Available online at www.sciencedirect.com
SCIENCE DIRECT
Mutation Research 587 (2005) 52–58
Genetic Toxicology and Environmental Mutagenesis
www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

A 1,4-dihydropyridine derivative reduces DNA damage and stimulates DNA repair in human cells in vitro

Nadezhda I. Ryabokon [a,1], Rose I. Goncharova [b], Gunars Duburs [c], Joanna Rzeszowska-Wolny [a,*]

[a] *Department of Experimental and Clinical Radiobiology, Centre of Oncology, M. Sklodowska-Curie Memorial Institute, Wybrzeze Armii Krajowej 15, 44-101 Gliwice, Poland*
[b] *Laboratory of Genetic Safety, Institute of Genetics and Cytology, National Academy of Sciences of Belarus, Akademichnaya 27, 220070 Minsk, Republic of Belarus*
[c] *Latvian Institute of Organic Synthesis, Aizkaukles 21, LV-1006 Riga, Latvia*

Received 29 April 2005; received in revised form 18 July 2005; accepted 30 July 2005
Available online 3 October 2005

Abstract

Compounds of the 1,4-dihydropyridine (1,4-DHP) series have been shown to reduce spontaneous, alkylation- and radiation-induced mutation rates in animal test systems. Here we report studies using AV-153, the 1,4-DHP derivative that showed the highest antimutagenic activity in those tests, to examine if it modulates DNA repair in human peripheral blood lymphocytes and in two human lymphoblastoid cell lines, Raji and HL-60. AV-153 caused a 50% inhibition of growth (IC_{50}) of Raji and HL-60 cells at 14.9 ± 1.2 and 10.3 ± 0.8 mM, respectively, but did not show a cytotoxic effect at concentrations <100 μM. Alkaline single-cell gel electrophoresis (comet) assays showed that AV-153 reduced the number of DNA strand breaks in untreated cells and also in cells exposed to 2 Gy of gamma-radiation, 100 μM ethylmethane sulfonate (EMS), or 100 μM H_2O_2. DNA damage was reduced by up to 87% at AV-153 concentrations between 1 and 10 nM, and a positive dose-effect relationship was seen between 0.01 and 1 nM. Comparison of the kinetics of DNA strand-break rejoining in the presence and absence of AV-153 revealed a considerable influence on the rate of repair. In view of the resemblance of this compound's structure to that of dihydronicotinamide, a substrate for poly(ADP-rybose)polymerase, the modulation of DNA repair by AV-153 could involve an influence on poly(ADP)ribosylation.

Keywords: 1,4-Dihydropyridine; Cytotoxicity; DNA damage and repair; DNA protection; Comet assay; Human lymphocytes; Lymphoblastoid cells

1. Introduction

Synthetic derivatives of 1,4-dihydropyridine (1,4-DHPs) possess important biochemical and pharmacological properties. They show modulating activity on cardiovascular and neuronal processes [1,2] and on corticosteroid regulatory circuits [3] and prevent inflammatory [4] and diabetic processes [5,6], and some show antineoplastic [7], geroprotective [8], radioprotective [9] and radiosensitizing effects [10]. Some of the positive effects of 1,4-DHPs are long-term [11,12], and thanks to their low or very low toxicity (glutapyrone showed an LD_{50} >3 g/kg when administered intravenously or >10 g/kg with oral administration) [2] this group of compounds appears to offer promise for

* Corresponding author. Tel.: +48 32 278 9677; fax: +48 32 231 3512.
E-mail address: jwolny@io.gliwice.pl (J. Rzeszowska-Wolny).
[1] Present address: Laboratory of Genetic Safety, Institute of Genetics and Cytology, National Academy of Sciences of Belarus, Akademichnaya 27, 220070 Minsk, Republic of Belarus.

1383-5718/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.mrgentox.2005.07.009

medical applications. Among 12 screened 1,4-DHPs that differed in chemical structure, six β-carbonyl-1,4-dihydropyridines that are analogues of dihydronicotinamide, the hydrogen- and electron-transferring moiety of the redox coenzymes NADH and NADPH, showed antimutagenic activity and significantly reduced spontaneous and alkylation-induced point mutations and chromosome breaks in germ cells of *Drosophila* [13,14], alkylation-induced micronuclei in mouse bone-marrow cells [15], and radiation-induced chromosome aberrations and other cytogenetic end-points in fish [16]. The reduction of mutation frequency reached 85% in some cases [14]. Studies on *Drosophila* suggested that the 1,4-DHPs inhibit chemical mutagenesis due to modulation of DNA repair [13–15], and in the present study we examined the effects of AV-153, a 1,4-DHP that showed the highest antimutagenic activity in animals [13,14], on spontaneous, chemically- and radiation-induced DNA damage and its repair in human cells in vitro.

2. Materials and methods

2.1. AV-153

Sodium 3,5-bis-ethoxycarbonyl-2,6,dimethyl-1,4-dihydropyridine-4-carboxylate (AV-153), synthesized in the Latvian Institute of Organic Synthesis, is an analogue of the active center of the reduced form of nicotinamide adenine dinucleotide (NADH) or its phosphate (NADPH) (Fig. 1). It is a yellow crystalline powder, resistant to temperature fluctuations, soluble in water and it passes the cell membrane. Stock solutions were prepared in phosphate-buffered saline (PBS) or culture medium and kept in the dark at 4 °C during a period of one month.

2.2. Cells and media

Venous blood from a young non-smoking healthy female was collected in heparinized tubes. Histopaque-1077 (ICN) was used to separate mononuclear lymphocytes, which were washed in RPMI 1640 (Sigma–Aldrich) at 4 °C and incubated in RPMI 1640 with 10% fetal bovine serum (FBS; Gibco), L-glutamine and 0.1% gentamicine at 37 °C in a humidified atmosphere with 5% CO_2. Human HL-60 (promyelocytic leukemia) and Raji (B-lymphoblastic leukemia) cells were cultured in the same conditions and used during exponential growth.

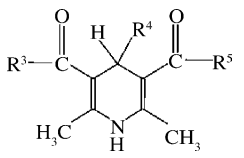

Fig. 1. Structure of AV-153. Positions R^3 and R^5 bear a OC_2H_5 group and position R^4 a COONa group.

2.3. Assessment of cell viability

We used the colorimetric methyl-thiazol-tetrazolium (MTT) assay [17] to study cell survival after incubation with AV-153. Briefly, cells in RPMI 1640 without phenol red (Sigma–Aldrich) were seeded in 96-well plates (NUNC, Denmark) using 1.5×10^4 cells/100 μl/well. Equal aliquots of PBS containing AV-153 at different concentrations were added 24 h later. Wells without cells or without AV-153 were used to determine baseline values. After further incubation for 24 h at 37 °C, MTT (Sigma–Aldrich) was added to all wells to a final concentration of 0.5 mg/ml and the plates were incubated for 3 h in the same conditions. The formazan crystals formed were dissolved by addition of 150 μl of DMSO and the optical density (OD) at 570 nm was measured with an ELx800 microplate reader (Bio-Tek Instruments, USA). The percentage cell survival was calculated by the equation [viable cells = $(OD_t - OD_{tb})/(OD_c - OD_{cb}) \times 100$], where OD_t is for the cell sample with AV-153, OD_{tb} for AV-153 alone (test blank), OD_c for control cells, and OD_{cb} for the control blank without cells and AV-153. All experimental series were performed at least three times with triplicate samples. The IC_{50}, i.e. the concentration at which 50% of cells showed inhibition of MTT processing, was calculated from the dose-response curve. The standard Trypan-blue exclusion test was also used according to the manufacturer's indications (Sigma–Aldrich) and blue (non-viable) cells were scored under the microscope.

2.4. Treatment with genotoxic factors

Cells were used at a concentration of 4×10^5/ml. Gamma-irradiation was performed on ice using a ^{60}Co radiotherapy source (Gammatron, Siemens) at a dose rate of 0.8 Gy/min to a total dose of 2 Gy. In other experiments cells suspended in ice-cold PBS were incubated with ethylmethane sulfonate (EMS, Sigma–Aldrich) or hydrogen peroxide (H_2O_2) at final concentrations of 100 μM for 1–3 min, washed in ice-cold PBS, and suspended in complete medium at 37 °C. Equal aliquots of AV-153 at different concentrations were added immediately after irradiation or after washing off the chemical agents.

2.5. Alkaline single-cell gel electrophoresis (comet) assay

DNA breaks were assessed by comet assays as described in [18–20] with all steps of preparation performed on ice. Images of 100 randomly selected cells were analyzed per experimental point. In initial experiments an image-analysis system (Lucia version 4.60, Laboratory Imaging Ltd.) was compared with visual scoring and a statistically significant correlation ($r \geq 0.72$, $p < 0.05$) between the results was obtained. Subsequently, only the less time-consuming visual scoring was used. Cells were classified into five categories (A_0–A_4) according to Collins et al. [21,22] and the DNA damage (D) in arbitrary units was calculated as $D = A_1 + 2A_2 + 3A_3 + 4A_4$, giving D values from 0 to 400 for 100 cells. To follow the kinetics of repair, irradiated cells were incubated in growth medium

at 37 °C and samples were taken at six time points from 0 to 180 min. The repair curves were modeled by the exponential function $[y(t) = a \times \exp(t/-\tau) + c]$, where y is DNA damage at time (t) during repair, a is the repaired fraction of damage, τ is a time constant inversely related to the rate of repair, and c is the residual unrepaired damage [20]. Slides were coded and evaluated by the same observer.

2.6. Statistical analyses

Experiments were repeated at least three times. Data were compared using the unpaired Student's t-test, Pearson's correlation, and Spearman's rank analysis and regression analysis.

3. Results

3.1. Effect of AV-153 on cell viability

We used two assays, MTT and Trypan-blue exclusion tests, to examine the toxic effect of AV-153 on Raji and HL-60 cells. Both tests showed that AV-153 had no influence on cell viability and mortality at concentrations <100 μM (Fig. 2A and B).

A significant increase in the number of non-viable cells was seen for AV-153 concentrations above 100 μM, and between 1 and 25 mM HL-60 and Raji cells differed in sensitivity (Fig. 3). In this concentration range the survival curves fitted the logarithmic function $[y = -a \times \ln(x) + b]$ with determination coefficient >0.92 and $p < 0.05$. The calculated values of IC_{50} were 10.3 ± 0.8 and 14.9 ± 1.2 mM for HL-60 and Raji cells, respectively; HL-60 cells were thus slightly more sensitive than Raji cells.

3.2. AV-153 diminishes the level of endogenous DNA damage

We tested the effect of exposure of cells to non-toxic concentrations of AV-153 on the level of DNA breaks.

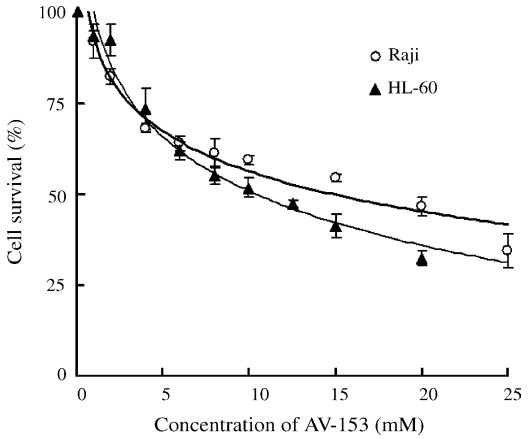

Fig. 3. Cell survival at AV-153 concentrations higher than 1 mM and calculation of IC_{50}. Survival was measured by MTT assays; means of three to four experiments ± S.E. are shown.

The level of spontaneously arising DNA single-strand breaks (SSBs) in peripheral blood lymphocytes or HL-60 cells was reduced by 13–67% after a 3 h incubation at concentrations varying by five orders of magnitude (1 nM to 10 μM) (Table 1).

For lymphocytes, AV-153 concentrations lower than 0.1 nM showed a non-significant reduction of endogenous SSBs but a significant effect was seen at 1 nM. Reduction of endogenous SSBs did not increase further at AV-153 concentrations higher than 1 nM (Table 1).

3.3. Protective effect of AV-153 against radiation- and chemically-induced SSBs

AV-153 was also active in protecting against DNA damage caused by exogenous factors such as ionizing radiation, H_2O_2 and ethylmethane sulfonate, which can be measured by the comet assay as DNA breaks. Lymphocytes or HL-60 cells exposed to 2 Gy of ionizing radiation and incubated for 3 h in the presence of ≥1 nM

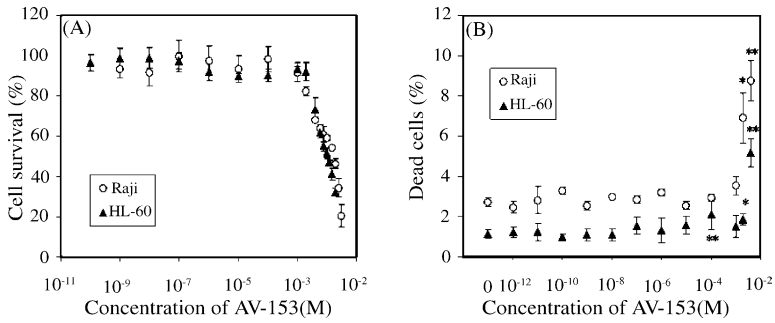

Fig. 2. Effect of AV-153 on the viability of Raji and HL-60 cells. Cells were incubated with AV-153 for 24 h: (A) cell survival measured using the MTT assay; (B), frequencies of dead cells assessed by the Trypan-blue exclusion test. Means of three to four independent experiments ± S.E. are presented. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with controls.

Table 1
Decrease of endogenous DNA SSB levels in lymphocytes and HL-60 cells after 3 h of incubation with AV-153

AV-153, M	DNA damage (a.u.), mean ± S.E.		Reduction factor (%), mean ± S.E.	
	Lymphocytes	HL-60	Lymphocytes	HL-60
0	7.71 ± 0.92	100.00 ± 1.00	–	–
10^{-11}	7.50 ± 0.50	–	2.73 ± 1.65	–
10^{-10}	7.00 ± 2.08	–	28.24 ± 1.62	–
10^{-9}	**2.75 ± 1.24**	**87.00 ± 2.00***	67.36 ± 19.26	13.44 ± 1.56
10^{-8}	**2.86 ± 0.59***	**56.00 ± 2.00***	52.36 ± 8.97	44.01 ± 1.44
10^{-7}	**2.90 ± 0.87***	**66.00 ± 12.00**	43.77 ± 17.99	38.73 ± 11.73
10^{-6}	**3.25 ± 0.85**	**65.00 ± 7.50***	40.38 ± 4.46	32.21 ± 9.21
10^{-5}	**3.25 ± 0.63***	**74.50 ± 9.50**	35.0 ± 14.17	25.17 ± 9.17

Bold values show $p < 0.05$ and $^{*}p < 0.01$ compared to untreated cells. The reduction factor (RF) was calculated as $((DD_i - DD_{av})/DD_i) \times 100$ (%), where DD_i is endogenous (or induced) damage and DD_{av} is damage after incubation with AV-153.

AV-153 showed a decreased level of SSBs in comparison with cells exposed to the same dose of ionizing radiation and incubated without AV-153. A similar effect was observed for Raji cells exposed to H_2O_2 and for lymphocytes incubated with EMS (Table 2).

The tendencies of the AV-153 dose-effect relationships in reducing exogenously induced oxidative damage were the same as those for endogenous DNA damage. H_2O_2 induced SSBs immediately, and after a 3 h repair period the level of SSBs was significantly lower in cells incubated in the presence of AV-153. At ≤1 nM the reduction of DNA damage increased with the concentration and showed a maximal value of 37%, but reduction of damage decreased with further increase of AV-153 concentration. In lymphocytes exposed to 100 μM EMS or 2 Gy of X-radiation and incubated for 3 h, SSBs were reduced by ∼50% and 90%, respectively (Table 2). Regression analysis revealed that the efficacy of AV-153 in reducing endogenous DNA breaks, or those induced by oxidizing or alkylating agents or ionizing radiation, gradually decreased with concentration from 1 nM to 10 μM and could be described by a logarithmic function.

3.4. Effect of AV-153 on the kinetics of DNA single-strand break rejoining

Analysis of the level of SSBs at different times after exposure to exogenous DNA-damaging factors allowed assessment of the influence of AV-153 on DNA repair.

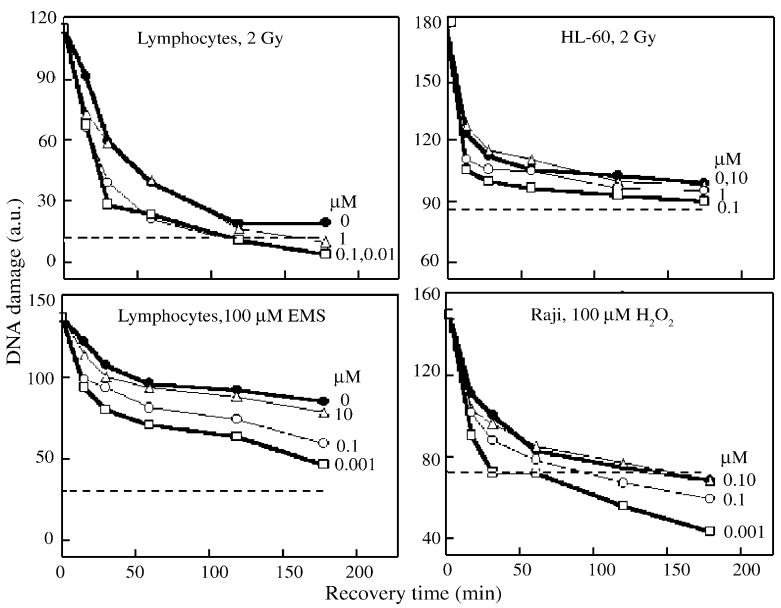

Fig. 4. Repair kinetics after exposure to different DNA-damaging agents and post-incubation with AV-153. Each point is the mean of at least three experiments. SE bars are not shown for clarity. Dashed line shows control.

Table 2
Effect of 3 h incubation with AV-153 on DNA damage induced by exogenous factors

	DNA damage (a.u.), mean ± S.E.				Reduction factor (%), mean ± S.E.			
	Raji	HL-60	Lymphocytes		Raji	HL-60	Lymphocytes	
Control	81.5 ± 8.8	84.5 ± 2.5	7.00 ± 2.9	31.00 ± 3.0	–	–	–	–
AV-153, M	H_2O_2	2 Gy	2 Gy	EMS	H_2O_2	2 Gy	2 Gy	EMS
0	110.6 ± 2.7	98.5 ± 1.5	18.7 ± 4.9	87.0 ± 4.6	–	–	–	–
10^{-11}	107.3 ± 2.5	–	–	–	4.0 ± 1.7	–	–	–
10^{-10}	**102.4 ± 1.3***	–	–	–	7.3 ± 2.2	–	–	–
10^{-9}	**69.1 ± 13.4***	–	–	**47.7 ± 11.7***	37.0 ± 12.2	–	–	46.4 ± 11.1
10^{-8}	–	**85.0 ± 2.0**	**3.0 ± 1.5**	–	–	13.7 ± 0.7	87.2 ± 5.3	–
10^{-7}	**95.0 ± 6.5***	89.0 ± 4.0	**3.7 ± 1.4**	60.7 ± 16.3	14.7 ± 3.7	9.7 ± 2.7	77.5 ± 10.0	31.9 ± 16.2
10^{-6}	–	94.0 ± 4.5	9.0 ± 2.3	–	–	4.6 ± 3.6	51.5 ± 9.8	–
10^{-5}	110.7 ± 5.0	97.0 ± 1.0	18.0 ± 4.6	80.3 ± 8.2	0.7 ± 4.0	1.5 ± 0.5	3.0 ± 1.6	8.2 ± 4.9

Values in bold show $p < 0.05$ and (*) shows $p < 0.01$ compared with irradiated cells or cells incubated with EMS (100 μM) or H_2O_2 (100 μM) in the absence of AV-153. The reduction factor was calculated as described in Table 1.

We studied the level of SSBs at 0, 15, 30, 60, 120 and 180 min after DNA-damage induction and incubation of cells in medium supplemented or not with AV-153 (Fig. 4).

The difference in SSB level between cells incubated with AV-153 at <10 μM was significant starting from 15 min of incubation. The repair kinetics could be modeled by an exponential function whose parameters were calculated (see Section 2). Table 3 shows the values of parameter τ that is inversely related to the rate of repair. AV-153 at 1 nM to 0.1 μM caused a significant increase of repair rate for all damaging agents and all cell types.

4. Discussion

These results demonstrate that AV-153 is non-toxic for human cells at concentrations <100 μM, like other derivatives of 1,4-dihydropyridine previously tested, such as nifedipine used in therapy of cardiovascular diseases [23] or the novel compound cerebrocrast with anti-inflammatory properties [4]. A toxic effect was seen at concentrations >100 μM and the IC_{50} differed slightly for the different cell types tested, HL-60 cells being more sensitive than Raji cells. HL60 cells are p53-deficient due to deletions in the gene coding for this protein [24], but nevertheless they undergo apoptosis readily and show a G2 checkpoint [25]. They have been found to be more sensitive than other cell lines in studies of anticancer drugs [26,27].

In the lower range of concentrations tested, AV-153 caused a decrease in the level of DNA SSBs as measured by alkaline comet assays, which detect single and double strand breaks including those associated with replication, incomplete excision repair of DNA damage, or alkali-labile (mostly apurinic and apyrimidinic, AP) sites [19]. The levels of both endogenous SSBs, which are common lesions caused by DNA oxidation and AP sites formed during metabolic processes (up to 10^4 depurinizations occur in human cells per day [28]), and of SSBs caused by ionizing radiation (oxidative damage to bases and cross-links) or alkylating agents like EMS (*N*-alkylation of purines and AP sites) were significantly reduced. These effects suggest that AV-153 may directly stimulate DNA repair, which is consistent with its influence on DNA-repair kinetics seen here and also with the results of Goncharova and Kuzhir [13,14] who showed that it reduced the frequency of spontaneous and EMS-induced point mutations in germ cells of *Drosophila* by 85% and 40%, respectively. AV-153 could influence cellular redox equilibria, because it may possess antioxidant activity [14] like several other 1,4-DHP derivatives [29–31], but this mechanism is improbable,

Table 3
Effect of AV-153 on DNA-repair rate after irradiation or exposure to H_2O_2 or EMS

AV-153, M	Parameter τ							
	100 μM H_2O_2				2 Gy γ-radiation		100 μM EMS	
	Raji		Lymphocytes		HL-60		Lymphocytes	
	Mean ± S.E.	p	Mean ± S.E.	p	Mean ± S.E.	p	Mean ± S.E.	p
0	27.0 ± 2.8		40.6 ± 3.3		12.7 ± 1.2		48.9 ± 14.5	
10^{-5}	21.1 ± 2.1	0.07	–	–	15.5 ± 2.2	0.24	39.2 ± 13.2	0.06
10^{-6}	–	–	40.3 ± 1.7	0.94	8.3 ± 2.3	0.11	–	–
10^{-7}	19.2 ± 6.3	0.07	25.8 ± 4.3	0.05	**7.7 ± 0.9**	**0.021**	26.9 ± 12.1	0.31
10^{-8}	–	–	**22.8 ± 2.0**	**0.04**	–	–	–	–
10^{-9}	**15.7 ± 1.4**	**0.0002**	–	–	–	–	26.2 ± 2.6	0.2

The experimental points were fitted to the exponential equation $y = a \times \exp(t/-\tau) + c$, where τ is a time constant inversely related to the rate of DNA repair. Values in bold show $p < 0.05$.

because it influences DNA damage induced by alkylating agents.

The structure of AV-153 resembles that of dihydronicotinamide, the hydrogen- and electron-transferring moiety of NADH and NADPH, suggesting two possible mechanisms for its protective effect against DNA damage. The oxidized form of NADH is a substrate for ADP-ribosyl cyclases, and cyclic ADP-ribose mobilizes calcium (reviewed in [32]). We therefore considered that AV-153 could influence the cell cycle through calcium signaling, which in turn could influence the SSB level. However, we observed that AV-153 had no influence on the cell cycle parameters of HL-60 cells at concentrations from 10^{-9} to 10^{-5} M as assessed by cytofluorometry (data not shown). A second hypothesis is that since NADH and NaDPH are substrates for poly(ADP-ribosyl)polymerase, which modifies proteins involved in DNA repair [32], a modulating effect of AV-153 on poly(ADP)ribosylation reactions could underlie its effects on DNA-repair kinetics. Elucidation of the details of the mechanism of action of AV-153 requires further studies.

Acknowledgements

The authors thank Ronald Hancock for critical reading of the manuscript and discussions. This work was carried out at the Department of Experimental and Clinical Radiobiology, Centre of Oncology, M. Sklodowska-Curie Memorial Institute, Gliwice in the framework of the scientific agreement between this institute and the Institute of Genetics and Cytology, National Academy of Sciences of Belarus, Minsk. The studies were supported by fellowship funds from the Association for the Support of Cancer Research, UNESCO (Polish Committee), and the National Cancer Institute (Bethesda, USA) and by a grant 4T11F01824 from the Polish State Committee for Scientific Research (KBN).

References

[1] J. Briede, K. Heidemanis, I. Dabina, G. Duburs, Effect of cerebrocrast on the function of human platelets and release of the arachidonic acid from plasma membrane, Cell Biochem. Funct. 20 (2002) 177–181.
[2] I. Misane, V. Klusa, M. Dambrova, S. Germane, G. Duburs, E. Bisenieks, R. Rimondini, S.O. Ögren, "Atypical" neuromodulatory profile of glutapyrone, a representative of a novel 'class' of amino acid-containing dipeptide-mimicking 1,4-dihydropyridine (DHP) compounds: in vitro and in vivo studies, Eur. Neuropsychopharmacol. 8 (1998) 329–347.
[3] E. Liutkevicius, A. Ulinskaite, R. Meskys, K. Kraujelis, G. Duburs, V. Klusa, Influence of different types of the 1,4-dihydropyridine derivatives on rat plasma corticosterone levels, Biomed. Lett. 60 (1999) 39–46.
[4] A. Klegeris, E. Liutkevicius, G. Mikalauskiene, G. Duburs, P.L. McGeer, V. Klusa, Anti-inflammatory effects of cerebrocrst in a model of rat paw edema and on mononuclear THP-1 cells, Eur. J. Pharmacol. 441 (2002) 203–208.
[5] J. Briede, D. Daija, E. Bisenieks, N. Makarova, J. Uldrikis, J. Poikans, G. Duburs, Effect of some 1,4-dihydropyridine Ca antagonists on the blast transformation of rat spleen lymphocytes, Cell Biochem. Funct. 17 (1999) 97–105.
[6] J. Briede, D. Daija, M. Stivrina, G. Duburs, Effect of cerebrocrast on the lymphocytes blast transformation activity in normal and streptozotocin-induced diabetic rats, Cell Biochem. Funct. 17 (1999) 89–96.
[7] L.P. Vartanian, E.V. Ivanov, S.F. Vershinina, A.B. Markochev, E.A. Bisenieks, G.F. Gornaeva, Iu.I. Pustovalov, T.V. Ponomareva, Antineoplastic effect of glutapyrone in continual gamma-irradiation of rats, Radiat. Biol. Radioecol. 44 (2004) 198–201.
[8] N.M. Emanuel, L.K. Obukhova, G.Ia. Duburs, G.D. Tirzit, Ia.R. Uldrikis, Geroprotective activity of 2,6-dimethyl-3,5-diethoxycarbonyl-l,4-dihydropyridine, Dokl. Akad. Nauk SSSR 284 (1998) 1271–1274.
[9] E.V. Ivanov, T.V. Ponomarjova, G.N. Merkusev, G.J. Dubur, E.A. Bisenieks, A.Z. Dauvorte, E.M. Pilscik, A new skin radioprotec-

tive agent Diethon (experimental study), Radiobiol. Radiother. (Berl.) 31 (1990) 69–78.
[10] E.V. Ivanov, T.V. Ponomareva, G.N. Merkushev, I.K. Romanovich, G.Ia. Dubur, E.A. Bisenieks, Ia.R. Uldrikis, Ia.Ia. Poikans, Radiation modulating properties of derivatives of 1,4-dihydropyridine and 1,2,3,4,5,6,7,8,9,10-decahydroacridine-1,8 dione, Radiat. Biol. Radioecol. 44 (2004) 550–559.
[11] R.I. Goncharova, T.D. Kuzhir, O.V. Dalivelya, G.Ya. Dubur, Ya.P. Uldrikis, 1,4-Dihydroisonicotinic acid derivatives, inhibitors of chemical mutagenesis, Vestnik RAMN 1 (1995) 9–19.
[12] V. Klusa, S. German, Alcoholized maternal rat offspring: model for testing of physical and psychoemotional neurodeficit, Scan. J. Lab. Anim. Sci. 23 (1996) 403–409.
[13] R.I. Goncharova, T.D. Kuzhir, A comparative study of the antimutagenic effects of antioxidants on the chemical mutagenesis in *Drosophila melanogaster*, Mutat. Res. 214 (1989) 257–265.
[14] T.D. Kuzhir, Antimutagens and chemical mutagenesis in Higher Eukaryotic Systems, Tecknologia, Minsk, 1999.
[15] R. Goncharova, S. Zabrejko, O. Dalivelya, T. Kuzhir, Anticlastogenicity of two derivatives of 1,4-dihydroisonicotinic acid in mouse micronucleus test, Mutat. Res. 496 (2001) 129–135.
[16] R.I. Goncharova, Remote consequences of the Chernobyl disaster: assessment after 13 years, in: E.B. Burlakova (Ed.), Low doses of radiation: are they dangerous?, Nova Science Publishers Inc., Huntington, New York, 2000, pp. 289–314.
[17] G. Mickisch, S. Fajta, G. Keilhauer, E. Schlick, R. Tschada, P. Alken, Chemosensitivity testing of primary human renal cell carcinoma by a tetrazolium based microculture assay (MTT), Urol. Res. 18 (1990) 131–136.
[18] M.H.L. Green, J.E. Lowe, S.A. Harcourt, P. Akinluyi, T. Rowe, J. Cole, A.V. Anstey, C.V. Arlett, UV-C sensitivity of unstimulated and stimulated human lymphocytes from normal and xeroderma pigmentosum donors in the comet assay: a potential diagnostic technique, Mutat. Res. 273 (1992) 137–144.
[19] R.R. Tice, E. Agurell, D. Anderson, B. Burlinson, A. Hartmann, H. Kobayashi, Y. Miyamae, E. Rojas, J.-C. Ryu, Y.F. Sasaki, Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing, Environ. Mol. Mutagen. 35 (2000) 206–221.
[20] O. Palyvoda, I. Mukalov, J. Polanska, A. Wygoda, L. Drobot, M. Widel, J. Rzeszowska-Wolny, Radiation-induced DNA damage and its repair in lymphocytes of patients with head and neck cancer and healthy donors, Anticancer Res. 22 (2002) 1721–1726.
[21] A. Collins, S. Duthie, V. Dobson, Direct enzymic detection of endogenous oxidative base damage in human lymphocyte DNA, Carcinogenesis 14 (1993) 1733–1735.
[22] A. Collins, I. Fleming, C. Gedik, In vitro repair of oxidative and ultraviolet-induced DNA damage in supercoiled nucleoid DNA by human cell extract, Biochem. Biophys. Acta 1219 (1994) 724–727.
[23] M.F. Kotturi, D.A. Carlow, J.V. Lee, H.J. Ziltener, W.A. Jefferies, Identification and functional characterization of voltage-dependent calcium channels in T lymphocytes, J. Biol. Chem. 278 (2003) 46949–46960.
[24] D. Wolf, V. Rotter, Major deletions in p53 tumor antigen cause lack of p53 expression in HL-60 cells, Proc. Natl. Acad. Sci. U.S.A. 82 (1985) 790–794.
[25] Z. Han, D. Chatterjee, D.M. He, J. Early, P. Pantazis, J.H. Wyche, E.A. Hendrickson, Evidence for a G2 ckeckpoint in p53-independent apoptosis induction by X-irradiation, Mol. Cell. Biol. 15 (1995) 5849–5857.
[26] C. Unger, H. Eibl, D.J. Kim, F.A. Fleer, J. Kotting, H.H. Bartsch, G.A. Nagel, K. Pfizenmaier, Sensitivity of leukemia cell lines to cytotoxic alkyl-lysophospholipids in relation to O-alkyl cleavage enzyme activities, J. Natl. Cancer Inst. 78 (1987) 219–222.
[27] D. Berkovic, E.A.M. Fleer, J. Breass, J. Pförtner, E. Schleyer, W. Hiddemann, The influence of 1-β-arabinofuranosylcytosine on the metabolism of phosphatidylcholine in human leukemic HL 60 and Raji cells, Leukemia 11 (1997) 2079–2086.
[28] S. Loft, H.E. Poulsen, Cancer risk and oxidative DNA damage in man, J. Mol. Med. 75 (1996) 67–68.
[29] A. Velena, J. Zilbers, G. Duburs, Derivatives of 1,4-dihydropyridines as modulator of ascorbate-induced lipid peroxidation and high-amplitudes swelling of mitochondria, caused by ascorbate, sodium linoleate and sodium pyrophosphate, Cell Biochem. Funct. 17 (1999) 237–252.
[30] D. Mantle, V.B. Patel, H.J. Why, S. Ahmed, I. Ratman, W. MacNee, W.S. Wassif, P.J. Richardson, V.R. Preedy, Effects of lisinopril and amlodipine on antioxidant status in experimental hypertension, Clin. Chim. Acta 299 (2000) 1–10.
[31] M. Inouye, T. Mio, K. Sumino, Nivadipine protects low-density lipoprotein cholesterol from in vivo oxidation in hypertensive patients with risk factors for atherosclerosis, Eur. J. Clin. Pharmacol. 56 (2000) 35–41.
[32] M. Ziegler, New functions of a long-known molecule. Emerging roles of NAD in cellular signaling, Eur. J. Biochem. 267 (2000) 1550–1564.