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AMIFOSTINE CAN DIFFERENTIALLY MODULATE DNA DOUBLE-STRAND BREAKS AND APOPTOSIS INDUCED BY IDARUBICIN IN NORMAL AND CANCER CELLS

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We have previously shown that amifostine differentially modulated the DNA-damaging action of idarubicin in normal and cancer cells and that the presence of p53 protein and oncogenic tyrosine kinases might play a role in this diversity. *Aim:* To investigate further this effect we have studied the influence of amifostine on idarubicin-induced DNA double-strand breaks (DSBs) and apoptosis. *Methods:* We employed pulse-field gel electrophoresis () for the detection of DSBs and assessment of their repair in human normal lymphocytes and chronic myelogenous leukaemia K562 cells lacking p53 activity and expressing the BCR/ABL tyrosine kinase. Apoptosis was evaluated by caspase-3 activity assay assisted by the alkaline comet assay and DAPI staining. *Results:* Idarubicin induced DSBs in a dose-independent manner in normal and cancer cells. Both types of the cells did not repair these lesions in 120 min and amifostine differentially modulated their level — decreased it in the lymphocytes and increased in K562 cells. In contrast to control cells, amifostine potentated apoptotic DNA fragmentation, chromatin condensation and the activity of caspase-3 in leukaemia cells. *Conclusion:* Amifostine can differentially modulate DSBs and apoptosis induced by idarubicin in normal and cancer cells. It can protect normal cells against drug-induced DNA damage and it can potentate the action of the drug in leukaemic cells. Further studies on link between amifostine-induced modulation of DSBs and apoptosis of cancer cells will bring a deeper insight into molecular mechanism of amifostine action.

Key Words: **DNA repair, DNA double strand breaks, amifostine, idarubicin, leukaemia, apoptosis.**

Idarubicin (Fig. 1, *a*) is an anthracycline antibiotic used in the treatment of acute leukaemias and other malignancies. Its primary anticancer effect is underlined by the ability to diffuse across the cellular membrane and intercalation between DNA base pairs, targeting topoisomerase II in proliferating cells, resulting in the induction of, usually lethal, DNA double-strand breaks (DSBs) [1–3]. Idarubicin may also generate reactive oxygen species (ROS), which contribute to its side effects in normal cells, especially in tissues, where free radical scavengers are scarce, e. g. heart [4, 5]. Apoptosis induced by the drug may be a consequence of DNA damage. Idarubicin may induce either apoptotic death, like in human leukaemia cells or non-apoptotic death, like in human MCF-7 breast tumour cells [1, 6, 7]. However, the molecular mechanism of idarubicin-induced cell death remains unclear.

Amifostine (Fig. 1, *b*) is a thiol derivative used to protect normal cells in chemo- and radiotherapy of numerous cancer including solid tumours, leukaemias and lymphomas [8]. It undergoes activation by the interaction with alkaline phosphatase, which is scarce in neoplastic cells. Therefore, different uptake of amifostine in normal and cancer cells may contribute to its diversity of action. Recently, we have shown that amifostine decreased the DNA-damaging effect of idarubicin in normal human lymphocytes, but increased the effect in murine growth factor-dependent pro-B lymphoid cell line BaF3 expressing TEL/ABL, an oncogenic fusion tyrosine kinase [9]. Additionally, amifostine did not affect the action of idarubicin in p53-deficient HL-60 cells. Therefore, it seems that the action of amifostine could depend on the expression of proteins, which can be involved in the reaction of the cell to DNA-damaging agents, like p53. We have also shown that oncogenic tyrosine kinases, including TEL/ABL, may stimulate DNA repair [10]. General mechanisms of the cytoprotective action of amifostine include stabilizing and protecting DNA by binding to it, scavenging of free radicals, forming of conjugates with electrophiles and reducing cell death [11–14].

In the present work we tried to take a closer look at the consequence of interaction of amifostine and idarubicin with DNA in normal and cancer cells. Because most chemotherapeutic agents exert cytotoxic effect via direct interaction with DNA, the mechanism of DNA damage and DNA repair should be primarily taken into account in searching for the mode of action of amifostine in cancer cells. Due to the great diversity of genetic changes in leukemias, it is unlikely that only one determines response of DNA to combined action of a chemotherapeutic agent and amifostine. One possible way to search for an insight into this problem is to choose leukemic cells with genetic aberrations connected with phenotypic features, which influence reaction of the cell to DNA damage. We chose normal human peripheral blood lymphocytes and myelogenous leukaemia K562 cells. The latter lack the p53 protein activity [15] and stably express the BCR/ABL oncogenic fusion tyrosine kinase, which can be involved in DSBs repair [10]. Additionally, K562 cells were reported to be resistant to apoptosis [16]. We used pulse-field gel electrophoresis (PFGE) as the most reliable method for detection and repair of DSBs.

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Abbreviations used: DSBs – double-strand breaks; FAR – fraction of activity released; PFGE – pulse-field gel electrophoresis; ROS – reactive oxygen species.

Apoptosis was evaluated by caspase-3 activity assay assisted by the alkaline comet assay to evaluate DNA fragmentation typical for apoptosis as well as DAPI staining of the cells for the detection of morphological changes in chromatin.

MATERIALS AND METHODS

Chemicals. Idarubicin (8-acetyl-10-[(3-amino-2,3,6-trideoxy-alpha-L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-(8S-*cis*)-5,12-naphthacenedion) was obtained from Pharmacia and Upjohn (Milan, Italy). Amifostine (WR2721, S-2-(3-aminopropylamino)-ethylphosphorothioic acid), RPMI 1640 medium, fetal bovine serum (FBS), streptomycin, penicillin, phytohemagglutinin, low-melting point (LMP) and normal-melting point (NMP) agarose, phosphate buffered saline (PBS), DAPI (4',6-diamidino-2-phenyl-indole), ethidium bromide and Bradford reagent were purchased from Sigma (St. Louis, MO, USA). CleanCut agarose, proteinase K reaction buffer, proteinase K, TBE buffer and wash buffer to PFGE assay were obtained from Bio-Rad (Hercules, CA, USA). ApoAlert Caspase Colorimetric Assay Kit was purchased from Clontech Laboratories Inc. (Palo Alto, CA, USA). All other chemicals were of the highest commercial grade available.

Cells. Blood was obtained from young (20–31 years old), male, healthy, non-smoking donors. Peripheral blood lymphocytes were isolated by centrifugation (15 min, 280 × g) in a density gradient of Gradisol L. The lymphocytes were cultured in RPMI 1640 medium with 2 mM L-glutamine supplemented with 20% heat-inactivated fetal bovine serum (FBS), streptomycin (100 μg/ml), penicillin (100 U/ml) and phytohemagglutinin (0.1 mg/ml) in the presence of a 5% CO_2 atmosphere at 37 °C. In the case of the lymphocytes each experiments was performed on the cells obtained from blood of three different donors. Human chronic myelogenous leukemia cells (K562 cells) were obtained from Prof. Jacques Robert of Department of Biochemistry, Institute Bergonie, Bordeaux, France. They were grown in RPMI 1640 medium with 2 mM L-glutamine supplemented with 10% heat-inactivated fetal bovine serum (FBS), streptomycin (100 μg/ml) and penicillin (100 U/ml) in the presence of a 5% CO_2 atmosphere at 37 °C.

Pulse-field gel electrophoresis (PFGE). The cells were pre-incubated with amifostine at 14 mM for 15 min and then exposed for 1 h to idarubicin at concentrations from the range 0.05–50 μM at 37 °C in RPMI 1640 medium without serum and antibiotic supplementation. An aliquot of 5 × 10^6 cells/ml after treatment was washed by centrifugation (1000 × g, 5 min, 4 °C) and mixed with 2% CleanCut agarose to a final concentration of 0.75% at 50 °C. The cells/agarose mixture was transferred to plugs moulds. After solidifying agarose plugs were lysed with proteinase K in a proteinase K reaction buffer and were incubated overnight at 50 °C. After lysis plugs were washed four times in 1 × wash buffer and stored at 4 °C in this buffer. Washed plugs were inserted into the wells of a 0.8% agarose gel prepared in 0.5 × TBE buffer. The gel was then placed horizontally in the gel box of the contour-clamped homogeneous electric field gel electrophoresis apparatus CHEF II (Bio-Rad) with 120° angle between fields. The voltage was 4 V/cm and the time-pulse gradient was 1–500 s for 60 h and 1500–1600 s for 31 h at 14 °C. After electrophoresis the gel was stained with 1 μg/ml ethidium bromide and examined under UV light. DNA concentration in samples before and after electrophoresis was determined using an Agilent spectrophotometer, model 8453 Hewlett Packard (Waldbronn, Germany). DNA double-strand breaks were measured as the fraction of activity released (FAR; ratio of DNA released in an electrophoretic band and total DNA) [17].

To examine DSBs repair using PFGE assay, the cells after treatment with idarubicin at 0.5 μM for 1 h, as well as control cells, were washed and resuspended in a fresh RPMI 1640 medium with 14 mM amifostine. Aliquots of the cell suspension were taken immediately and 30, 60 and 120 min later. The samples were then processed as described above.

Alkaline comet assay. The alkaline version of the comet assay was performed essentially according to the procedure of Singh et al [18] as described previously [9]. The cells were pre-incubated with amifostine at 14 mM for 15 min and then incubated for 12 h with idarubicin at concentrations from the range 0.05–50 μM at 37 °C in RPMI 1640 medium with serum and antibiotics supplementation. A freshly prepared suspension of the cells (1–3 × 10^5 cells/ml) in 0.75% LMP agarose dissolved in PBS was spread onto microscope slides pre-coated with 0.5% normal agarose. The cells were then lysed for 1 h at 4 °C in a buffer consisting of 2.5 M NaCl, 100 mM EDTA, 1% Triton X-100, 10 mM Tris, pH 10. After the lysis, the slides were placed in an electrophoresis unit, DNA was allowed to unwind for 20 min in the solution containing 300 mM NaOH and 1 mM EDTA, pH > 13. Electrophoresis was conducted in the electrophoretic solution containing 30 mM NaOH and 1 mM EDTA, pH > 13 at ambient temperature of 4 °C for 20 min at electric field strength 0.73 V/cm (28 mA). The slides were then neutralized with 0.4 M Tris, pH 7.5, stained with 2 μg/ml DAPI and covered with cover slips.

The slides were observed at 200 × magnification in a Eclipse fluorescence microscope (Nikon, Tokyo, Japan) attached to a COHU 4910 video camera (Cohu, San Diego, CA, USA) equipped with an UV filter block consisting of an excitation filter (359 nm) and a barrier filter (461 nm) and connected to a personal computer-based image analysis system Lucia-Comet v. 4.51 (Laboratory Imaging, Praha, Czech Republic). Fifty images were randomly selected from each sample and the percentage of DNA in the comet tail was measured. Two parallel tests with aliquots of the same sample of cells were performed for a total of 100 cells and the mean percentage of DNA in the comet tail was calculated. The percentage of DNA in the comet tail is posi-

tively correlated with the level of DNA breakage in a cell and its mean value from all samples in each experiment was taken as an index of DNA fragmentation.

DAPI nuclear staining assay. The cells were pre-incubated with amifostine at 14 mM for 15 min and then incubated for 12 h to idarubicin at concentrations from the range 0.05–50 μM at 37 °C in RPMI 1640 medium with serum and antibiotics supplementation. After treatment the cells were washed with PBS and mixed with DAPI at a final concentration of 50 ng/ml. After 30 min of staining, the cell samples were placed on glass slides and covered with cover slips. Apoptotic chromatin condensation was observed under the Eclipse fluorescence microscope. The changes in a chromatin condensation were evaluated qualitatively in 100 cells. The experiment was prepared one time.

Caspase-3 activity. The cells were pre-incubated with amifostine at 14 mM for 15 min and then incubated with idarubicin at concentrations from the range 0.5–20 μM for 48 h at 37 °C in RPMI 1640 medium with serum and antibiotics supplementation. An aliquot of cell suspension (2×10^6/ml) after treatment was washed with PBS and centrifuged (400 × *g*, 10 min, 4 °C). The cells were then lysed in 50 μl lysis buffer (ApoAlert Caspase Colorimetric Assay Kit). Undissolved proteins and nuclei were removed by centrifugation at 1000 × *g* for 3 min at 4 °C. 50 μl of aliquots of 2 × reaction buffer/DTT mix and 5 μl of 1 mM caspase-3 substrate (DEVD-pNA) were added to supernatants to a final concentration of 50 μM and final mixtures were incubated at 37 °C for 60 min. The samples were read at 405 nm with calibration against a standard linear regression curve of *p*-nitroaniline (pNa). Caspase activity was defined as nmol pNA released per hour per mg of protein (nmol/h/mg protein). The protein concentration was determined using Bradford reagent.

Data analysis. The data were analysed using Statistica package (StatSoft, Tulsa, OK, USA). If no significant differences between variations were found, as assessed by Snedecor-Fisher test, the differences between means were evaluated by applying the Student's *t*-test. Otherwise, the Cochran-Cox test was used.

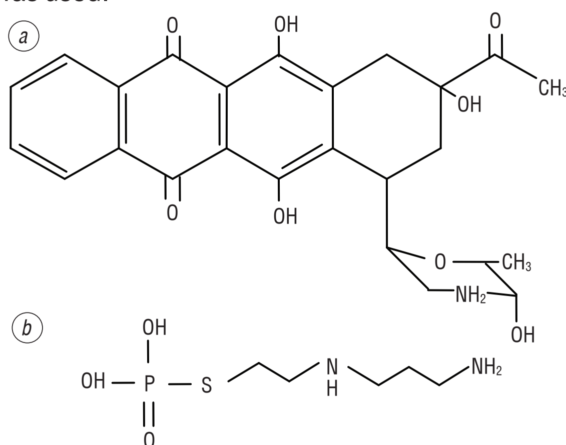

Fig. 1. Chemical structure of idarubicin (*a*) and amifostine (*b*)

RESULTS

Effect of amifostine on idarubicin-induced DSBs and their repair

Fig. 2 shows DNA damage measured by PFGE in lymphocytes (*a*) and K562 cells (*b*) pre-treated with amifostine at 14 mM for 15 min and then exposed for 1 h to idarubicin in comparison with the cells treated with idarubicin singly. Idarubicin induced DSBs in a dose-independent manner. In the case of lymphocytes we observed the highest level of DSBs after incubation with idarubicin at 0.5 μM (Fig. 2, *a*, lane 2), in K562 cells the maximum was reached after incubation with idarubicin at 5 μM (Fig. 2, *b*, lane 3). Amifostine exerted different effect on this type of DNA damage in idarubicin-treated normal and cancer cells: it decreased the level of DNA damage in lymphocytes and it increased the extent of DNA damage in K562 cells. We also calculated the values of FAR for the control cells and the cells pre-incubated with amifostine, which confirmed our observations (Fig. 3).

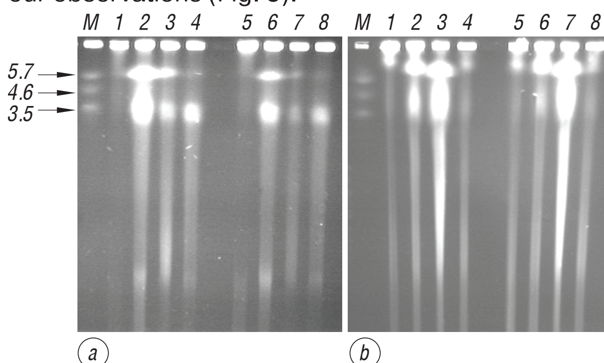

Fig. 2. PFGE assay of DNA double strand breaks in lymphocytes (*a*) and K562 cells (*b*) after treatment with amifostine and idarubicin. M — *S. pombe* molecular weight marker (Mbp); 1 — control; 2–4 — idarubicin at 0.5, 5 and 50 μM, respectively; 5 — amifostine at 14 mM; 6–8 — 14 mM amifostine with idarubicin at 0.5, 5 and 50 μM, respectively

Fig. 4 presents DNA damage in lymphocytes (*a*) and K562 cells (*b*) immediately after exposure to idarubicin at 0.5 μM as well as 30, 60 and 120 min thereafter. Post-incubation was performed in the presence of amifostine at 14 mM. In the case of the lymphocytes, the level of DNA damage in the control cells (Fig. 4, *a*, lanes 1–4) and in the cells post-incubated in the presence of amifostine (Fig. 4, *a*, lanes 5–7) was very low. Lymphocytes exposed to idarubicin at 0.5 μM did not repair DSBs during a 120-min post-treatment incubation (Fig. 4, *a*, lanes 8–11). However, in the presence of 14 mM amifostine we observed a significant decrease ($P < 0.001$) of DNA damage during repair incubation in lymphocytes (Fig. 4, *a*, lanes 12–14). In the case of control K562 cells (Fig. 4, *b*, lanes 1–4) and the cells post-incubated with amifostine (Fig. 4, *b*, lanes 5–7), we detected a high level of DNA fragmentation. Therefore, the preparation of these cells must have introduced damage to their DNA. Additionally, we calculated the values of FAR for the control cells and the cells post-incubated with amifostine, which confirmed our observations (Fig. 5). Similarly to lymphocytes, K562 cells exposed to idarubicin at 0.5 μM did not re-

pair DSBs during a 120-min post-treatment incubation (Fig. 4, *b*, lanes 8–11). In the presence of amifostine at 14 mM, we observed an increase in the level of DNA fragmentation for 60 and 120 min of repair incubation (Fig. 4, *b*, lanes 13–14) (less DNA in samples incubated during repair with amifostine for 60 and 120 than in samples incubated without amifostine).

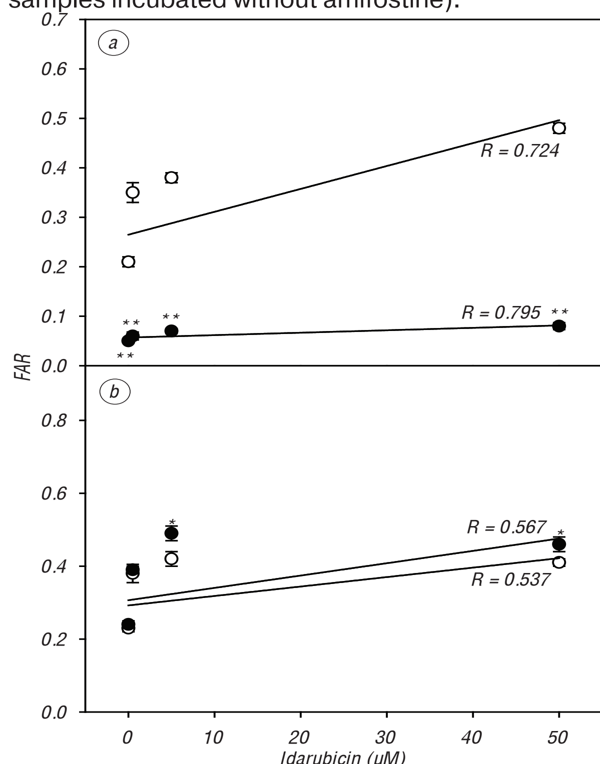

Fig. 3. DNA damage measured as fraction of activity released (FAR) in PFGE assay in lymphocytes *(a)* and K562 cells *(b)* exposed at 37 °C to idarubicin in the absence (O) or in the presence (●) of 14 mM amifostine. Regression lines were calculated by the means of the least square methods. Figures next to each curve indicate the regression coefficiant (R) for that curve. Error bars denote SD. Data presented are mean results from two independent experiments. *$P < 0.05$, **$P < 0.001$.

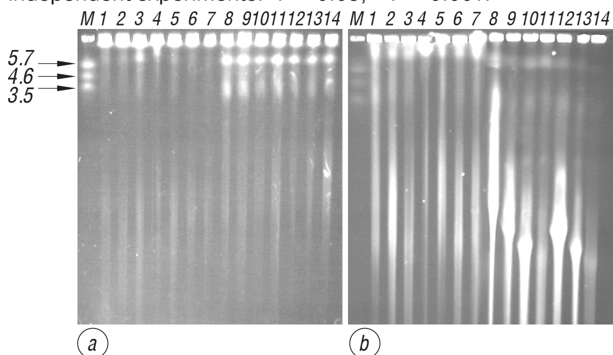

Fig. 4. PFGE assay of DNA double strand breaks in lymphocytes (*a*) and K562 cells (*b*) after various time of repair incubation of idarubicin-induced DNA damage in the presence of 14 mM amifostine. M — *S. pombe* molecular weight marker (Mbp); 1–4 — control at 0, 30, 60 and 120 min, respectively; 5–7 — 14 mM amifostine at 30, 60 and 120 min, respectively; 8–11 — 0.5 μM idarubicin at 0, 30, 60 and 120 min, respectively; 12–14 — 14 mM amifostine and 0.5 μM idarubicin at 30, 60 and 120 min, respectively

Effect of amifostine on apoptotic DNA fragmentation and chromatin condensation induced by idarubicin. The comet assay can be applied to distinguish apoptotic from non-apoptotic cells on the basis of their characteristic image and DNA fragmentation pattern [19]. Apoptotic cells form structures with fan-like tails and small heads, as normal or less damaged cells form smaller structures with large heads and narrow tails. Idarubicin induced apoptotic DNA fragmentation and chromatin condensation in both types of the cells (Fig. 6). At the maximal used concentration of idarubicin, 50 μM, we observed majority of such changes in K562 cells (Fig. 6, *b*). The changes observed were less pronounced in the lymphocytes exposed to idarubicin, especially at 0.5 and 5 μM, in the presence of amifostine in comparison with the cells incubated with idarubicin only (Fig. 6, *a*). In K562 cells we observed an increase of the cell number with chromatin condensation and apoptotic DNA fragmentation after co-treatment with idarubicin and amifostine (Fig. 6, *b*).

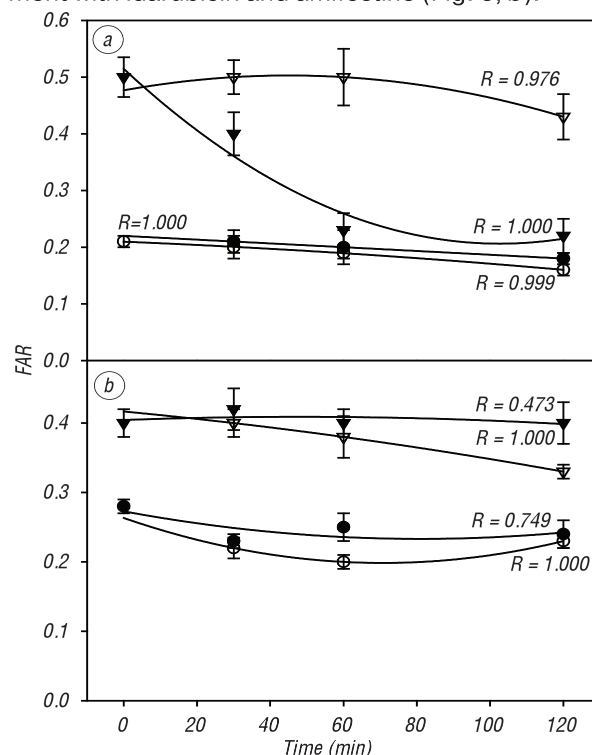

Fig. 5. Time course of the repair of DNA damage in human lymphocytes (*a*) and K562 cells (*b*) treated with idarubicin at 0.5 μM in the absence (▽) or in the presence (▼) of 14 mM amifostine compared with untreated control (O) and amifostine alone (●) and evaluated by PFGE assay. Regression lines were calculated by the means of the least square methods. Figures next to each curve indicate the regression coefficiant (R) for that curve. Error bars denote SD. Data presented are mean results from two independent experiments

Effect of amifostine on idarubicin-induced activity of caspase-3. The activation of caspase-3 was determined by the cleavage of specific substrate (DEVD-pNA) and the product of the cleavage was measured by a colorimetric assay. After 48-h incubation idarubicin increased the rate of the activation of caspase-3 in the lymphocytes and K562 cells (Fig. 7). Amifostine at 14 mM decreased this rate in normal cells in the case of idarubicin at 5 μM and 50 μM ($P < 0.05$ and $P < 0.01$, respectively, Fig. 7, *a*) and increased it in cancer cells after incubation with the drug at 0.5 μM and 5 μM ($P < 0.01$ and $P < 0.05$, respectively, Fig. 7, *a*).

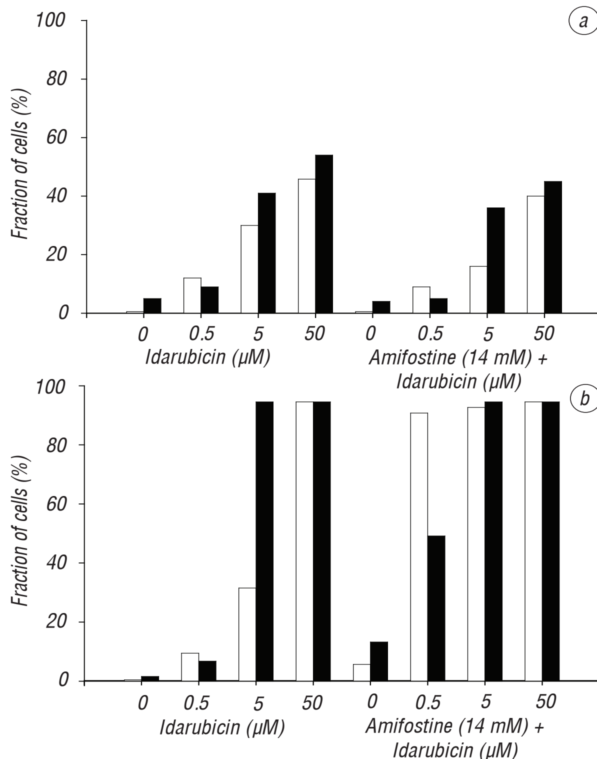

Fig. 6. DNA fragmentation (white bars) and apoptotic morphological changes in chromatin (black bars) in lymphocytes (*a*) and K562 cells (*b*) exposed to idarubicin without or with amifostine at 14 mM. Apoptotic changes in chromatin were evaluated qualitatively into 100 cells with a fluorescent microscope after staining with DAPI. DNA fragmentation was evaluated into 100 cells by the alkaline comet assay

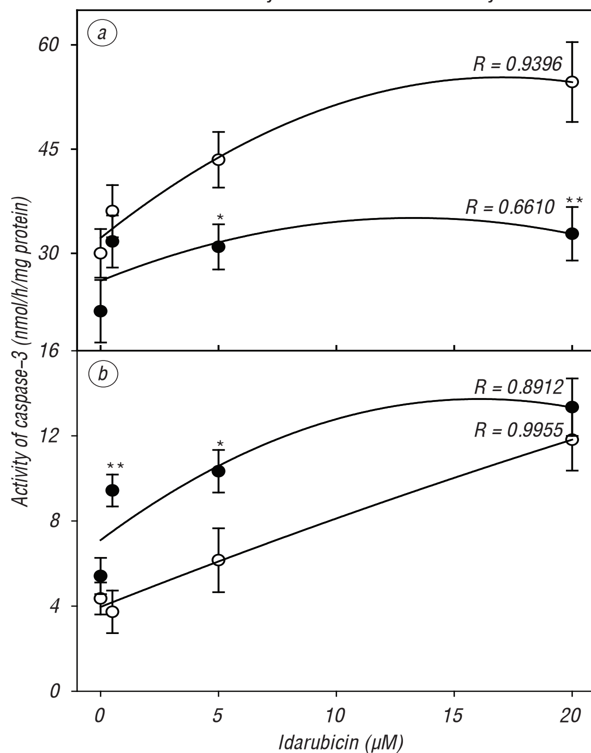

Fig. 7. Effect of amifostine on idarubicin-induced activity of caspase-3 in the lymphocytes (*a*) and K562 cells (*b*). The cells were incubated with idarubicin in the absence ( ) or in the presence of amifostine at 48 h ( ). Activity of caspase-3 was determined by ApoAlert Caspase Colorimetric Assay as described in the text. Regression lines were calculated by the means of the least square methods. Figures next to each curve indicate the regression coefficiant (R) for that curve. Error bars denote SD. Data presented are mean results from three independent experiments. *$P < 0.05$, **$P < 0.01$.

DISCUSSION

The primary mode of action of idarubicin underlying its anti-tumour activity is interaction with topoisomerase II leading to continuous production of DSBs in proliferating cells. Metabolism of idarubicin in the cell can also lead to the production of reactive oxygen species (ROS), which can damage DNA [4, 5]. The accumulation of unrepaired DNA damage may induce the process of apoptotic cell death. It is unclear whether the generation of ROS is actually associated with the apoptotic process induced by idarubicin. We observed that idarubicin induced DSBs in both types of cells, normal lymphocytes and leukaemia K562 cells (Fig. 2). Because we used non-proliferating lymphocytes, we presume that DSBs, which we observed by PFGE assay, resulted from the action of ROS rather than of topoisomerase II. DNA damage observed at low concentration of idarubicin in lymphocytes could be related to the action of ROS. Similarly, the clear action of amifostine in lymphocytes in contrast to K562 cells (Fig. 2) might indicate on ROS-mediated mechanism of DNA damaging. These DNA lesions were not repaired in either kind of cells (Fig. 3). Amifostine modulated the DNA-damaging potential of idarubicin. In lymphocytes pre-incubated with amifostine we observed decrease in the level of DSBs (Fig. 2, *a*, lanes 6–8). It was shown that amifostine might decrease the DNA-damaging potential of idarubicin through formation of conjugates with it and scavenging ROS induced by idarubicin [5, 13, 20]. Amifostine may also decrease the amount of this type of DNA damage, arising from inactivation of topoisomerase II, in proliferating cells [2]. In contrast with normal cells, amifostine caused increase in the level of DSBs in K562 cells (Fig. 2, *b*, lanes 6–8). We speculate that this effect, in part, could result from different uptake of amifostine in normal and cancer cells [8].

We showed that amifostine could modulate the extent of DNA fragmentation induced by idarubicin in K562 cells (Fig. 4, *b*, lanes 12–14). It is possible by its interaction with DNA repair proteins [21, 22].

High molecular weight DNA fragments resulting from DSBs are characteristic to the early stage of apoptosis. We observed that idarubicin apart from induction such DNA fragmentation, caused morphological changes in chromatin (Fig. 6) and increased activity of caspase-3 (Fig. 7) in normal and cancer cells. These changes are associated with apoptosis [19, 23–25]. Idarubicin and other anthracyclines can activate caspases in the response to DNA damage or through oxidative stress and mitochondrial membrane potential collapse [26, 27]. However, it was shown that the generation of ROS is not involved in idarubicin-induced apoptosis in human leukaemia cells [28]. Moreover, recent data have shown that caspase-3/7 may be dispensable for idarubicin-induced internucleosomal DNA cleavage during apoptosis in human leukaemia cells [29]. Our results indicate that the level of idarubicin-induced DSBs correlate with apoptotic DNA fragmentation, chromatin condensation as well as activation of caspase-3. These effects were esspecially pronounced in K562 cells, where more cells with apop-

totic DNA fragmentation and chromatin condensation in comparison to normal cells were observed (Fig. 6). Interestingly, amifostine reduced DNA fragmentation and chromatin morphological changes (Fig. 6, *a*) and decreased caspase-3 activity (Fig. 7, *a*) in lymphocytes. Similar effects were also reported previously [30, 31]. The activation of caspase-3 can course via H_2O_2-mediated mechanism in cells incubated with amifostine.

In K562 cells, amifostine in combination with idarubicin increased not only the level of DSBs, but also cellular changes typical for apoptosis [32]. However it was also observed that the continuous exposure of a human myelodysplastic cell line to amifostine was cytotoxic and associated with an induction of apoptosis independent of alterations in p53 expression [33]. In our previously studies we showed that amifostine increased the viability of murine growth factor-dependent pro-B lymphoid cells BaF3 and their BCR/ABL-transformed counterparts [34].

The results obtained suggest that amifostine can differentially modulate DSBs and apoptosis induced by idarubicin in normal and cancer cells. It can protect normal cells against drug-induced such DNA damage and it can potentate the action of the drug in leukaemic cells. Taking into account the genetic constitution of the cells, which we used in these studies, we can speculate that different response to amifostine might follow from the interaction between amifostine and BCR/ABL protein and interaction between caspase-3 and BCR/ABL protein.

Further studies on link between amifostine-induced modulation of DSBs and apoptosis of cancer cells will bring a deeper insight into molecular mechanism of amifostine action.

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АМИФОСТИН МОЖЕТ ДИФФЕРЕНЦИАЛЬНО МОДУЛИРОВАТЬ ДВУХНИТЕВЫЕ РАЗРЫВЫ ДНК И АПОПТОЗ, ВЫЗВАННЫЙ ИДАРУБИЦИНОМ В НОРМАЛЬНЫХ И ОПУХОЛЕВЫХ КЛЕТКАХ

Ранее нами было показано, что амифостин дифференциально модулирует ДНК-повреждающее действие идарубицина в нормальных и злокачественных клетках, и что наличие белка p53 и онкогенных тирозин киназ может иметь значение для этих различий. *Цель:* изучить влияние амифостина на идарубицин-опосредованные двухнитевые разрывы ДНК (DSBs) и апоптоз. *Методы:* мы применили гель-электрофорез в пульсирующем поле (PFGE) для выявления DSBs и изучения их репарации в нормальных лимфоцитах человека и клетках K562 хронической миелоидной лейкемии, у которых p53 неактивен и экспрессирована BCR/ABL-тирозин киназа. Апоптоз оценивали с помощью реактивов для выявления активности каспазы-3, проведения щелочного гель-электрофореза одиночных клеток и DAPI-окрашивания. *Результаты:* идарубицин вызывает образование DSBs в нормальных и злокачественных клетках независимо от дозы. Оба типа клеток не репарировали эти повреждения за 120 мин, при этом амифостин дифференциально молулировал уровень DSBs — уменьшал в лимфоцитах и увеличивал в K562-клетках. В отличие от контрольных клеток амифостин потенциировал апоптическую фрагментацию ДНК, конденсацию хроматина и активность каспазы-3 в лейкемических клетках. *Выводы:* амифостин может дифференциально модулировать DSBs и апоптоз, вызванные идарубицином в нормальных и злокачественных клетках. Он может защитить нормальные клетки от повреждения ДНК, вызванного химиопрепаратом, и в то же время потенциировать действие препарата на лейкемические клетки. Дальнейшие исследования связи между вызванной амифостином модуляцией DSBs и апоптоза опухолевых клеток позволит лучше понять молекулярные механизмы действия амифостина.

Ключевые слова: **ДНК репарация, двухнитевые разрывы ДНК, амифостин, идарубицин, лейкемия, апоптоз.**

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