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Communication

Hyperthermia can differentially modulate the repair of doxorubicin-damaged DNA in normal and cancer cells★✪

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Hyperthermia can modulate the action of many anticancer drugs, and DNA repair processes are temperature-dependent, but the character of this dependence in cancer and normal cells is largely unknown. This subject seems to be worth studying, because hyperthermia can assist cancer therapy. A 1-h incubation at 37°C of normal human peripheral blood lymphocytes and human myelogenous leukemia cell line K562 with 0.5 μM doxorubicin gave significant level of DNA damage as assessed by the alkaline comet assay. The cells were then incubated in doxorubicin-free repair medium at 37°C or 41°C. The lymphocytes incubated at 37°C needed about 60 min to remove completely the damage to their DNA, whereas at 41°C the time required for complete repair was shortened to 30 min. There was also a difference between the repair kinetics at 37°C and 41°C in cancer cells. Moreover, the kinetics were different in doxorubicin-sensitive and resistant cells. Therefore, hyperthermia may significantly affect the kinetics of DNA repair in drug-treated cells, but the magnitude of the effect may be different in normal and cancer cells. These features may be exploited in cancer chemotherapy to increase the effectiveness of the treatment and reduce unwanted effects of anticancer drugs in normal cells and fight DNA repair-based drug resistance of cancer cells.

Heat treatment of tumors, either alone or in combination with anticancer drugs and radiation, is a widely accepted procedure in fighting cancer. Therefore, the two main obstacles

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of cancer therapy, unwanted side effects and resistance of cancer cells to drugs and radiation, should be taken into account in using heat as a therapeutic factor. Ideally, heat should potentiate the DNA-damaging effect of DNA-targeted anticancer drugs in cancer cells and reduce it in normal cells. Moreover, cancer cells showing resistance to these drugs should become more sensitive after heat treatment.

The rate of the repair of DNA damage evoked by anticancer drugs may indicate the effectiveness of these drugs in cancer cells and determine the unwanted effect in normal cells. On the other hand, increased efficiency of DNA repair may significantly reduce the sensitivity of cancer cells to therapeutic drugs and radiation and we have recently shown that it may be stimulated by oncogenic fusion tyrosine kinases, such as BCR/ABL, which is a hallmark of some leukemias (Slupianek *et al.,* 2002; Majsterek *et al.,* 2002). Because DNA repair can depend on temperature, heat can differentially modulate sensitivity to DNA-damaging drugs of normal and cancer cells, including those displaying drug-resistance.

In the present work we investigated the kinetics of the repair of DNA damage induced by doxorubicin in normal human lymphocytes and in human myelogenous leukemia K562 cells in normal (37°C) and hyperthermic (41°C) conditions. K562 cells express BCR/ABL fusion tyrosine kinase (Lozzio & Lozzio, 1975) which can be involved in modulation of drug resistance by stimulation of DNA repair we used two variants of these cells: doxorubicin-sensitive and resistant ones.

MATERIALS AND METHODS

Lymphocytes were isolated from human peripheral blood by centrifugation in a sucrose density gradient. The human myelogenous leukemia cell line K562 and its doxorubicin-resistant variant, K562doxS and K562doxR, respectively, were obtained from Dr. Jacques Robert of the Institute Bergonie (Boredaux, France). Doxorubicin (Sigma Chemicals, St. Louis, MO, U.S.A) was added to a suspension of the cells in RPMI 1640 medium to give a final concentration of 0.5 μM. The cells were incubated with the drug for 1 h at 37°C. Each experiment included a positive control, which was hydrogen peroxide at 10 μM applied for 10 min at 4°C. To examine DNA repair, the cells after the treatment with doxorubicin, as well as control samples, were washed and resuspended in fresh, drug-free RPMI 1640 medium preheated either to 37°C or 41°C. Aliquots of the suspension were taken immediately and 15, 30, 60 and 120 min later. Comet assay was performed under alkaline conditions essentially according to the procedure of Singh *et al.* (1988) with some modifications (Klaude *et al.,* 1996) as described previously (Blasiak & Kowalik, 2000). The slides were observed at 200 × magnification in an Eclipse fluorescence microscope (Nikon, Tokyo, Japan) attached to a COHU 4910 video camera (Cohu Inc., San Diego, CA, U.S.A.) equipped with a UV-1A filter block and connected to the 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 tail DNA (%) was measured. Two parallel tests with aliquots of the same sample of the cells were performed for a total of 50 cells and the mean tail DNA was calculated. The amount of the tail DNA is positively correlated with the level of DNA breakage in a cell (Singh *et al.*, 1988). The mean value of the tail DNA in a particular sample was taken as an index of DNA damage in the sample. All the values in this study were expressed as means ± S.E.M. from two separate experiments, i.e. data from two experiments were pooled and the statistical parameters were calculated. The differences between variations were calculated by Fisher-Snedecor test. If no significant differences were found, the differences between the

means were evaluated by applying the Student's *t*-test. Otherwise, the Corchan-Cox test was used.

RESULTS AND DISCUSSION

Doxorubicin at 0.5 μM evoked DNA damage measured as a significant increase in the percentage of DNA in the comet tail in all kinds of cells (Figs. 1–3). Normal lymphocytes repaired damage to their DNA during a 120-min period at 37°C (Fig. 1A), but the level of DNA damage in K562doxS (Fig. 2A) and K562doxR cells (Fig. 3A) increased with time. All kinds of cells were able to repair the damage induced by 10 μM hydrogen peroxide (positive control) at the normal and elevated temperatures. A relatively large difference between the initial values of the level of DNA damage for lymphocytes and K562 cells might follow from the fact, that the former were isolated from whole blood and the latter were derived from the cell culture. The procedure of isolation always gives rise to additional DNA damage; the level of damage may depend also on the endogenous DNA damage of the blood donors.

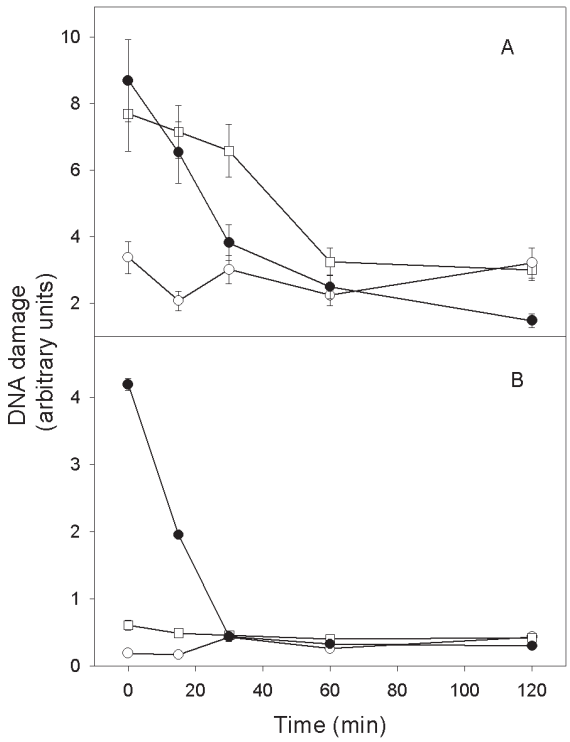

Figure 1. Time course of the repair of DNA damage in human lymphocytes exposed for 1 h at 37°C to 0.5 μM doxorubicin (●) and incubated in a drug-free medium at 37°C (A) or 41°C (B) as compared with negative (○) and positive control, which was hydrogen peroxide at 10 μM (❑).

The number of cells scored in each treatment was 100. The figure shows mean results from three independent experiments. Error bars denote S.E.M.; in some cases they were smaller than the symbol radius.

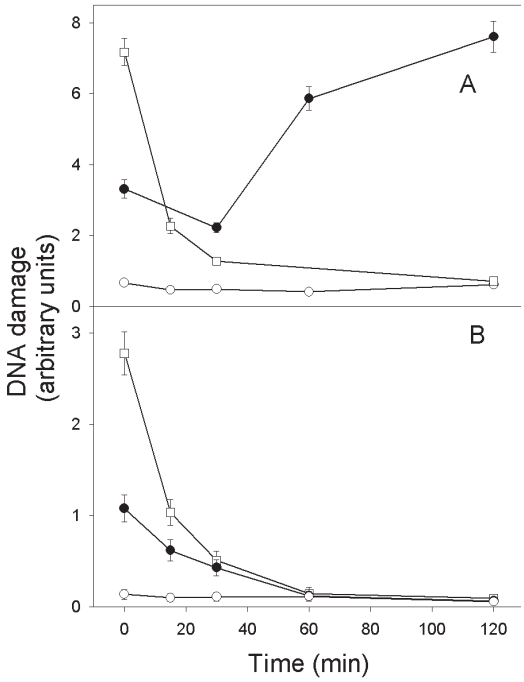

Figure 2. Time course of the repair of DNA damage in human myelogenous leukemia K562 cells sensitive to doxorubicin.

See the legend to Fig. 1 for explanation of symbols and conditions of measurement.

We do not know why we observed an accumulation of DNA damage in the course of repair incubation at 37°C in K562 cells (Figs. 2A and 3A). It can probably be linked with genomic instability in these cells associated with their neoplasmic transformation; the action of 0.5 μM doxorubicin might have further affected this instability by disturbing DNA repair processes. The impaired repair could lead

to the inability to remove DNA damage generated by doxorubicin and/or its metabolites still present in the cells.

Hyperthermia brought about an ability of K562doxS and K562doxR cells to repair DNA damage in a period shorter than 60 min. The lymphocytes needed less than 30 min to recover at 41°C.

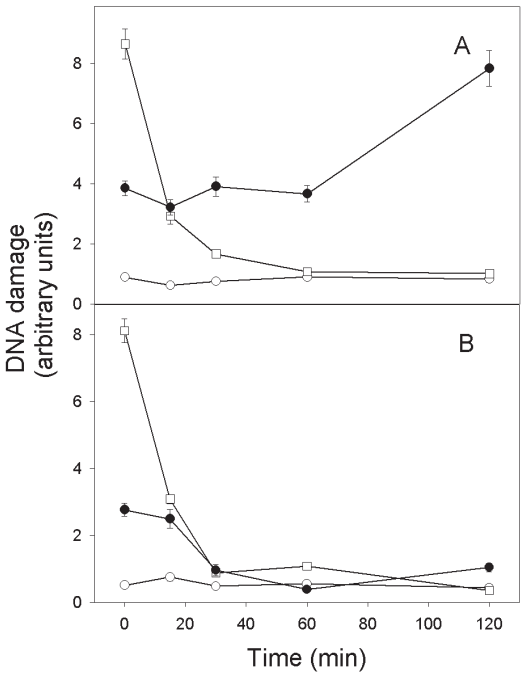

Figure 3. Time course of the repair of DNA damage in human myelogenous leukemia K562 cells resistant to doxorubicin.

See the legend to Fig. 1 for explanation of symbols and conditions of measurement.

To assess quantitatively the effect of hyperthermia on DNA repair we compared two parameters: the time needed for removing half of the DNA damage and the time required for total recovery. The results (Table 1) indicated that doxorubicin-resistant K562 needed almost twice as long to remove half of the damage to their DNA at 41°C than their doxorubicin-sensitive counterparts and normal lymphocytes. However, the resistant cells completed the repair in 30 min, whereas the sensitive ones — in 60 min. Therefore, the kinetics of the repair of DNA damaged by doxorubicin were different in the wild-type and resistant cells. Moreover, the difference concerned also a kinetic components of the repair. The kinetics of enzymatic repair of DNA single and double strand breaks generally show a fast component with time constants of the order of minutes as well as a slow component of the order of one hour (Woods, 1981). The DNA-damaging effect of doxorubicin, like that of other anthracyclines, originates from its ability to diffuse across the cell membrane

Table 1. Time needed at 41°C to decrease the level of DNA damage evoked by 1 h exposure at 37°C to 0.5 μM doxorubicin by half (1/2 repair) or to the unexposed control level (complete repair) in human lymphocytes, human myelogenous leukemia K562 cells sensitive to doxorubin (K562doxS) or resistant to doxorubicin (K562doxR)

Cells	Time (min) needed for	
	1/2 repair	complete repair
Lymphocytes	13	30
K562doxS	11	60
K562doxR	23	30

and to intercalate between DNA base pairs and target topoisomerase II, resulting in DNA double strand breaks, but we have also shown that free radicals produced by anthracyclines induce DNA single strand breaks, alkali labile sites and oxidized DNA bases (Blasiak *et al.*, 2002). Consequently, we can consider two components of the repair process in our experiment: a slow and a fast one and we can conclude that hyperthermia may primarily affect the fast component in doxorubicin-sensitive cells and the slow component in the resistant cells. Because increased efficacy of DNA repair may contribute to the cellular resistance to drugs, hyperthermia could be considered to fight the resistance. Altogether, our results suggest that increased temperature can diversely modulate the action of anticancer drugs (1) in normal and cancer cells and (2) in drug sensitive and resistant cells and so it can be considered to modulate cancer therapy based on DNA-damaging drugs.

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