp-fluoromethyl ketone (Z-IETD-fmk), did not inhibit the ΔΨm reduction. While the reduction of ΔΨm induced by etoposide was inhibited by Z-VAD-fmk (Fig. 7) and also by Z-IETD-fmk (unpublished data), indicating that caspase-8 and/or other caspases are involved in the collapse of ΔΨm in etoposide-induced apoptosis. The activation of the mitogen-activated protein kinase (MAPK) and stress-activated protein kinase pathways including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) or p38 kinase, has also been shown to reduce the ΔΨm in a variety of cells after apoptotic stimulation (14, 28). Then, we examined the effect of the inhibitors of MAPK/ERK kinase (MEK) 1/2 and p38 kinase, PD98059 and SB203580, respectively. None of them inhibited the reduction of ΔΨm or DNA fragmentation (unpublished data). These results indicate that both FasL/Fas-ligation and the MAPK/stress-activated protein kinase pathways were not involved in the reduction of ΔΨm and progression of apoptosis in lidocaine-treated cells. Arita *et al.* (3) reported that dibucaine as well as lidocaine induced apoptosis with the collapse of ΔΨm in promyelocytic leukemia cells (HL-60 cells), which depended on the activation of caspase-8 and caspase-3. Thus, caspase-8 may be involved in the lidocaine-induced collapse of ΔΨm in HL-60 cells, but not in U937 cells.

It has been shown that local anesthetics interact with phospholipids in the cellular membranes, resulting in the dysregulation of the activities of membrane enzymes such as protein kinase C and phospholipase A_2, and with mitochondrial energy metabolism (31). Kim *et al.* (22) showed that dibucaine-induced apoptosis in neuroblastoma cells might be due to membrane damage with the formation of oxygen radicals or due to increased intracellular calcium ions. It has been reported that local anesthetics reached to the mitochondria in cell culture to induce collapse of the ΔΨm (13), and inhibited adenine nucleotide transport because of their high lipophilicity (20). Recently, Johnson *et al.* showed that lidocaine at 18–37 mM induced mitochondrial injury followed by caspase activation in neuronal cells with inhibition of mitochondrial respiration. Arai *et al.* (1) reported that enhancement of hyperthermia-induced apoptosis by lidocaine was associated with the ΔΨm reduction in U937 cells through the increased intracellular Ca^{2+} concentration. It is uncertain at present whether the ΔΨm reduction in U937 cells is due to direct or indirect action by lidocaine.

At higher concentrations above 15 mM, lidocaine did not induce DNA fragmentation; rather it induced rapid disruption of the membrane integrity revealing an increase in PI positive cells (Fig. 8). Thus, lidocaine at higher concentrations may have direct toxic effects to induce necrosis in U937 cells as observed in rat neurons (12).

The present study showed that lidocaine induced apoptosis and necrosis in U937 cells depending on its concentration, indicating that lidocaine has some adverse effects on tissues depending on its concentration.

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