Effect of cytosine arabinoside and hydroxyurea on micronucleus formation induced by model clastogens in Chinese hamster V79 cells*

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Post-cultivation of treated cells in the presence of DNA repair inhibitors has been proposed as a new methodological approach of the micronucleus (MN) assay to increase the sensitivity of this technique. In order to assess the advantages and limitations of this promising methodological approach, several genotoxic/clastogenic agents with different mechanisms of activity were chosen to assess the effect of DNA repair inhibitors on the level of micronuclei (MNi) induced by particular agent using Chinese hamster V79 cells. Both UV light (UV) and benzo(a)pyrene (BaP) increased significantly the micronucleus level in V79 cells ($p<0.01–0.001$). In contrast, only at cytotoxic concentration (>0.8 mM) a slight but statistically significant rise of MNi was determined in cells exposed to *N*-methyl-*N*-nitroso urea (MNU). However, post-cultivation of MNU-treated cells in the presence of DNA repair inhibitors (cytosine arabinoside, AraC and hydroxyurea, HU) led to an additional rise of MNi. While AraC had a synergistic effect on MN formation (0.4 mM and 0.8 mM, DS=2.14 and 1.13, respectively), HU had less than additive effect (DS=0.86 and 0.66) and the combined treatment of cells with AraC and HU was least effective (Cf=0.36 and 0.28). On the other hand, post-cultivation of UV- and BaP-treated cells in the presence of AraC did not result in any synergistic effect on MN formation. No effect or even a decrease of MNi was measured particularly due to HU or combined treatment of HU and AraC. Incubation of control untreated cells with AraC gave rise to a significant increase of MN formation (2- to 2.5-fold) as well. Hydroxyurea or the combined treatment of HU with AraC had lower effect on the spontaneous level of MNi. Our study shows, that the combination of MNU treatment with DNA repair inhibitors increased the number of MNi on well proliferating V79 cells; in case UV light and BaP treatment, the involvement of DNA repair inhibitors did not contribute to an increase of sensitivity of MN assay. On the basis of our results we suppose that the AraC/CBMN assay might be a promising approach in genetic toxicology applied only to lymphocytes.

Key words: micronucleus assay, cytosine arabinoside, hydroxyurea, N-methyl-N-nitroso urea, UV-light, Chinese hamster V79 cells

Over the past 30 years the micronucleus technique has undergone an extensive progress and at present, it belongs to one of the most commonly used short-term tests for assessing chromosome breakage and mitotic spindle dysfunction *in vitro* and *in vivo* [6]. MN assay is frequently used in human biomonitoring studies to measure the impact of environmental, occupational or lifestyle factors on human health; in the screening of genotoxic potential of new chemicals produced by the agrochemical and pharmaceutical industries and for special purposes such as prediction of the inter-individual variation in radiosensitivity to optimize the radiotherapy in clinical oncology. On the basis of a high concordance of results (up to 90%) between the *in vitro* micronucleus assay and the *in vitro* chromosome aberration technique [29, 30], the MN assay has been proposed as a faster, simpler and less expensive alternative to *in vitro* metaphase analysis [28, 31].

Owing to various modifications in the methodology, MN assay can provide multiple and complementary measures of genotoxicity and cytotoxicity within one system including chromosome breakage, chromosome loss, chromosome non-disjunction, chromosome rearrangement (nucleoplasmic bridges), cell division inhibition, necrosis, apoptosis, and HPRT variant detection [5, 7, 8, 23]. The involvement of several immunofluorescent and molecular approaches

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including the use of antikinetochore antibodies, PRINS (primed *in situ* DNA synthesis), SKY (spectral karyotyping) and FISH (fluorescent *in situ* hybridization) technology allows to characterize in more details the origin and chromosomal contents of MNi [1, 2, 4, 26, 38]. Even an automation of MNi scoring has been already introduced to speed up the MNi evaluation [13]. It is, however, necessary to realize that MN assay is unable to provide a measure of a subtle chromosome changes such as balanced translocation and therefore cannot replace the detailed analysis of metaphase chromosomes.

The use of cytochalasine B, a microfilament-assembly inhibitor proposed by FENECH and MORLEY [10], increased the accuracy of the MN assay. The cytokinesis block makes the technique more precise by restricting scoring to cells deriving from a single mitotic division; therefore, the data obtained are not confounded by altered cell division kinetics caused by cytotoxicity of agents tested or sub-optimal cell culture conditions. At present, the cytokinesis-block micronucleus assay (CBMN) is an obligatory procedure for biomonitoring purposes applied to peripheral blood lymphocytes [9, 24]. The addition of cytochalasine B to well proliferating cell lines cultivated *in vitro* is, however, still considered optional because there are no definitive data showing a clear advantage or disadvantage of its use [20, 24, 29, 32, 36]. Moreover it was shown that cytochalasine B itself may induce MNi or mask the MN inducing effect [32].

Micronuclei formed on account of exposure to genotoxic agents originate either from acentric chromosome fragments or from whole chromosomes/chromatides that are not incorporated into daughter nuclei at the time of cell division [3]. FENECH and NEVILLE [11] showed that MN assay is relatively insensitive to detect those mutagens/carcinogens that induce predominantly rapidly repaired DNA lesions. In order to overcome this limitation, the authors proposed to use DNA repair inhibitors, which stalled the gap-filling step during excision repair of DNA damage. They presumed that unfilled gaps could be converted to double strand breaks after S phase and afterward to micronuclei at completion of cell division. Later on, they demonstrated that post-cultivation of treated cells in the presence of AraC and HU led to the conversion of excision repairable DNA lesions to MNi [12]. Using the immunofluorescent approach, they revealed that MNi formed due to AraC and HU post-incubation, originated from acentric chromosome fragments, thus the MNi were probably derived from unrepaired DNA lesions. This methodology was later applied to assess the clastogenicity of 10 pesticides [37], which had manifested contradictory results using the standard MN assay and SCE technique.

Although the AraC/CBMN assay may represent an additional significant improvement of the MN methodology, further laboratory and field studies are required to fully evaluate possible drawbacks and limitations of this promising approach for genetic toxicology. The aim of our study was to evaluate the role of DNA repair inhibitors on MN formation, using Chinese hamster V79 cells. Various model genotoxic agents (*N*-methyl-*N*-nitrosourea, benzo(a)pyrene and ultraviolet light) with known mechanism of activity were used to study the effect of AraC and HU alone or in combination on the level of MNi.

Material and methods

Chemicals. *N*-Nitroso-*N*-methylurea (CAS No.:684-93-5, MNU), benzo(a)pyrene (CAS No.:192-97-2, BaP), cytosine arabinoside (CAS No.:147-94-4, AraC), 2'-deoxycytidine hydrochloride (CAS No.:3992-42-5, DC) and hydroxyurea (CAS No.:127-07-1, HU) were purchased from Sigma, USA.

Cell line. Chinese hamster V79MZ cell line was obtained from prof. J. Doehmer, Institut für Toxikologie und Umwelthygiene, Technische Universität München, Germany. Cells were grown in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA), supplemented with 10% foetal bovine serum (Gibco, USA), 4.5 mg/ml glucose (Sigma, USA), 1 mM sodium pyruvate (Gibco, USA) and antibiotics (penicillin 100 U/ml, streptomycin and kanamycin 100 μg/ml, Gibco, USA). Cells were maintained at 37 °C in ahumidified atmosphere containing 10% CO_2.

Treatment of cells with model genotoxins. Cells were seeded into two microscope slides-containing Petri dishes (approx. $1x10^5$ to $1.2x10^5$ cells per slide) or several Petri dishes ($5x10^4$, ∅=60 mm) and incubated for 24 hours. Exponentially growing cells were exposed to mutagens/carcinogens or irradiated with UV light. Before treatment cells were washed twice with phosphate saline buffer (PBS).

Stock solution of MNU was prepared in dimethyl sulphoxide (DMSO, Serva, Germany) at concentration of $4x10^{-1}$ M freshly before use. Appropriate dilutions were made in sterile phosphate saline buffer (PBS, with Ca^{2+} and Mg^{2+}) prior to addition to the cultures to reach the final concentration 0.4 mM and 0.8 mM. Cells were treated for 15 minutes.

The stock solution of BaP in DMSO (2000 μM) was added to DMEM to reach the final concentration. The S9 fraction was prepared according to KUROKI et al [25]. The S9 fraction (4%) was added along with glucoso-6-phosphate (4.2 μM) and NADP(H) (1.8 μM). Cells were treated with BaP for 2 h in DMEM in the presence of S9 fraction.

Cells were irradiated with a dose of 10 J/m^2 and 20 J/m^2. The source of ultraviolet light (254 nm) was a germicidal lamp PHILIPS TUV 15W and the dose rate was estimated using photometer model IL 1400A (International Light Inc., Massachusetts, USA).

After finishing the treatment, the cells were washed twice with culture medium and incubated either in fresh medium or in a medium with DNA repair inhibitors.

DNA repair inhibitors and 2'-deoxycytidine hydrochloride. The stock solution of cytosine arabinoside (AraC, $2x10^{-2}$ M) was prepared in sterile distil water and kept at –20 °C. The stock solution of hydroxyurea (HU, 1M) and 2'-deoxycytidine hydrochloride (DC, 1 mg/ml) were prepared immediately before use in sterile distil water. All stock solutions were added directly to the medium to reach the final concentration (AraC – $4x10^{-6}$ M or $4x10^{-5}$ M; HU – $5x10^{-3}$ M, and DC – 0.5 μg/ml or 1 μg/ml). Genotoxin-exposed cells were post-cultivated in the presence of DNA repair inhibitors for 16 or 24 h. Then the cells were washed and processed for MN scoring or cultivated in fresh medium to assess the proliferating activity.

The effect of 2'-deoxycytidine on cell viability and MN formation was investigated as well. After exposure of the cells to AraC (0, 2, 4, 6 h) cells were washed and post-cultivated in the presence or absence of DC in fresh medium for additional 24, 22, 20 or 18 h, respectively.

Proliferating activity of cells. The proliferating activity of V79 cells was measured at different time intervals (24, 48, 72 h) after post-cultivation of exposed cells with DNA repair inhibitors. At 24 h time interval, cells were trypsinized and counted using the hemocytometer.

Micronucleus assay. After 16 or 24 h post-cultivation of exposed cells in the presence or absence of DNA repair inhibitors, cultures were washed with 0.9% NaCl, incubated in mild hypotonic solution (0.075 M KCl/0.9% NaCl, 1:19) for 10 minutes at 37 °C and then fixed in methanol/acetic acid 3:1 for 1 hour at 4 °C. The slides were finally air dried and stained with 3% Giemsa solution.

Cell scoring and statistical analyses of data. MNi were identified according to the following criteria: clearly surrounded by nuclear membrane, area less than one-third of the area of the main nucleus, nonrefractility, and the location within the cytoplasm of the cells [32]. At least 500 cells per microscope slide were analysed for the presence of micronuclei. Each column represents the mean value $\pm$SD of at least two parallel slides from one experiment with at least 1000 cells analysed for the presence of micronuclei from at least 3 independent experiments. The data from all experiments were combined and the significance of differences between treated samples and untreated control sample were evaluated using Student's t-test.

A degree of synergism (DS) and the "combination factor" (Cf) effect was calculated according to SURRALLÉS et al [37] using the formulas:

$$DS = \frac{(MN_{t+i} - MN_c)}{[(MN_t - MN_c) + (MN_i - MN_c)]}$$

where MN_c is the MN frequency of the control, MN_{t+i} is the MN frequency found in the cultures receiving both treatment and inhibitor, MN_t is the MN frequency found in cultures receiving only treatment, and MN_i is the MN frequency found in cultures receiving only the inhibitor;

$$Cf = \frac{(MN_{i1+i2} - MN_c)}{[(MN_{i1} - MN_c) + (MN_{i2} - MN_c)]}$$

where MN_c is the control frequency of MNi, MN_{i1+i2} is the MN frequency in cultures receiving the combined treatment of AraC and HU, MN_{i1} is the frequency of MNi in cultures treated with AraC alone and MN_{i2} is the frequency of MNi in cultures treated with HU alone.

When the DS or Cf=1 there is no synergism but an additive effect.

Results

Proliferating activity of cells. Growth activities of V79 cells irradiated with UV light (10 and 20 J/m^2) or exposed to MNU (0.4 mM) and post-cultivated 24 h in the presence or absence of DNA repair inhibitors are presented in Figure 1 and 2, respectively. Immediately after finishing the cultivation of control untreated V79 cells in the presence of DNA repair inhibitors, no significant reduction of cell proliferation was determined (Fig. 1A, 24 h), however, a cytostatic and cytotoxic effect was determined at later time intervals. Irradiation of V79 cells with a dose 10 J/m^2 (Fig. 1B) resulted in a mild reduction of proliferating activity, though at the dose 20 J/m^2 (Fig. 1C) a significant decrease of growth activity was determined 48 h after irradiation. Similarly to control cells, 24 h post-cultivation of irradiated cells in the presence of repair inhibitors did not result in any substantial inhibition of cell proliferation as compared to cells exposed to UV light only immediately after finishing the treatment with AraC and HU or combination AraC and HU (Fig. 1B, C). Removal of DNA repair inhibitors from the culture medium, however, did not lead to cell growth recovery; in contrast, a significant inhibition of cell growth was detected as in control cells.

Cell exposure to MNU was less cytotoxic (Fig. 2B); only a slight reduction of cell proliferating activity was found. Likewise as in the case of UV light, no substantial reduction of cell growth activity was found due to post-cultivation of cells in the presence of repair inhibitors (Fig. 2B, 24 h). However, later on a cytotoxic effect was determined.

Cell cultivation in the presence of deoxycytidine had no inhibitory effect on cell growth (Fig. 2C). Conversely, the addition of deoxycytidine to the culture medium after AraC-removal resulted in a cell growth recovery both in control and MNU-treated cells (Fig. 2C).

Effect of AraC and HU on the spontaneous level of micronuclei. Preliminary experiments were performed with AraC

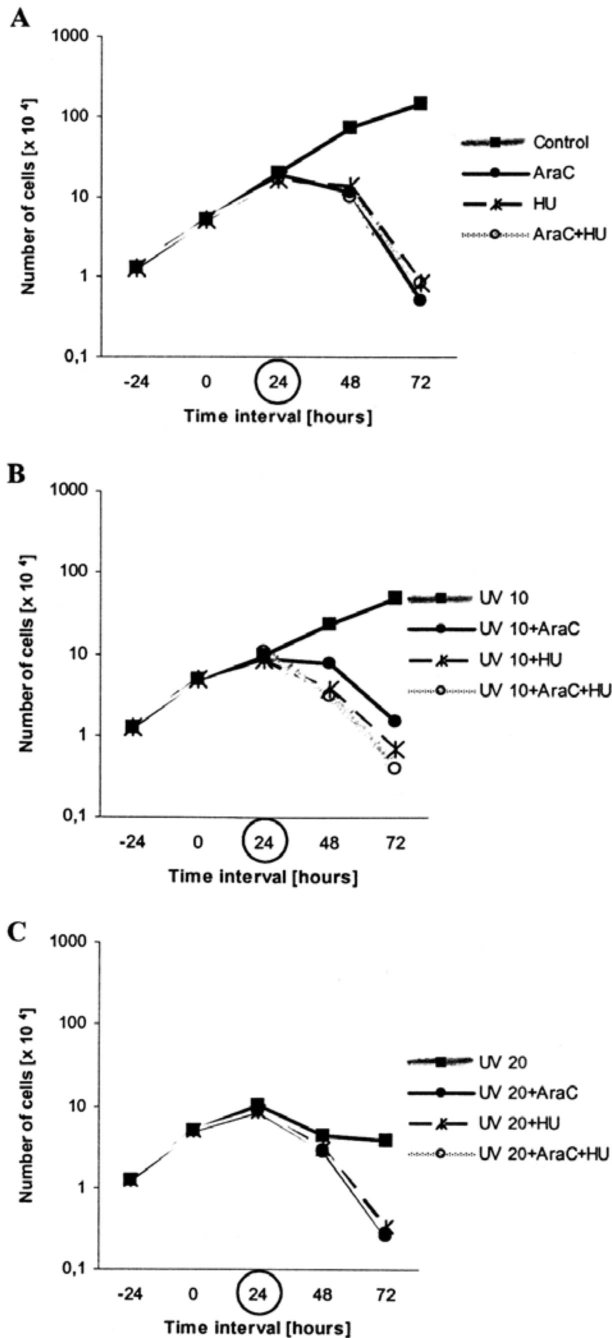

Figure 1. The proliferating activity of V79 cells post-cultivated in the presence and absence of DNA repair inhibitors. Untreated cells (A), cells irradiated with UV light, 10 J/m^2 (B) and 20 J/m^2 (C). The cells were irradiated with UV light at 0 time interval and post-cultivated 24 hours in the presence of AraC (4x10^{-6} M), HU (5x10^{-3} M) or combined treatment AraC + HU (4x10^{-6} M and 5x10^{-3} M, respectively). In 24 h intervals cells were counted.

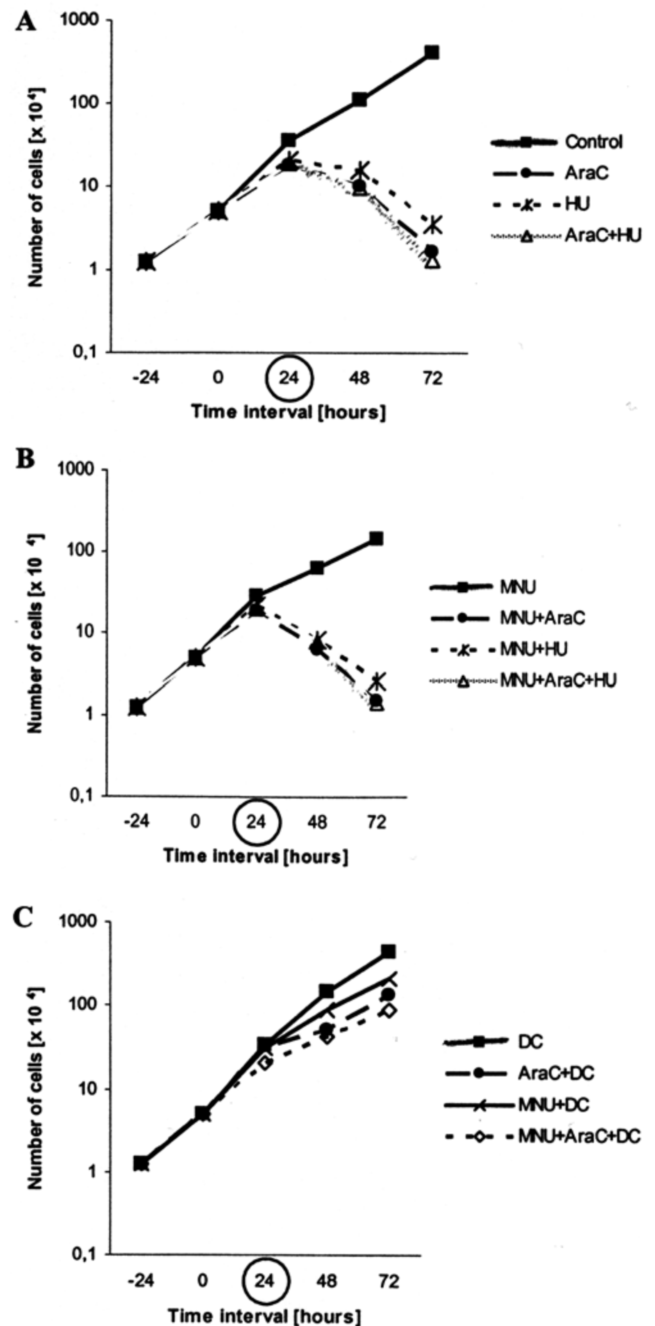

Figure 2. The proliferating activity of V79 cells post-cultivated in the presence and absence of DNA repair inhibitors. Untreated cells (A), cells treated with MNU (B), incubation in the presence of DC (C). The cells were exposed to MNU for 15 min at 0 time interval and post-cultivated 24 hours in the presence of AraC (4x10^{-6} M), HU (5x10^{-3} M) or combined treatment AraC + HU (4x10^{-6} M and 5x10^{-3} M, respectively). Cultivation of cells in the presence of DC took 20 h, AraC treatment was reduced to 4 h.

and HU in order to find suitable treatment conditions (concentrations and time interval) for the application of DNA repair inhibitors. Two AraC concentrations (4x10^{-6} M and 4x10^{-5} M) and one HU concentration (5x10^{-3} M) alone or in a combination were tested. A dramatic increase (~2- to 2.5-fold) in MN numbers was elicited in cells exposed to AraC (4x10^{-6} M) alone regardless the exposure period (Tab. 1). The presence of 2'-deoxycytidine (0.5 μg/ml and 1 μg/ml)

Table 1. The level of MNi in control cells after 16- and 24-h post-cultivation with DNA repair inhibitors

	16h			24h		
	Average	STDEV	t-test	Average	STDEV	t-test
No inhibitor	18.60	±4.33		20.35	±7.90	
AraC [$4x10^{-6}$ M]	48.00	±13.23	***	48.89	±15.87	***
AraC [$4x10^{-5}$ M]	31.33	±1.15	***	30.67	±4.13	***
HU [$5x10^{-3}$ M]	34.50	±8.06	**	26.92	±8.89	*
AraC [$4x10^{-6}$ M] + HU [$5x10^{-3}$ M]	41.00	±4.76	***	38.25	±11.03	**

The data were analyzed statistically by Student's t-test. Significantly different from control group (without inhibitor) at *p<0.05, **p<0.01, ***p<0.001.

Table 2. The effect of post-cultivation in the presence of 2'-deoxycytidine on the level of MNi induced by AraC ($4x10^{-6}$ M) in control untreated cells

	DC 0 μg/ml		DC 0.5 μg/ml		DC 1 μg/ml	
	Average	STDEV	Average	STDEV	Average	STDEV
AraC 24 h and DC 0 h	40.67	±7.64	–	–	–	–
AraC 6 h and DC 18 h	34.34	±10.26	51.17	±6.81	72.17	±1.15
AraC 4 h and DC 20 h	45.17	±5.51	55.84	±8.50	54.84	±5.69
AraC 2 h and DC 22 h	50.00	±3.61	47.34	±1.53	49.84	±8.50
AraC 0 h and DC 0 h	20.84	±6.81	–	–	–	–

Table 3. The level of MNi induced by UV light (UV), benzo(a)pyrene (BaP), and *N*-methyl-*N*-nitroso urea (MNU)

	Average	STDEV	t-test
Control	20.65	±7.22	
UV [10 J/m^2]	70.00	±14.45	***
UV [20 J/m^2]	64.33	±13.04	***
BaP [4 μM]	49.33	±4.16	***
MNU [0.4 mM]	24.00	±5.08	
MNU [0.8 mM]	29.23	±8.35	*

The data were analysed statistically by Student's t-test. Significantly increased from control untreated cells at *p<0.05, ***p<0.001.

after AraC exposure, in order to replace any residual AraC, did not result in any MN reduction (Tab. 2). AraC was more potent inducer of MNi than HU. Although the higher concentration of AraC ($4x10^{-5}$ M) induced relatively less MNi, an increased cytotoxicity was observed under these treatment conditions (data not shown). A combined exposure to AraC ($4x10^{-6}$ M) and HU did not manifest any additive effect on the MN formation. On the basis of these results a 24-hour post-incubation period with DNA repair inhibitors after cell exposure to genotoxins was used in further experiments.

Level of micronuclei induced by model genotoxins. Several known genotoxic agents with different mechanism of activity were used to assess their clastogenicity *in vitro* (UV light, direct and indirect chemical carcinogens). Based on previous experiments with V79 cells, only one sampling time interval, 24 h after finishing the exposure was used to detect the MN level in treated cells. UV light (two doses) and BaP, model mutagens/carcinogens increased highly significantly (p<0.01 and p<0.001) the MN level in exposed cells (Tab. 3). In contrast, MNU, a direct mutagen, at concentration 0.4 mM did not induce any MN formation in V79 cells. Only a slight, however, significant rise of MNi was followed in cells exposed to higher MNU concentration (0.8 mM) (p<0.05). In addition, this concentration reduced significantly the viability of treated cells (data not shown).

Effect of AraC and HU on the level of micronuclei induced by model genotoxins. Post-incubation of MNU treated cells in the presence of AraC resulted in a synergistic increase of MN formation at both 0.4 mM and 0.8 mM concentrations (DS=2.14 and 1.13, respectively) (Fig. 3). Hydroxyurea stimulated also significantly MN formation induced by MNU at both concentrations (p<0.001 and p<0.01), though, this effect was less than additive (DS=0.86 and 0.66, respectively). The combined treatment of AraC and HU at both MNU concentrations was least evident (Cf=0.36 and 0.28, respectively). At the higher MNU concentration this combined treatment resulted in stronger cytotoxicity (data not shown).

Post-incubation of UV- and BaP- treated V79 cells in the presence of individual DNA repair inhibitors or the combined treatment of AraC and HU did not lead to any additional rise of MNi, as in the case of MNU (Fig. 4 and 5). In contrast, no or even an evident decrease of MNi was found. Hydroxyurea reduced the MN number most effectively.

Discussion

The assessment of the genotoxic potential of new chemicals produced by agrochemical and pharmaceutical industries or a risk assessment of occupational and environmental exposure requires accurate, sensitive and if possible, easy and not too time-consuming methodologies. One of the most promising techniques for the estimation of DNA damage *in vivo* and *in vitro* is the micronucleus assay [6, 23],

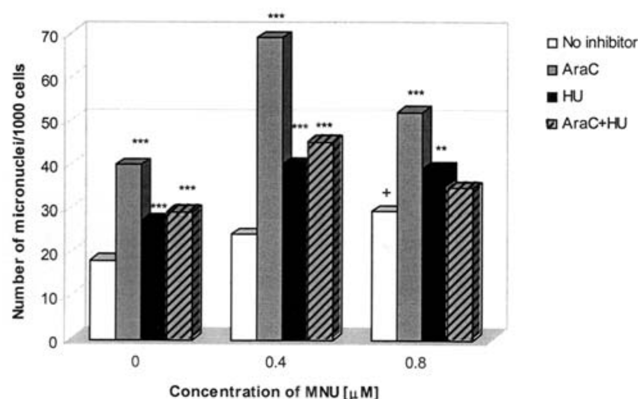

Figure 3. The effect DNA repair inhibitors on the level of MNi induced by MNU. The data were analyzed statistically by Student's t-test. Significantly increased from MNU at $^{**}p<0.01$, $^{***}p<0.001$; from control untreated cells at $^{+}p<0.5$.

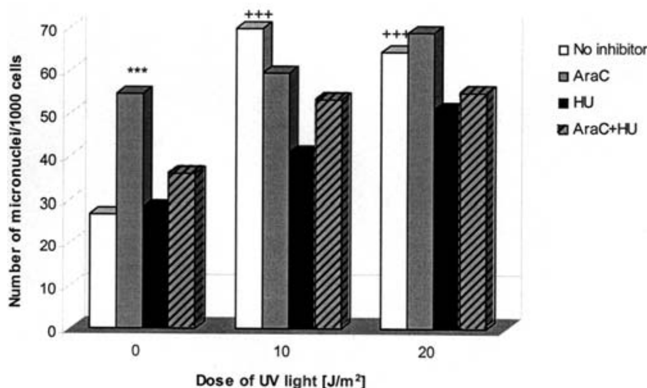

Figure 4. The effect DNA repair inhibitors on the level of MNi induced by UV light. The data were analyzed statistically by Student's t-test. Significantly increased from UV light at $^{***}p<0.001$; from control untreated cells at $^{+++}p<0.001$.

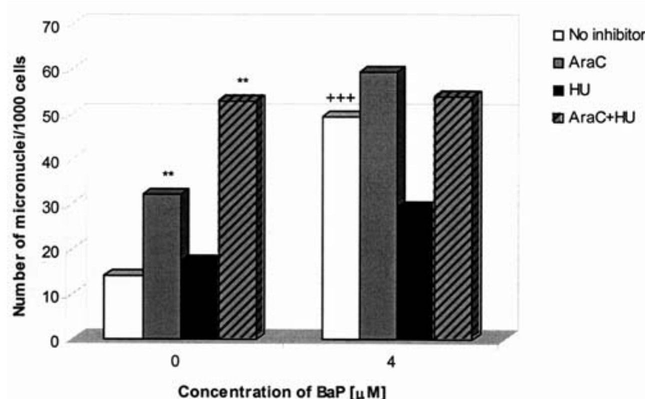

Figure 5. The effect DNA repair inhibitors on the level of MNi induced by BaP. The data were analyzed statistically by Student's t-test. Significantly increased from BaP at $^{**}p<0.01$, from control untreated cells at $^{+++}p<0.001$.

though its position within the battery of screening assays for determination of genotoxic potential is still discussed [9, 24, 27].

Since the early 1970s, important improvements of the standard MN assay have been achieved in order to increase the sensitivity of this assay. Among them, the inhibition of a gap-filling step by DNA repair inhibitors represents one of the promising modifications of the CBMN [11]. Generally, lymphocytes when treated with genotoxins that mainly produce DNA adducts and not strand breaks, do not efficiently induce MN formation in once-dividing cells. However, a significant increase of MN formation was shown in lymphocytes exposed to UV and MNU due to post-incubation of exposed cells in the presence of cytosine arabinoside [12]. This AraC/CBMN methodology has been already successfully applied to assess the clastogenicity of 10 pesticides [37]. Although the AraC/CBMN assay may represent an additional significant improvement of the MN methodology, further laboratory studies are needed to evaluate possible drawbacks and limitations of this promising approach for genetic toxicology.

In addition to peripheral blood lymphocytes, also established cell lines are frequently used for screening of possible genotoxic agents or quick assessment of chromosomal mutation induction [17, 21, 30, 33]. Chinese hamster V79, CHO or CHL/IU cells are most frequently used cell lines in the *in vitro* MN assay; they have a stable karyotype, a short generation time and are easy to maintain and handle [17, 19, 20, 29]. Therefore this study was focused to assess the effect of DNA repair inhibitors on MN levels induced by model genotoxins.

Treatment of V79 cells with model genotoxins UV light and BaP led to a variable but statistically significant enhancement of MN formation (Tab. 3). UV light was the most potent inductor of MNi (3.3-fold increase). The clastogenicity of UV radiation demonstrated also KESHAVA et al [22] using the V79 cells. Our data clearly manifested that proliferating cells in contrast to lymphocytes are able to detect clastogenic activity of various genotoxic agents, regardless the type of DNA lesion they induce. MNU treatment resulted in a slight (1.4-fold) but statistically significant rise of MNi only at concentration 0.8 mM. However, a significant decrease of cell viability was measured at this concentration at the same time (data not shown). The main damage induced by MNU in DNA is methylation of guanine at the position N^7 and O^6 and adenine at the position N^3 [34]. This type of DNA damage is rapidly removed by the base excision repair (BER) mechanism. FENECH and NEVILLE [11] did not find any significant increase of MNi formation due to MNU exposure at concentration 60 μg/ml using the lymphocytes *in vitro*. However, STEPHANOU et al [35] measured a 2.3-fold increase of MNi at the concentration 125 μg/ml. The presence of AraC and HU in the post-incubation period after MNU treatment (Fig. 3) re-

sulted in a synergistic enhancement of MNi at both concentrations (DS=2.14 and 1.13, respectively). HU alone had less than additive effect (DS=0.86 and 0.66) and the combined treatment of AraC and HU was least effective (Cf=0.36 and 0.28). These results were in a good agreement with the data obtained by FENECH et al [12] and SURRALLÉS et al [37]. On the other hand, AraC or HU post-incubation followed after UV- and BaP-exposure did not result in an additional rise of MNi formation. Moreover, no or even reduction of the MNi number was detected (Fig. 4, 5). The most significant reduction of MNi was found in samples post-incubated in the presence of HU ($p<0.001$), regardless the genotoxic agent was used. Hydroxyurea is a specific inhibitor of ribonucleotide reductase (RR), an essential enzyme involved in the formation of deoxyribonucleotidediphosphates (dNDPs) from ribonucleotidediphosphates (rNDPs), precursors are required for DNA synthesis and repair [18]. Survival of exposed cells depends on rapid removal of DNA damage from dividing cells; therefore dNDP starvation due to HU presence probably resulted in DNA break accumulation and cell death. At the sampling time for MN detection (24 h), no strong inhibition of cell growth was determined in untreated or exposed cells as a consequence of cell post-cultivation in the presence of DNA repair inhibitors (Fig. 1 and 2). However, negative effect of AraC and HU or their combination on the cell proliferation was found at later time intervals (48 and 72 hour after treatment).

A significant elevation of MN level was found in control, untreated cells after exposure to AraC and HU alone or in combination. AraC alone increased the spontaneous level of MNi 2.6-fold, while the effect of HU alone was less significant (Tab. 1). A combined treatment of AraC and HU has less than additive effect. AraC, an inhibitor of DNA-polymerase α (or β) [14], is frequently used as a cytostatic drug in the treatment of acute myeloid leukemia. After phosphorylation to AraC triphosphate (AraCTP) it is incorporated into DNA and causes DNA fragmentation, strand breaks and apoptosis [15, 37]. It was shown that DNA inhibitors are potent inducers of chromosome breaks and MNi when cells are exposed during S phase [16], probably due to interaction of these agents with DNA replication and transcription. FENECH [12] and SURRALLÉS et al [37] also showed the negative impact of repair inhibitors on the level of MNi in control untreated cells. Although they found out that supplementation of the culture medium with 2'-deoxycytidine after exposure to AraC decreased the level of MNi, in our experiments DC did not dramatically influence the number of MNi (Tab. 2), though a significant effect on cell growth recovery was found (Fig. 2C).

The role of DNA repair inhibitors in MN assay has not been properly cleared up; therefore we attempted to clarify the relevance of their use in well proliferating cell lines. Exponentially growing cells are able to detect clastogenic activity also of such type of genotoxins which induce mostly DNA adducts instead of direct strand breaks. The involvement of DNA repair inhibitors did not contribute to an increase of sensitivity of the MN assay. The positive effect was found only in the case of MNU; post-incubation of MNU-exposed cells in the presence of AraC resulted in a significant increase of MNi. On the basis of our results we suppose that the AraC/CBMN assay might be a promising approach in genetic toxicology applied to lymphocytes. Using the stable cell lines, this approach might be applied to prove the negative results obtained under standard treatment conditions.

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