Introduction

Neutrophils are destined to undergo either necrotic cell death or apoptotic cell death, according to the circumstances of their location. When these phagocytic cells encounter microbes, they phagocytose them inside the cells to digest with lysosomal proteases and to destroy with superoxide generated by NADPH (nicotinamide adenine dinucleotide phosphate, reduced form) oxidase, and finally are driven to necrotic cell death, accompanied by loss of integrity of the nuclear and plasma membranes. Apoptosis, by contrast, is characterized by the orderly and non-inflammatory process. It appears to be the preferred means of cell death in normal physiological situations, and plays an important role in various development processes, such as organogenesis and cell homeostasis.

Keywords: neutrophil, phenoxazine, apoptosis, mitochondria, pHi
by the inflammation of tissues and their surroundings (1–3). The lifespan of these cells is very short in the circulating blood (48 h) (4, 5), because they are obliged to undergo another mode of cell death, that is, the apoptotic cell death that is regulated by an intrinsic mechanism (6, 7) in which the mitochondria play pivotal roles (6) and/or an extrinsic mechanism, where the ligation of Fas or TNF (tumor necrosis factor)-related apoptosis-inducing ligand is involved (8, 9). Different from necrotic cell death, apoptosis occurring in neutrophils does not induce inflammation because it does not accompany the release of the potentially toxic intracellular contents (4–6). In this context, the agents to induce apoptotic cell death, in particular, in neutrophils will be useful for preventing inflammation (10, 11), but such agents have not yet been developed.

We recently found that oxidative phenoxazines such as Phx-1 and Phx-3 induce apoptotic cell death of various cancer cells, causing mitochondrial depolarization (12–14) and extensive reduction of intracellular pH (pHi) (14–17), within 6 h and 30 min, respectively, after administration. It has been demonstrated that mitochondrial depolarization is associated with the apoptosis in neutrophils (6) and that intracellular acidification precedes apoptosis in these cells (18). In addition, cancer cells and neutrophils are similar in that the energy requirement relies heavily on glycolysis (19–22) and in that the mitochondria seem to operate more significantly in regulating apoptotic cell death than in producing ATP via the oxidative phosphorylation, in both these cells (23, 24). This view prompted us to investigate the effects of these phenoxazines on human neutrophils in terms of apoptosis induction. We noticed that Phx-1 and Phx-3 caused the apoptosis of neutrophils, without causing apoptotic cell death in lymphocytes and cell lysis in erythrocytes, when whole blood was treated with these phenoxazines. In the present study, we investigated extensively how Phx-1 and Phx-3 affect human neutrophils, examining apoptotic cell death, the apoptosis-related events including intracellular acidification, depolarization of the mitochondria, and superoxide generation in the cells.

Materials and Methods

Reagents

Phx-1 (2-amino-4,4α-dihydroxy-4α-7H-phenoxazine-3-one) and Phx-3 (2-amino-phenoxazine-3-one) were obtained according to the method of Nakachi et al. (25). Briefly, 2-amino-5-methylphenol or o-aminophenol which was dissolved in 0.9% NaCl solution was added to the suspension of bovine erythrocytes and 0.9% NaCl solution. The mixture was incubated at 37°C for 17 h. Phx-1 or Phx-3 was extracted by adding methanol to this mixture and could be purified by Sephadex LH-20 (GE Healthcare Bio-Sciences, Uppsala, Sweden) column chromatography, which yielded a purity of nearly 99% or even more, as calculated by the millimolar extinction for Phx-1 and Phx-3 (25). Each of these phenoxazines was dissolved in dimethylsulfoxide (DMSO) to make a 20 mM stock solution, which was diluted appropriately with phosphate-buffered saline (PBS). The chemical structure of these oxidative phenoxazines is illustrated in Fig. 1. Ferricytochrome c and phorbol myristate acetate (PMA) was obtained from Sigma Chemical (St. Louis, MO, USA).

Preparation of human neutrophils

Blood was freshly drawn from a healthy adult after obtaining their informed consent. It was then treated with different concentrations of Phx-1 and Phx-3 or used for isolating neutrophils (synonymous with polymorphonuclear cells, PMN). Human neutrophils from peripheral blood were isolated by dextran sedimentation and then purified on a Ficoll gradient according to the method of Boyum (26). Briefly, after the erythrocytes were eliminated by dextran sedimentation, the cell suspension was layered on a Ficoll–sodium isothalamate gradient and centrifuged (400 × g, for 30 min at room temperature) to separate the neutrophils from the lymphocytes, monocytes, and platelets. After the remaining erythrocytes were eliminated by a brief hypotonic lysis, the isolated neutrophils were washed twice with ice-cold PBS (pH 7.4) and then re-suspended in RPMI-1640 medium containing 10% heat-inactivated fetal calf serum (FCS) for a cell culture assay or in a Hepes-buffered salt solution containing 120 mM NaCl, 5 mM KCl, 5 mM glucose, 1 mM MgCl2, 0.5 mM CaCl2, 0.04% bovine serum albumin, and 17 mM Hepes (pH 7.4) for pHi determination.

Cell culture condition

Isolated human neutrophils were immediately cultured in RPMI-1640 medium containing 10% FCS, in the pres-
ence of Phx-1 or Phx-3. The cells were cultured at 37°C in a 5% CO₂ humidified atmosphere. For whole blood culture, heparinized blood from healthy adults was centrifuged (800 x g, for 15 min at room temperature), and the whole blood cells were isolated from the plasma. The plasma was stored at 4°C until use. Cells of whole blood were re-suspended in RPMI-1640 medium and diluted to ten times the original blood volume by the medium. The diluted whole blood was cultured in the presence or absence of Phx-1 and Phx-3 for 18 h at 37°C in a 5% CO₂ incubator. In the present study, DMSO was used as the vehicle of Phx-1 or Phx-3.

*Morphological study of whole blood or isolated neutrophils treated with Phx-1 or Phx-3*

Following a cell culture with Phx-1 or Phx-3, the isolated neutrophils were mounted on a glass slide by using the Shandon Cytospine II (Shandon, Pittsburg, PA, USA), and the mounted cells were viewed with either Pappenheim staining or May-Grünwald-Giemsa staining. For whole blood culture samples, blood cells were harvested and centrifuged (800 x g, for 15 min at 4°C), and then the cell pellet was re-suspended with the autologous plasma. Morphological observation of leukocytes in cultured blood was conducted by using blood smears. Blood smear samples were viewed with the Pappenheim’s staining.

*Apoptosis and necrosis detection*

Apoptotic and necrotic cells were quantitatively evaluated by flow cytometry using an annexin V-fluorescein staining Kit (Wako Pure Chemicals, Osaka). Isolated human leukocytes (1 x 10⁶) were treated with 0 (vehicle) or 50 μM Phx-1 or Phx-3 for 6 h. The cells were harvested and washed twice with phosphate-buffered saline (PBS, pH 7.4). An aliquot of 100 μl cell suspension was stained with 2 μl of fluorescein isothiocyanate (FITC)-labeled annexin V (annexin V–FITC) and propidium iodide (PI). The samples were incubated for 15 min at room temperature, after which 1 ml of binding buffer was added. The level of fluorescent staining of the cells was analyzed using a flow cytometer (Partec PAS; Partec, Münster, Germany) to estimate the population of apoptotic cells in neutrophils treated with Phx-1 or Phx-3.

*Influence of Phx-1 and Phx-3 on pHi in human neutrophils*

For neutrophils, pHi was determined according to the method described by Litman et al. (27). Briefly, the cells (4 x 10⁷/ml) were loaded with the pH-sensitive fluorescent probe BCECF-AM (3 μM) (Dojin Chemical, Kumamoto) in HEPES buffer (153 mM NaCl, 5 mM KCl, 5 mM glucose, 20 mM Hapes, pH 7.4) at 37°C for 30 min. After being washed once with Hapes buffer, the cells were resuspended in HEPES buffer. The cells (3 x 10⁶) were treated with 0 (vehicle), 25, 50, and 100 μM of Phx-1 or Phx-3 for 20 min. Fluorescence was measured at an excitation wavelength of 500 nm and an emission wavelength of 530 nm, using a FP750 microplate fluorescence reader (Jasco Co., Ltd., Tokyo). To calibrate fluorescence, BCECF-AM-loaded cells (3 x 10⁶) were suspended in pH 6.6, 7.2, 7.4, 7.8, and 8.2 calibration buffer (130 mM KCl, 10 mM NaCl, 1 mM MgSO₄, and 10 mM Na-MOPS) and 10 μg/ml nigericin was added to equilibrate the external and internal pH. The relative fluorescence ratio values were plotted against corresponding pHi values, in order to determine the unknown pHi. A linear calibration curve for pHi was obtained (data not shown): the optical density of the solution including BCECF-AM increased linearly with an increase of the pH. Therefore, it was possible to estimate the pHi of cells loaded with BCECF-AM.

*Detection of the loss of mitochondrial membrane potential in human neutrophils*

Reduced mitochondrial membrane potential is considered as the initial and irreversible step towards apoptosis. Therefore, the loss of mitochondrial membrane potential in human neutrophils treated with Phx-1 or Phx-3 was examined by 5,5’,6,6’-tetrachloro-1,1’,3,3’-tetracyanomethidazolylcarbocyanine iodide (JC-1) in order to detect the apoptosis of these cells. The cells (1 x 10⁶) were seeded in 12-well plates and treated with a vehicle (control) or 50 μM Phx-1 or Phx-3 for 6 h. At the end of the cell culture, the cells were stained with 1 ml 10% RPMI-1640 medium containing 5 μM JC-1 (Molecular Probes, Eugene, OR, USA) for 30 min at 37°C. Cells were then rinsed twice with ice-cold PBS, resuspended in 1 ml ice-cold PBS. Mitochondrial depolarization in the cells was analyzed by flow cytometry. JC-1 monomer in cytosol (a marker of cell containing depolarized mitochondria) and J-aggregates in mitochondria (a marker of cell containing normal mitochondria) were monitored using the FITC (FL1, 520 nm) and phycoerythrin emission (FL3, 590 – 650 nm) signal detectors, respectively.

*Detection of superoxide generated by human neutrophils with or without administration of Phx-1 or Phx-3*

The assay mixture (1.0 ml) consisted of 75 μM cytochrome c and neutrophils (1 x 10⁶ cells) in the assay buffer containing 120 mM NaCl, 5 mM KCl, 5 mM glucose, 1 mM MgCl₂, 0.5 mM CaCl₂, and 17 mM Hepes (pH 7.4), with 0.04% bovine serum albumin. Cells were incubated in the presence or absence of final concentration of 0 to 100 μg/ml Phx-1 or Phx-3, at 37°C for 10 min. The production of O₂⁻ was initiated by adding PMA.
(200 μg/ml) and measured by determining the rate of superoxide dismutase (SOD)-inhibitable reduction of cytochrome c at 550 – 540 nm using a dual-wavelength spectrophotometer (Hitachi 557; Hitachi Co., Ltd., Tokyo). The O₂⁻ release was calculated using a molar absorption coefficient of 19,100 M⁻¹·cm⁻¹.

Statistical analysis
Experimental data were presented as mean ± S.D. The data obtained from three independent experiments in Fig. 5 to Fig. 8 were analyzed by Student’s t-test to estimate statistical significance. The differences were considered significant when P was less than 0.01 or 0.05. In Fig. 7, statistical analysis was performed for hydrogen ion concentrations, because pH is defined as −log [H⁺].

Results
First, we studied the morphological changes in neutrophils, lymphocytes, and monocytes in freshly obtained blood incubated in the presence or absence of various concentrations of Phx-1 or Phx-3, at 37°C for 18 h (Figs. 2: A – C, Fig. 3: A and B). There was little change in morphology in the neutrophils and lymphocytes in the blood without these phenoxazines (Fig. 2A). When freshly obtained blood was treated with 50 μM Phx-1 or Phx-3 for 18 h, condensation of the nucleus was indicated in neutrophils (Fig. 2: B and C), but not in lymphocytes and monocytes (Fig. 2B). There was no hemolysis of the erythrocytes in blood treated with these phenoxazines for 18 h. These results indicate that Phx-1 and Phx-3 selectively induced apoptotic cell death in human neutrophils.

Figure 3A gives the population of the apoptotic neutrophils in the freshly obtained blood treated with different concentrations of Phx-1 or Phx-3. The population of neutrophils with characteristic apoptotic morphology increased with the dose of these phenoxazines, reaching up to 90% or 50% in the cells treated with 100 μM Phx-1 or 100 μM Phx-3, respectively. However, there were few apoptotic features in any lymphocytes, when whole blood was treated with 100 μM Phx-1 or Phx-3, at 37°C for 18 h (Fig. 3B).

Thus, we examined whether the isolated neutrophils are driven to apoptotic cell death, when they were treated with 50 μM Phx-1 or Phx-3 for 24 h. The left column in

![Fig. 2. Typical morphological features of human neutrophils, lymphocytes, and monocytes in whole blood treated with Phx-1 or Phx-3. Freshly drawn blood was treated with or without DMSO (as a vehicle control), 100 μM Phx-1, or Phx-3 at 37°C for 18 h. The morphology of each blood cell including neutrophils, lymphocytes, and monocytes was examined microscopically. Original magnification: × 1,000. A: Morphology of lymphocytes and neutrophils in the control blood (negative control and vehicle control). B: Morphology of lymphocytes, neutrophils, and monocytes in the blood with 100 μM Phx-1 or Phx-3. C: Features of apoptotic neutrophils (indicated by arrows) and non-apoptotic cells in the blood with 100 μM Phx-1 or Phx-3.](image-url)
Fig. 4 presents a microscopic photo of neutrophils (PMN) just after isolation from freshly obtained blood and indicating normal morphology without apoptotic features. Six hours after the administration of 50 μM Phx-1 or Phx-3, the population of neutrophils with typical apoptotic morphology including chromatin condensation of the nucleus (as shown by the arrows) increased significantly, compared with the negative control neutrophils (with saline) and the vehicle control neutrophils (with DMSO) (the middle four columns in Fig. 4). However, almost all the neutrophils underwent apoptosis (as shown by the arrows) and lost their morphology by 24 h after the addition of 50 μM Phx-1 and Phx-3, respectively, while more than half of the neutrophils still maintained their normal morphology in the negative and positive controls (the right four columns in Fig. 4). These results clearly demonstrate that Phx-1 and Phx-3 have the capacity of driving isolated neutrophils to apoptotic cell death (4, 5). When the cells were treated with 50 μM Phx-1 (Phx-3) for 6 h, the population of the apoptotic cells increased up to 7% (10%). The proapoptotic effect of Phx-3 was stronger than that of Phx-1, which is in good agreement with the morphological results in isolated neutrophils presented in Fig. 4.

Since mitochondrial depolarization has been shown to occur prior to cellular apoptosis (6), we studied whether mitochondrial depolarization may be caused in isolated neutrophils treated with Phx-1 or Phx-3 for 6 h (Fig. 6). We found that mitochondrial depolarization was significant in neutrophils treated with 50 μM Phx-1 or Phx-3 (population with depolarized mitochondria: control with saline, 40%; control with DMSO, 50%; with Phx-1, 78%; with Phx-3, 73%).

Since it has been indicated that cellular acidification precedes apoptotic cell death in neutrophils (18), we examined whether pH decrease in isolated neutrophils with the oxidative phenoxazines, Phx-1 or Phx-3, 30 min after administration. Figure 7 indicates a decrease of pH in neutrophils treated with different concentrations of Phx-1 or Phx-3. Phx-1 and Phx-3 caused a dose-dependent decrease of pH in the isolated neutrophils, although the pH-reducing effect of Phx-3 was far greater than that of Phx-1 (ΔpHi = 0.2 for 100 μM Phx-1; ΔpHi = 0.8 for 100 μM Phx-3).

The quick and abundant release of reactive oxygen species such as hydrogen peroxide and its precursor
superoxide from neutrophils has been characterized as the respiratory burst (1, 2), which has been demonstrated to be inhibited by acidification in these cells (28). Thus, we studied whether or not the release of superoxide, which can be detected by the reduction of ferricytochrome c, may be prevented by Phx-1 or Phx-3 or not, in isolated neutrophils in the presence of PMA, a potent activator of the respiratory burst. Figure 8 illustrates the effects of different concentrations of Phx-1 or Phx-3 on superoxide generation in neutrophils. It was found that the release of superoxide was extremely suppressed, in isolated human neutrophils treated with 25 μg/ml or more Phx-1 and Phx-3 (25 μg/ml is equivalent to 103 and 118 μM for Phx-1 and Phx-3, respectively) or more Phx-3. These results suggest that these oxidative phenoxazines inhibit the production of reactive oxygen species in human neutrophils.

Discussion

In this study, we demonstrated that apoptotic cell death occurred in neutrophils, but not in lymphocytes and monocytes, when whole blood was exposed to 50 μM or more Phx-1 or Phx-3 at 37°C for 18 h (Figs. 2 and 3). These findings were confirmed by microscopic analysis (Fig. 4) and flow cytometric analysis (Fig. 5) for isolated human neutrophils with 50 μM Phx-1 or Phx-3. In related work, Miyano-Kurosaki (29) demonstrated that treatment of peripheral blood mononuclear cells (PBMC) obtained from healthy adult donors with 40 μM Phx-1 and Phx-3 did not cause apoptotic cell death in lymphocytes. These results show that Phx-1 and Phx-3 have the capacity to
selectively induce apoptotic cell death in neutrophils among the blood cells. The drugs that target human neutrophils alone have not previously been identified hitherto, although Actinomycin D and cycloheximide are known to exert proapoptotic effects on neutrophils and other hematopoietic cells, including monocytes (30, 31). In this sense, oxidative phenoxazines such as Phx-1 and Phx-3 seem to be distinctive compounds that exert proapoptotic effects on human neutrophils alone, without causing apoptotic cell death in human lymphocytes and monocytes.

Neutrophils are susceptible to apoptotic cell death and therefore have a shorter lifespan in blood circulation (4,
5), although the mechanism for this susceptibility of neutrophils to apoptosis has not been sufficiently clarified. Depolarization of the mitochondria may be one of the major determinants that direct apoptotic cell death in normal and cancer cells (23, 32, 33) and has been known to be associated with the apoptotic cell death of neutrophils as well (6). Our previous study demonstrated that apoptosis could be caused in many human cancer cell lines treated with Phx-1 or Phx-3, where depolarization of the mitochondria had been induced prior to apoptotic cell death (12 – 14, 17). Our present results indicated that 6 h after 50 μM Phx-1 or Phx-3 was administered to neutrophils, the population with the depolarized mitochondria increased by approximately 40% (Fig. 6), while that of the apoptotic cells increased to some extent (a 7% to 10% increase) in these cells (Fig. 5). This result strongly suggests that depolarization of the mitochondria precedes the apoptotic cell death in neutrophils, when the cells have been treated with Phx-1 or Phx-3. The mitochondrial depolarization in neutrophils may be possibly caused by Bax and Bad, proapoptotic proteins of the Bcl-2 family, which are shown to be constitutively expressed in these cells (34, 35). However, it is unclear currently to what extents these proteins are involved in the mitochondrial depolarization in human neutrophils treated with Phx-1 and Phx-3. The decreased levels of Mcl-1 (myeloid cell leukemia-1), another member of the Bcl-2 family proteins that acts for the survival of neutrophils (35, 36), may be associated with the apoptosis induction of these cells. The contribution of this protein in the apoptotic cell death in human neutrophils treated with Phx-1 or Phx-3 may become an interesting theme in the future.

We recently reported that depolarization of the mitochondria is seldom caused in normal cells such as human umbilical vein endothelial cells (HUVECs) and the human embryonic lung fibroblast cell line HEL, when treated with Phx-1 or Phx-3 at the concentrations that extensively suppressed the growth of these cells (14). However, it is currently unclear why mitochondrial depolarization was not induced by Phx-1 or Phx-3 in these normal cells or what discriminates the susceptibility of the mitochondria to these phenoxazines among neutrophils and other normal cells. Thus, neutrophils may be unique among hematopoietic cells and may be rather analogous to cancer cells, in that the energy requirements of neutrophils and cancer cells are closely dependent on the glycolysis (19 – 22) and that apoptosis in these cells, when treated with Phx-1 or Phx-3, is preceded by depolarization of the mitochondria (Figs. 5 and 6 and Ref. 14).

We found that pH i was significantly decreased in neutrophils treated with Phx-1 or Phx-3 within the initial 30 min after the administration to the cells, although the pH i-reducing activity of Phx-3 was far stronger than that of Phx-1 (Fig. 7). Similar results were seen in cancer cells because pH i was decreased significantly in many cancer cell lines treated with Phx-1 and Phx-3, where the pH i-decreasing activity of Phx-3 was much stronger than that of Phx-1 (14). Gottlieb et al. (18, 37) showed that intracellular acidification is critical to the apoptosis of neutrophils because endonuclease II, which is responsible for the DNA fragmentation, is activated at acidic pH i, specifically, below pH 6.8. Therefore, it is probable that the drastic decrease in pH i from 7.2 to less than 6.8 during 30 min incubation in neutrophils with 50 μM or more Phx-3 (Fig. 7) may be reflected in the activation of endonuclease II to cause the apoptosis in the cells (Fig. 4). However, with regards to Phx-1, endonuclease II contributes to apoptosis in neutrophils only partly, because only a small decrease of pH i, from 7.2 to 7.1 (7.2 to 7.0), was indicated in cells treated with 50 μM (100 μM) Phx-1 (Fig. 7). In this case, the contribution of mitochondrial depolarization (Fig. 6) to the induction of apoptosis in these cells may be rather greater in cells treated with Phx-1.

The decrease of pH i in neutrophils disturbs the intracellular metabolism including the glycolytic and hexose monophosphate pathways, resulting in an insufficient supply of NADPH and inhibition of superoxide-producing NADPH oxidase (1, 2, 38). Superoxide generation was extremely inhibited in neutrophils treated with 100 μM or more Phx-1 or Phx-3 (Fig. 8). The inhibition may involve an insufficient supply of NADPH, which could be caused by an extensive decrease in pH i in neutrophils with Phx-1 or Phx-3 (Fig. 7). Alternatively, Phx-1 or Phx-3 may block the phosphorylated Akt (B kinase)-mediated activation of the oxidase regulator p47phox, an event required for superoxide generation (39). This is because the phosphorylation of Akt was shown to be greatly inhibited in rat basophilic leukemia RBL-2H3 cells treated with Phx-1 (40) and in A549 cancer cells treated with Phx-3 (our unpublished data) and because it was strongly inhibited by the chemically synthesized phenoxazines including N10-substituted phenoxazines such as 10-[4’-(N-diethylamino) butyl]-2-chlorophenoxazine and 10-[4’-(β-hydroxyethyl)piperazino]buthyl]-chlorophenoxazine (41). This view should be confirmed by further investigation for Akt in neutrophils.

Neutrophils play critical roles in phagocytosing invading microbes, releasing the reactive oxygen species (ROS) and granule enzymes and finally inducing inflammation of the tissues during the conflict between phagocytes and microbes or the tissues nearby. In addition, it has been recognized that when neutrophils infiltrate into the tissues under inflammatory and injury conditions,
their lifespan is greatly prolonged due to the delay of their apoptotic cell death, thereby preventing the resolution of inflammation (9, 10). A delay of apoptosis in circulating neutrophils has been seen in patients with sepsis and thermal injury (42, 43), as well as in pulmonary neutrophils in patients with acute respiratory distress syndrome (44), resulting in the aggravation of these diseases. There are several factors in delaying the apoptosis of neutrophils, including the gram-negative bacterial product (LPS), granulocyte-monocyte colony-stimulating factor (GM-CSF), and glucocorticoids (18). Conversely, our present results are unique in the observation that the oxidative phenoxazines such as Phx-1 and Phx-3 extensively accelerate the apoptosis of human neutrophils alone in the blood or in an isolated state and extremely suppress the production of superoxide, a sort of ROS in these cells, suggesting that these phenoxazines may prevent the development of inflammation, when applied to the patients with severe inflammatory conditions caused by activated neutrophils with prolonged lifespan. Since Kohno et al. (45) indicated that Phx-3 effectively suppressed the function of macrophages, another type of inflammatory cell, by inhibiting their COX-1 activity and their release of prostaglandin E1 and interleukin-8, the proapoptotic effects of Phx-3 on human neutrophils would be advantageous for suppressing inflammation.

In addition to the above, Tabuchi et al. (46, 47) reported that when neutrophils were removed from circulating blood of rabbits with tumors or patients with recurrent metastatic tumors by apheresis, the cancers were extensively undersized, suggesting that neutrophils are relevant to the development of cancer. Currently, neutrophils are recognized as the cells to modify tumor growth, angiogenesis, and metastasis (48). Therefore, the promotion of apoptosis in neutrophils may be advantageous for preventing cancer development and metastasis. Applying Phx-1 and Phx-3 to cancer development and metastasis related to neutrophil apoptosis may be a target for future investigation, although these phenoxazines also exert strong anticancer activity against various cancer cells in vitro and in vivo (14, 49 – 51).

In conclusion, Phx-1 and Phx-3 may be beneficial anti-inflammatory agents that cause the apoptosis of neutrophils, preceded by a decrease of pHt, promotion of mitochondrial depolarization, and inhibition of superoxide generation in the cells.

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References


