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Hydrogen Peroxide Produced Inside Mitochondria Takes Part in Cell-to-Cell Transmission of Apoptotic Signal

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Abstract—In monolayer of HeLa cells treated with tumor necrosis factor (TNF), apoptotic cells formed clusters indicating possible transmission of apoptotic signal via the culture media. To investigate this phenomenon, a simple method of enabling two cell cultures to interact has been employed. Two coverslips were placed side by side in a Petri dish, one coverslip covered with apoptogen-treated cells (the inducer) and another with non-treated cells (the recipient). TNF, staurosporine, or H_2O_2 treatment of the inducer cells is shown to initiate apoptosis on the recipient coverslip. This effect is increased by a catalase inhibitor aminotriazole and is arrested by addition of catalase or by pre-treatment of either the inducer or the recipient cells with nanomolar concentrations of mitochondria-targeted cationic antioxidant MitoQ (10-(6′-ubiquinolyl)decyltriphenylphosphonium), which specifically arrests H_2O_2-induced apoptosis. The action of MitoQ is abolished by an uncoupler preventing accumulation of MitoQ in mitochondria. It is concluded that reactive oxygen species (ROS) produced by mitochondria in the apoptotic cells initiate the release of H_2O_2 from these cells. The H_2O_2 released is employed as a long-distance cell suicide messenger. In processing of such a signal by the recipient cells, mitochondrial ROS production is also involved. It is suggested that the described phenomenon may be involved in expansion of the apoptotic region around a damaged part of the tissue during heart attack or stroke as well as in "organoptosis", i.e. disappearance of organs during ontogenesis.

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It is well established that programmed cell death is often accompanied by the generation of large amounts of the reactive oxygen species (ROS). On the other hand, ROS can also initiate apoptosis (for review see [1-3]). These dual effects suggest that ROS may, at least in some cases, play a role in transmission and amplification of apoptotic signals [1, 4]. Among the many types of ROS produced *in vivo*, hydrogen peroxide stands out as being chemically stable and able to penetrate biomembranes. Furthermore, its concentration can be regulated by H_2O_2-decomposing enzymes like catalase and peroxidases. The above features make H_2O_2 a good candidate for involvement in intercellular signaling. Addition of H_2O_2 to the cell cultures is well known to induce apoptosis. In 1998, a hypothesis was put forward by one of us (V. P. S.) that apoptogenic activity of hydrogen peroxide is used by organisms to form a "dead" zone around a virus-infected cell (like in tobacco leaf infected by the tobacco mosaic virus), thus preventing expansion of the infection [5]. It was suggested that in a tissue the front of elevated concentration of H_2O_2 produced by an infected cell spreads significantly faster than the front of viral particles formed in the very same cell.

In this paper, transmission of apoptotic signal(s) was studied in a HeLa cell monolayer in a model where direct cell-to-cell contacts were excluded. Long-distance transmission of the signal was demonstrated in various cases of apoptosis. Catalase arrests the transmission as well as very

Abbreviations: FCCP) carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone; MitoQ) 10-(6′-ubiquinolyl)decyltriphenylphosphonium; ROS) reactive oxygen species; TNF) tumor necrosis factor.

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low concentrations of antioxidant MitoQ (10-(6′-ubiquinolyl)decyltriphenylphosphonium), which was electrophoretically accumulated in mitochondrial matrix. It is concluded that it is mitochondrially-produced H_2O_2 that is responsible for the long-distant intercellular transmission of apoptotic signal. A short preliminary communication of the data has been published [6].

MATERIALS AND METHODS

Human cervical carcinoma cells HeLa were maintained in DMEM supplemented with gentamicin sulfate (0.08 mg/ml) and 10% fetal calf serum at 37°C and 5% CO_2. In some experiments, Bcl-2-overexpressing HeLa cells were studied. In these cases, the human *bcl-2* gene was introduced into HeLa cells using the pLPC-bcl-2 vector for transfection. A control clone was similarly prepared using an empty vector. These cell lines were kindly provided by Dr. G. A. Belov (Chumakov Institute of Poliomyelitis and Viral Encephalitis, Moscow). Before the experiments (if not specially indicated), cells were cultivated for two days in the same medium with low glucose content (5 mM) up to 50% confluency. In the experiments depicted in Fig. 1, cells were grown to more than 80% confluency to obtain a large area of the cell monolayer. To visualize apoptotic cells, they were stained with Hoechst 33342 (1 μg/ml, 40 min). The cells with condensed and fragmented chromatin usually were stained with Annexin V-Cy3 (Molecular Probes, USA) fluorescent conjugates indicating phosphatidylserine externalization and were not stained with propidium iodide indicating intactness of the plasma membrane. This staining confirmed apoptotic death of the cells without significant secondary necrosis in our models. Apoptotic cells stained with Hoechst 33342 were counted among 500 cells (minimal) in every assay.

To analyze clustering of apoptotic cells, after treatment and staining with Hoechst 33342, large areas of monolayers were photographed and mapped (Fig. 1). In the obtained graph, the number of contacts between apoptotic cells (C_{exp}) was computed. In the same graph, N random choices (usually $N = 10{,}000$) of the same number of apoptotic cells were performed and the number of contacts between such cells (C_{trial}) was computed in each trial. The ratio $P = N_0/N$ (where N_0 is a number of trials with $C_{trial} > C_{exp}$) is an estimation of the probability that the distribution of dead cells in the experimental image is random. Clustering index (D) is defined as $1 - P$. Thus, for example, in the case presented in Fig. 1, 327 cells were analyzed and 104 contacts between apoptotic cells were observed while random distribution predicted ~65 contacts. Calculated $D > 0.999$ indicates clustering of apoptotic cells with $P < 0.001$.

For vital observations (Fig. 2), cells were grown in a special chamber with parallel coverslip walls. They were analyzed using an Axiophot microscope (Carl Zeiss, Germany) equipped with a camera with time-lapse videotaping (Hamamatsu C 2400, Japan).

In experiments on signal transmission between cell cultures, cells were grown on coverslips (9 × 18 mm). Inducer cells were treated with tumor necrosis factor (TNF) (10-50 ng/ml) or staurosporine (2 μM) for 3 h, washed carefully, and placed into a dish (35 mm diameter) side-by-side with the coverslip covered with untreated cells (the recipient). One milliliter of the medium was added to create the layer of ~1 mm over the coverslips. In case of TNF-induced apoptosis, the inducer coverslip was treated in the presence of inhibitors of protein synthesis (1 μM emetine or 10 μM cycloheximide) and the same inhibitors were added to the medium during co-culturing. In the experiments on signal transmission from H_2O_2-treated cells, the inducer was prepared by treatment of cells (grown at low density (20-30%) on the bottom of the dish) with 50 μM H_2O_2 added 3 times with 1 h interval. The recipient coverslip was placed into this dish over the growing cells. All the results are presented as averages with standard deviation from 3-5 experiments.

H_2O_2 was measured fluorimetrically using Amplex Red (Molecular Probes) and horseradish peroxidase (excitation, 530 nm; emission, 590 nm).

Immunostaining and staining with the membrane potential-sensitive and ROS sensitive fluorescent dyes were performed as described early [7].

TNF and monoclonal anti-TNF antibodies were kindly provided by Dr. L. N. Shingarova (Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Moscow). DMEM and fetal calf serum were from Gibco (USA). All the other reagents were from Sigma (USA).

RESULTS

In the first series of experiments, we have addressed the question whether apoptotic HeLa cells studied in our experiments are competent in sending apoptogenic signal(s) to adjacent cells (i.e., bystander killing). HeLa cells are a good model system in which to test this as they lack gap junctions that can propagate certain types of the bystander killing in some cell lines [8, 9]. To induce apoptosis, we treated the cells with TNF and emetine (the latter was added to arrest the protein synthesis involved in the NF-κB-mediated anti-apoptotic effect of TNF). After 3-4 h, the apoptotic cells were revealed in the cell monolayer by staining their nuclei with Hoechst 33342 (Fig. 1a). Then the number of dead-to-dead cell contacts was counted and compared with the theoretical number of contacts calculated assuming a random distribution of apoptotic cells in the monolayer (Fig. 1b). To this end, a computer program Cluster Detector was elaborated.

The results presented in Fig. 1b clearly showed clustering of apoptotic cells. Ninety-three dead cells found in

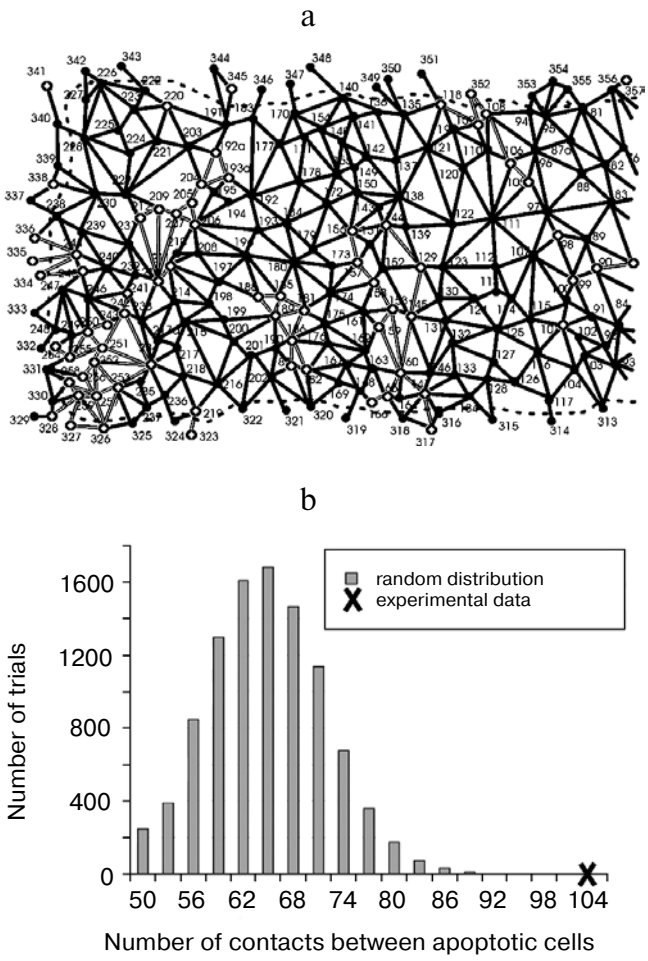


Fig. 1. Clustering of apoptotic cells in monolayer cell culture. HeLa cells were treated with TNF (10 ng/ml) and emetine (1 μM) for 3.5 h. Then cells were fixed with 4% formaldehyde and stained with Hoechst 33342. a) Mapped image of distribution of the apoptotic cells (white circles) in the monolayer. Only a part (about 50%) of the image used in calculations is shown (surrounded by dashed line). b) Statistical analysis. The distribution of the numbers of "dead-dead" contacts in 10,000 random trails and the observed number of dead-dead contacts (indicated with X) in the mapped image. A typical experiment of three is presented.

the field of 327 cells were shown to form 104 dead-to-dead contacts whereas occasional dead cell distribution predicted 63.8 ± 7.0 contacts. Statistical analysis indicated that the probability that the distribution of dead cells in the experimental image is random is less than 0.001. It should be stressed that the size of the clusters is much larger than the possible size of the clones formed by divisions of a single cell during the experiment (two days, 2-3 divisions). Thus it was concluded that intercellular apoptotic signals were transmitted in monolayer of HeLa cells.

The next question was whether direct cell-to-cell contacts (even in the absence of gap junctions) are necessary for this phenomenon. To investigate this problem a less dense culture which formed isolated islands, each of them being composed of several cells, was used. A typical result is shown in Fig. 2 where two cell islands after treatment with TNF are seen. It is obvious that each cell makes its "personal decision" when it commits suicide. The time required for such a "decision" varies from 1 h 20 min to 5 h 10 min (in this particular experiment) and shortens if there is an apoptotic cell nearby. This observation confirmed the suggestion of transmission of a bystander signal produced by an apoptotic cell without direct contact.

To test this suggestion, a simple method of bringing two cell cultures grown separately on two coverslips close together in the same Petri dish was employed. The cells on inducer coverslip were treated by apoptogen (TNF, staurosporine, or H_2O_2) and after 3 h were washed with a medium and placed side by side to the second coverslip with non-treated (recipient) cells. Figure 3 shows an experiment when TNF was used as an inducer of apoptosis and the results were analyzed after 17 h of co-culturing. Apoptotic cells were identified by chromatin condensation (staining with Hoechst 33342). It was revealed that massive apoptosis occurs not only among the inducer but also among the recipient cells (Fig. 3a). The percentage of apoptotic cells on the recipient coverslip does not in

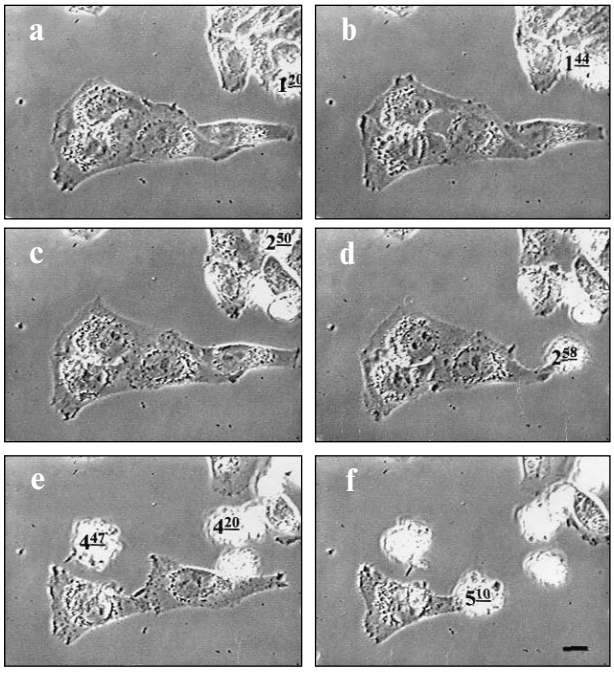


Fig. 2. Vital observation of development of apoptosis in the cell culture with low density. At zero time, HeLa cells were treated with TNF (5 ng/ml) and emetine (1 μM). Selected frames from the videotape taken at 1 h 20 min (a), 1 h 44 min (b), 2 h 50 min (c), 2 h 58 min (d), 4 h 20 min and 4 h 47 min (e), and 5 h 10 min (f) are presented. The individual cells are marked with the time corresponding to the beginning of the rapid morphological changes (rounding, blebbing) of apoptotic cells. Bar, 10 μm.

fact depend on distance from the inducer coverslip (not shown). This percentage decreased when thickness of the layer of the growth medium above the growing cell increased (not shown in figures) supporting the suggestion of signal transmission via solution. To exclude possibility of migration of TNF from the inducer to recipient cell, the medium for co-culturing was supplemented with TNF-specific monoclonal antibodies and no inhibition of apoptosis of recipient cells was found (Fig. 3b) indicating that the molecules of TNF were not involved in signal transmission. The efficiency of the antibodies was confirmed by prevention of TNF-induced apoptosis. One more indication that the observed effect is not due to a TNF redistribution between the inducer and recipient cells was obtained when the effects of oligomycin was tested. As was previously found in our group [7], this inhibitor arrested TNF apoptosis but did not affect some other types of apoptosis. Arrest took place if oligomycin was added not later than in 1.5 h after TNF. To exploit this feature of oligomycin, we placed inducer cells treated with TNF for 3 h to the recipient cells growing in the oligomycin-containing medium.

As is seen in Fig. 3b, oligomycin, like TNF antibodies, does not affect the recipient cells. On the other hand, it strongly inhibited apoptosis in the inducer cells when added together with TNF. Migration of the cells between the inducer and the recipient coverslip was excluded by a direct observation when HeLa cells transfected with a plasmid bearing fluorescent protein (EYFP) gene was used as the inducer. No fluorescent cells were found on

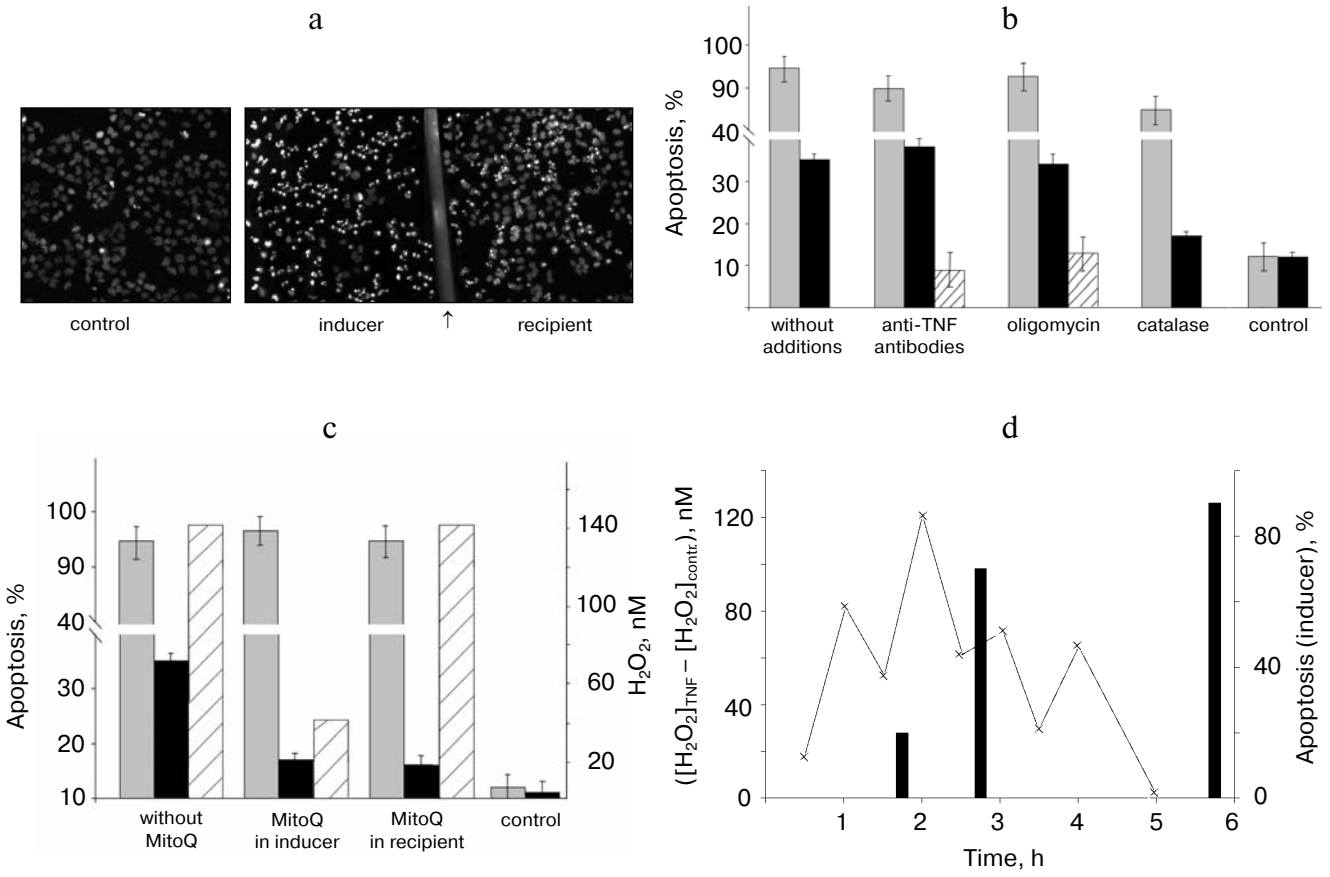


Fig. 3. Transmission of apoptotic signal from the cells treated with TNF. An inducer coverslip covered with HeLa cells was treated with TNF (10 ng/ml) and emetine (1 μM) or cycloheximide (10 μM) for 3 h, washed, and placed side by side for 17 h with the recipient coverslip covered by non-treated cells (see "Materials and Methods"). Cells were stained with Hoechst 33342. Concentration of H_2O_2 in the incubation medium was measured using Amplex Red and horseradish peroxidase. a) Typical distribution of apoptotic cells on the inducer and the recipient coverslips. Arrows indicate the area where two coverslips are in contact. b) Monoclonal anti-TNF antibodies (700 ng/ml) were added to the inducer coverslip simultaneously with TNF (hatched column) or when two coverslips were placed together (black column). Oligomycin (5 μg/ml) was added either 30 min before TNF (hatched column) or 3 h after TNF when the coverslips were placed together (black column). Catalase (2500 U/ml) was added when the coverslips were placed together. Control, the inducer glass was treated with emetine without TNF. c) Where indicated, the inducer or the recipient cells were grown with 20 nM MitoQ for 6 or 4 days. Concentration of H_2O_2 in the incubation medium (hatched columns) and also apoptosis of inducer (gray columns) and recipient cells (black columns) was measured after 24 h of co-culturing. d) Concentration of H_2O_2 in the incubation medium (solid line) and the level of apoptosis of the inducer cells (columns) at various durations of co-culturing. The concentration of H_2O_2 in the medium with emetine but without TNF was subtracted.

the recipient coverslip at the end of the experiment (not shown in figures).

If it is hydrogen peroxide that plays the role in transmission of apoptotic signal, addition of catalase, an enzyme decomposing H_2O_2, should suppress the apoptosis on the recipient coverslip. The experiment shows that this is the case (Fig. 3b). Catalase, at the concentration used being without measurable effect on the TNF-induced apoptosis of inducer cells. Higher concentrations of catalase were shown to decrease slightly (less than two-fold) the apoptosis level on the inducer coverslip (not shown on figures).

To study the role of the mitochondrial interior as a possible place for generation of apoptosis-inducing hydrogen peroxide, we have employed MitoQ, an antioxidant selectively targeted to mitochondria. This compound is composed of the quinone residue of coenzyme Q and decyltriphenylphosphonium cation, which allow its electrophoretic accumulation in the matrix of mitochondria [10, 11]. In fact, the cationic part of MitoQ is identical to that of phosphonium derivatives suggested by Liberman et al. in 1969 [12] to measure mitochondrial membrane potential (for more details see [13-15]). MitoQ is electrophoretically accumulated by a factor of around 1000 in the matrix [10] and reduced by the respiratory chain. In the reduced form, this antioxidant rescued the cells from oxidative stress and H_2O_2-induced apoptosis but not from apoptosis caused by TNF or staurosporine [11].

In the experiments presented at Fig. 3c, the recipient or the inducer cells were grown for 4-6 days with 20 nM MitoQ and the inducer-to-recipient death signal transmission was strongly suppressed in the both cases. On the other hand, MitoQ was almost without effect on the TNF-induced apoptosis on the inducer coverslip (Fig. 3c). The level of H_2O_2 measured in the medium after 24 h of co-incubation was much lower if the inducer cells were pre-treated with MitoQ, while pre-treatment of the recipient cells was without effect (Fig. 3c). Thus, it can be suggested that H_2O_2 produced by mitochondria in TNF-treated cells was critical for intercellular transmission of apoptotic signal. On the other hand, apoptosis of the recipient cells also strongly depended on H_2O_2 production in their mitochondria.

Measurable increase in $[H_2O_2]$ in the medium was observed after 1-4.5 h of co-incubation (Fig. 3d). Such an increase took place in spite of very high rate of consumption of the added H_2O_2 (1-10 μM) by HeLa cells (not shown). The concentration of H_2O_2 in the maximum was still lower than necessary for induction of apoptosis in HeLa by single addition of H_2O_2 (50-100 μM). Prolonged increase of $[H_2O_2]$ was probably critical for induction of apoptosis in the recipient cells.

The major features of apoptosis of the recipient cells were typical of apoptosis induced by various stressors in HeLa cells [7]. It was accompanied by collapse of the mitochondrial membrane potential, BAX translocation from cytosol to mitochondria, cytochrome *c* translocation in the opposite direction, and a burst in ROS formation (measured with intracellular fluorescent dye). Apoptosis was suppressed if a HeLa cell line overproducing Bcl-2 was used as a recipient (not shown in figures).

Apoptosis with similar features was observed among the recipient cells when staurosporine instead of TNF was used to cause apoptosis of the inducer cells. The qualitative

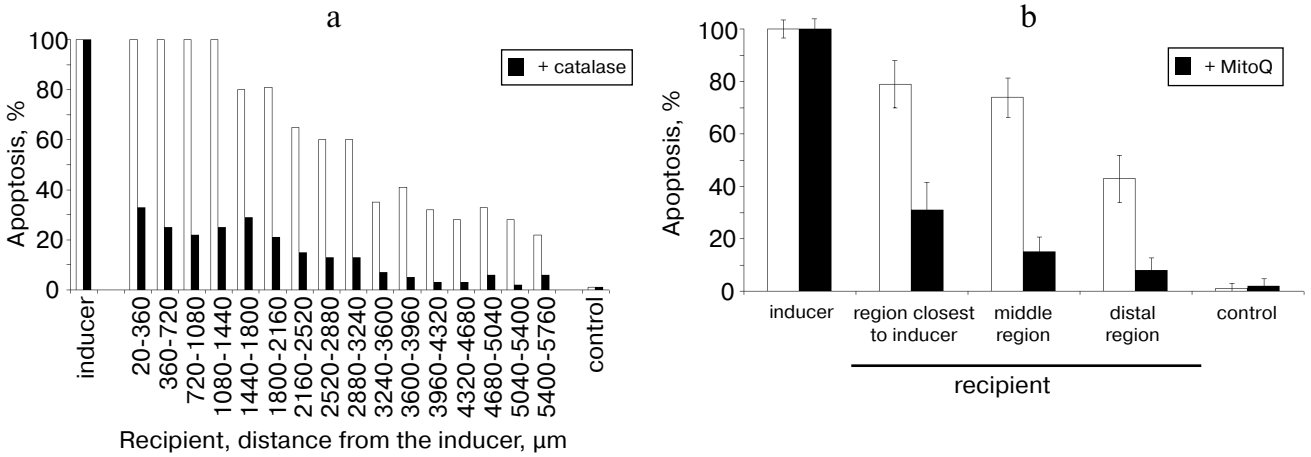


Fig. 4. Transmission of apoptotic signal from cells treated with staurosporine. HeLa cells were treated with staurosporine (2 μM) for 3 h, washed, and placed side by side with recipient cells (see "Materials and Methods") for 17 h. Apoptotic cells were counted after staining with Hoechst 33342. a) Distribution of apoptotic cells on the recipient coverslip. Strips parallel to the border between the coverslips of ~360 μm width (the view field) were analyzed; 300-500 cells were counted in the each strip; in a separate assay (black columns) catalase (2500 U/ml) was added. b) 20 nM MitoQ was added to the recipient cells 1.5 h before placing the coverslips together (black columns) and apoptosis was analyzed in the region closest to inducer (strips *1-4*), middle region (strips *7-10*), and in the distal region (strips *13-16*). Control, inducer cells were not treated with staurosporine.

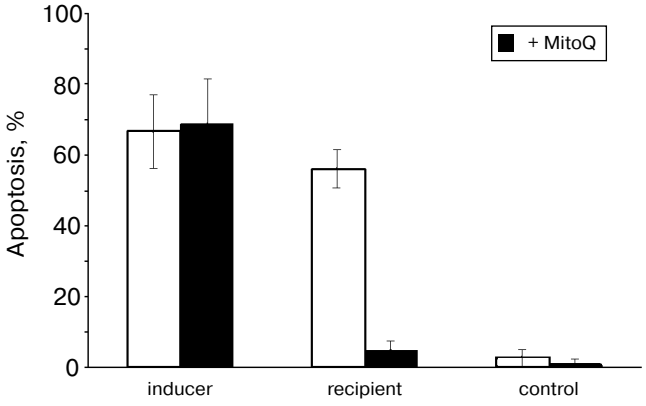


Fig. 5. Transmission of apoptotic signal from the cells treated with H_2O_2. A culture of HeLa cells with low density (less then 50% of confluence) in a dish was treated with H_2O_2 (50 μM H_2O_2 was added 3 times during 3 h) and washed. The recipient coverslip was placed in the same dish (see "Materials and Methods") and aminotriazole (7 mM) was added for 18 h. MitoQ (14 nM) was added to the recipient cells 1 h before they were placed to the dish. MitoQ was also present during 18 h of co-culturing (black columns).

difference was that the effect of staurosporine on the inducer cells, in contrast to that of TNF, proved to be oligomycin-resistant in line with earlier data [7]. It seems that staurosporine, in contrast to TNF and H_2O_2, cannot be effectively washed out before co-culturing. This resulted in formation of a gradient of apoptotic signal between cells occupying proximal and distal parts of the recipient coverslip relative to the inducer coverslip. The cells in the proximal area were killed by the combined action of staurosporine and hydrogen peroxide and were not fully protected by catalase (Fig. 4a). At the same time, catalase strongly protected (Fig. 4a) and the catalase inhibitor aminotriazole stimulated (not shown) apoptosis in the distal region of the recipient coverslip confirming the role of H_2O_2 in signal transmission. Similarly, the inhibitory effect of MitoQ proved to be higher in the distal than in the proximal region (Fig. 4b). This is in agreement with strong antiapoptotic effect of MitoQ against H_2O_2-induced apoptosis, but not against staurosporine-induced apoptosis [11].

In the next series of experiments, apoptosis in the inducer cells was caused by addition of H_2O_2 (Fig. 5). The half-life of H_2O_2 in the presence of the cells did not exceed 10-15 min, and to improve the effect three additions of 50 μM H_2O_2 with 1 h intervals were applied. Apoptosis was analyzed after 18 h of co-culturing in the presence of 7 mM aminotriazole (an inhibitor of catalase). Pretreatment of the recipient cells with MitoQ and its addition during co-culturing almost completely prevented apoptosis of the recipient cells while apoptosis of the inducer cells remained unaffected (Fig. 5).

Since MitoQ was very important as a tool for studies of intercellular signal transmission, the mechanism of its action was analyzed in more details. It was shown that when HeLa cells were cultivated during seven days (two passages) with MitoQ they became resistant to apoptosis induced by 50 μM H_2O_2. When cells were cultured with MitoQ in the presence of protonophorous uncoupler FCCP (carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone) (1 μM), the protective effect of MitoQ was not pronounced (Fig. 6). These data indicated the critical role of electrophoretic accumulation of MitoQ in mitochondria for its antiapoptotic activity.

DISCUSSION

The data presented above indicate that HeLa cells treated by three different apoptogens (TNF, staurosporine, and H_2O_2) generate an apoptotic signal that can be transmitted to intact cells via the medium without direct contact. H_2O_2 is employed as a major intercellular messenger for transmission of the signal. Apoptosis in recipient cells was strongly suppressed either by catalase or by mitochondria-targeted antioxidant, MitoQ and enhanced by a catalase inhibitor. These agents however did not affect apoptosis caused by TNF or by staurosporine in the inducer cells. The concentration of H_2O_2 measured in the medium during co-culturing was diminished by pre-treatment of the inducer cells with MitoQ (Fig. 3c). The same treatment made the recipient cells resistant to exogenous H_2O_2. These data indicated that H_2O_2 was produced by mitochondria in the inducer cells and then penetrated into the recipient cells and caused secondary ROS production in mitochondria, inducing apoptosis. Probably, H_2O_2-induced excessive ROS production was mediated by opening of the permeability transition pore in the mitochondrial membrane as shown

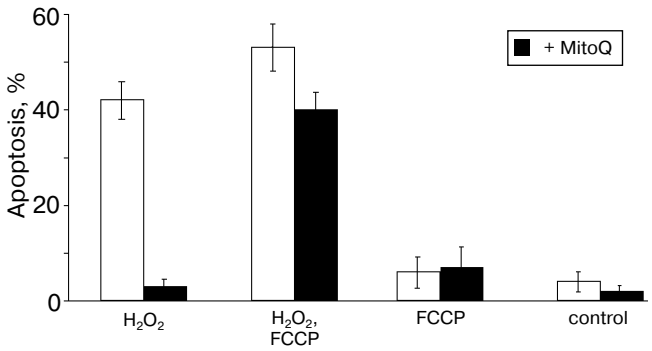
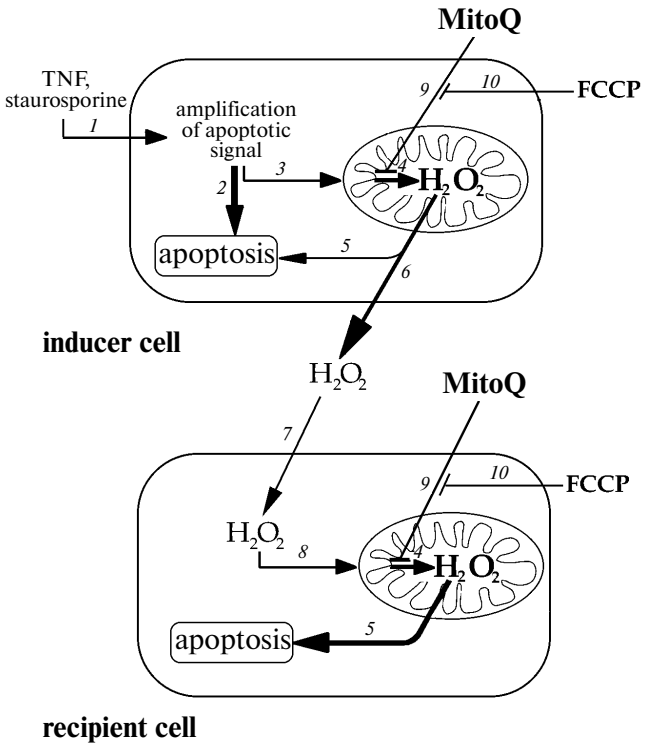


Fig. 6. Uncoupler FCCP abolishes the protective action of MitoQ against H_2O_2-induced apoptosis. MitoQ (20 nM) was added for 7 days; then the cells were washed and cultivated for 24 h without MitoQ (black columns). FCCP (1 μM) was added 1 h before MitoQ (when indicated) and was present for all 7 days of incubation; 50 μM H_2O_2 was added 24 h before measuring apoptosis.

by Zorov et al. [16] in the case of photodynamic treatment of cardiomyocytes. The pore opening could cause severe depletion of mitochondrial antioxidant systems (glutathione-dependent, first of all) and following decrease in general antioxidant potency of the cell. It seems possible also that H_2O_2 stimulated ROS production in Complex I and Complex III of the respiratory chain, as it was shown for isolated mitochondria [17]. The general scheme of H_2O_2-mediated intercellular transmission of the apoptotic signal is presented on scheme.

Intercellular transmission of the signal using H_2O_2 was predicted by one of us (V. P. S.) in 1998 [5] and was supported in 2000 by Reznikov et al. [18] in experiments with monolayer cell cultures. The studied spontaneous apoptosis in culture of osteosarcoma and described non-random distribution of apoptotic cells similar to the clustering described above (Fig. 1). Very low level of apoptosis in these experiments did not allow measuring of H_2O_2 production in the medium and transmission of the signal at significant distance (the size of the clusters in [18] did not exceed 3-5 cells). At the same time, catalase prevented formation of clusters under these conditions, indicating a key role of H_2O_2 in transmission of apoptotic signal. Suggestions on spreading of apoptosis in the absence of direct cell-to-cell contacts and on the role of ROS and NO in the process in the other cellular models were also discussed (see [19] for a review). In particular, it was suggested that transmission of ROS-dependent signal resulted in bystander apoptosis of cells surrounding a cell damaged by ionizing radiation [20, 21]. While our paper was in preparation, an interesting model was described [22] indicating a role of H_2O_2-mediated intercellular transmission of the apoptotic signal in a pathophysiological process. Production of H_2O_2 by myofibroblasts stimulated with TGF-β transforming growth factor caused cell death in lung epithelium placed in the same medium at several millimeters distance. Cell death in epithelium had the same combined features of apoptosis and autophagy as in physiological fibrotic damage of lungs.



Generation, transmission, and perception of H_2O_2-mediated apoptotic signal. It is assumed that induction of apoptosis by TNF or staurosporine in the inducer cell (stages *1-3*) resulted in H_2O_2 generation in the mitochondrial interior (stage *4*). H_2O_2 generated inside the cell stimulates apoptosis (stage *5*) and releases from the cell (stage *6*) to attack the recipient cell (stage *7*). In the recipient cell H_2O_2 penetrates into mitochondria (stage *8*) and stimulates there H_2O_2 generation (analogous to stage *4*) and H_2O_2-dependent apoptosis (analogous to stage *5*). MitoQ inhibits production of H_2O_2 in mitochondria (stage *9*) and FCCP prevents accumulation of MitoQ in mitochondria, inhibiting its action (stage *10*)

Scheme

Special attention should be paid to the possible physiological function of the phenomenon of H_2O_2-mediated long-distance transmission of an apoptotic signal. As originally suggested [5], it can be involved in formation of a barrier of dead cells impermeable for pathogens and toxic products around centers of infection or inflammation. Because expansion of the H_2O_2-mediated apoptosis does not require direct cell-to-cell contacts, it can spread faster and to large areas, leaving behind the infection. This phenomenon is apparently involved in the H_2O_2-mediated "hypersensitive response" to pathogens in plants (see, for example, [23, 24]). Moreover, H_2O_2 seems to mediate "organoptosis" [25], i.e., self-elimination of organs during ontogenesis such as the disappearance of the tail as the tadpole converts into a frog. It was shown [26] that in this classic example of "organoptosis" the process was accompanied by massive H_2O_2-induced apoptosis of the tail cells.

Stimulation of the long-distance cell killing might be of therapeutic importance for treatment of tumors. Chemotherapy or ionizing radiation could induce apoptosis only in a part of the tumor and killing of the rest of the cancer cells will be caused by H_2O_2. This mechanism can kill even those forms of cancer cells which are known to be resistant to apoptosis induced by chemotherapy (due to defects in p53- and Rb-mediated apoptotic pathways, as in the HeLa cell line, for example). Fortunately, the H_2O_2-induced apoptosis is not dependent on these pathways. Intercellular spreading of apoptosis was observed in tumors treated with ionized radiation [26], radioactive drugs [27], genotherapy [28], and the antitumor cytokine TRAIL [29].

On the other hand, H_2O_2-mediated transmission of apoptotic signal may play a negative role during myocardial infarct, stroke, or septic shock. Propagation of a front of apoptosis around a region of tissue damage could be

critical for these pathologies. In these cases, use of MitoQ and other mitochondria-targeted antioxidants seems promising. It was suggested [3] that H_2O_2-mediated spreading of apoptosis is an important event during the program for aging. If so, the mitochondria-targeted antioxidants could become a new generation of geroprotectors.

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