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Effects of acetylsalicylic acid and a new pyrazine derivative *PD-101* on sister chromatid exchange frequency and cell kinetics in cultured human lymphocytes

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Abstract. Acetylsalicylic acid (ASA) and α-2-pyrazylidene-α-cyano N-butyl acetamide (*PD-101*), a new antiaggregatory pyrazine derivative were tested for their genotoxicity in human lymphocytes in vitro using the sister chromatid exchange (SCE) technique. Both compounds were found to be inactive in inducing SCE in concentration from 1 μM up to 1000 μM. The agents displayed inhibitory effect on cell kinetics.

Key words: acetylsalicylic acid, cell cycle kinetics, clastogenicity in vitro, pyrazine non-carboxylic acid derivative, sister chromatid exchange.

Introduction

Recently anti-inflammatory, analgesic and antithrombotic properties were observed in a new group of compounds: non-carboxylic acid derivatives of pyrazine characterised by an active methylene moiety. These properties of the agents were predicted according to their structure based on quantitative structure activity relationships (QSAR) (KALISZAN et al. 1985). Antiaggregation effects of new pyrazine acids were compared with the mode of action of ASA as a reference agent (PETRUSEWICZ et al. 1995). The compound marked as *PD-101* (α-2-pyrazylidene-α-cyano N-butyl acetamide) was found to have especially high antiaggregation activity (KALISZAN et al. 1985, PETRUSEWICZ

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et al. 1995). The pharmacological mechanism of action of the new pyrazine derivative is unknown and it has not been tested for mutagenicity so far.

There were contradictory results of cytogenetic analysis of different cell lines exposed to ASA when chromosome aberrations were investigated (MAUER et al. 1970, SANKAR et al. 1971, WATANABE 1982, MULLER et al. 1991). Mutagenetic test SCE was done only once in vivo on bone marrow cells of mice (GIRI et al. 1996). In that experiment only the highest dose of ASA showed a significant increase in SCE when compared with the control. On the other hand a single oral dose of ASA up to 350 mg kg^{-1} did not cause a significant increase in the frequency of SCE. In another experiments on cultured human lymphocytes, obtained from patients treated with ASA, there were no observed differences in frequency of SCE before and after treatment (OZKUL et al. 1996).

The aim of this work was to ascertain whether ASA and a new pyrazine derivative display the mutagenetic effect on human lymphocytes in vitro and have an influence on their proliferation kinetics.

Material and methods

The tested compounds were first dissolved in dimethylsulfoxide (DMSO) so that the planned amount per 5 mL of cell culture was contained in 10 µL. The working concentrations were: 1000, 500, 100, 10 and 1 µM (both compounds are known to elicit pharmacological effects at concentrations about 300 µM). Acetylsalicylic acid was obtained from Polpharma, Starogard Gdański, Poland and *PD-101* (α-2-pyrazylidene-α-cyano N-butyl acetamide) was synthesised and patented by PILARSKI and FOKS (1986).

Cultures of human lymphocytes were prepared from fresh heparinized peripheral blood (0.5 mL) suspended in a medium containing 3.5 mL Eagle's medium (GIBCO, Gaithersburg, USA), 1 mL inactivated calf serum (Wytwórnia Surowic i Szczepionek, Lublin, Poland), 300 µL phytohaemagglutinin (Biomed, Cracow, Poland), 15 µM bromodeoxyuridine (BrdU) (Serva Feinbiochemicals, Heidelberg, Germany). During the manipulations and culture the tubes were protected from light. After 24 hrs of incubation (37°C) 10 µL of solutions of the tested compounds were added.

Every compound was tested twice (experiment A and B) in similarly done set cultures, but with 10 µL of pure DMSO added instead of a solution of the tested compound to those which served as the negative control. Mitomycin C (BDH Chemicals Ltd. Pool, England) at the concentration 10^{-7} M was used

as the positive control (KATO, SHIMADA 1975). Two hours before the end of the culture time (70 hrs) 50 μL of Colcemid (GIBCO, Gaithersburg, USA) were added. Then cultures were continued until 72 hrs. The cultures were harvested according to standard protocols. Chromosomes were stained by the method described by WOLFF and PERRY (1974). SCE were counted in 60 completed metaphases after 2nd cell cycle (M_2) and 10-30 metaphases after 3rd cycle (M_3) from each culture.

Sister chromatid exchange values were calculated as a frequency of SCEs per cell. Additionally, high frequency cells (HFC) (10% of cells with the highest frequency of SCE) and base line (BL) (remaining 90% of cells) were counted. To determine the cell kinetics, the proliferation index (PI) was calculated according to the formula (PRESTON et al. 1987):

$$PI = (M_1 + 2M_2 + 3M_3) / \text{Total number of metaphases,}$$

where M_1, M_2, M_3 are number of metaphases after 1st, 2nd and 3rd cell cycle, respectively. The results of PI were analysed by the χ^2 test.

Results

The frequency of SCE in controlled cultures was: 5.1 ± 1.2 in the negative control (with DMSO) and 14.10 ± 3.46 in the positive control (with mitomycin C). The average numbers for SCE were 4.19 ± 1.08 for ASA and 4.57 ± 1.37 for *PD-101*. There was no statistically significant difference in SCE numbers in M_2 metaphases as well as in HFC and BL in comparison with control cultures.

The results of cell kinetics of lymphocytes in vitro exposed to ASA and *PD-101* are summarised in Tables 1 and 2.

In the cultures treated with ASA and *PD-101* a decrease in M_2 and especially M_3 metaphases in comparison with the control cultures was observed. These inhibition effects on cell proliferation were recorded even at the lowest doses for both compounds (ASA and *PD-101*) but were not dose-dependent.

Discussion

The results obtained from cytogenetic mutagenic tests of ASA which have been reported so far are contradictory. One research group reported a positive result in the chromosomal aberration (CA) test using CHL cell line and human lymphocytes in vitro at concentrations 0.5-5.5 μM (JARVIK, KATO 1968).

Table 1. Number of metaphases and proliferation index in human lymphocytes cultured in vitro after the exposure to acetylsalicylic acid

Concentration [μM]	Number of metaphases						Proliferation index (χ^2, P)	
	Exp. A			Exp. B			Exp. A	Exp. B
	M_1*	M_2*	M_3*	M_1	M_2	M_3		
1	76	23	1	78	22	0	1.25 (11.25, P<0.01)	1.22 (19.91, P<0.001)
10**	67	12	1	84	15	1	1.18 (18.27, P<0.001)	1.17 (26.64, P<0.001)
100	81	17	2	85	14	1	1.19 (13.73, P<0.01)	1.16 (27.88, P<0.001)
500	86	13	1	72	28	0	1.15 (20.82, P<0.001)	1.28 (14.18, P<0.001)
1000	nd	nd	nd	76	22	2	nd	1.26 (14.93, P<0.001)
Control	58	32	10	50	43	7	1.52	1.57

* M_1, M_2, M_3 – metaphase after 1st, 2nd and 3rd cell cycle; nd – no data

** For concentration 10 μM (experiment A) it was not possible to find requested 100 metaphases

However, in other experiments, when ASA concentration varied from 0.4 to 1.65 mM, no chromosomal aberrations in human lymphocytes were observed (MAUER et al. 1970, WATANABE 1982). Negative results were also obtained in the CA test using rat cells, mouse lymphocytes and V79 cell lines (SANKAR et al. 1971, MULLER et al. 1991).

The SCE test in vivo was used only once for bone marrow cells of mice. ASA was administered *i.p.* (25, 50, 100 mg kg^{-1}) and only the highest dose caused a significant increase in SCE frequency as compared to the control (GIRI et al. 1996). In cultured human lymphocytes SCE technique was used in patients treated with ASA for two weeks. Results obtained from that experiment indicate that treatment with ASA does not induce SCE (OZKUL et al. 1996). Our study confirmed a lack of mutagenic effect of ASA in SCE test on chromosomes derived from human lymphocytes in vitro. In addition, a new pyrazine derivative *PD-101* did not induce SCE in the same experimental model.

Table 2. Number of metaphases and proliferation index in human lymphocytes cultured in vitro after the exposure to α-2-pyrazylidene-α-cyano N-butyl acetamide

Concentration [μM]	Number of metaphases						Proliferation index (χ^2, P)	
	Exp. A			Exp. B			Exp. A	Exp. B
	M_1*	M_2*	M_3*	M_1	M_2	M_3		
1	93	7	0	76	21	3	1.07 (31.28, P<0.001)	1.27 (8.47, P<0.05)
10	64	34	2	83	14	3	1.38 (4.72, P<0.1)	1.20 (15.25, P<0.001)
100	76	21	3	78	21	1	1.27 (6.81, P<0.01)	1.23 (12.59, P<0.01)
500	nd	nd	nd	76	21	3	nd	1.27 (8.47, P<0.05)
Control	60	31	9	58	32	10	1.49	1.52

*M_1, M_2, M_3 – metaphase after 1st, 2nd and 3rd cell cycle; nd – no data

The analysis of cell cycle kinetics of lymphocytes by calculating the proliferation index (PI), showed that there was an inhibitory effect of both substances. This effect occurred even at the lowest concentrations used (1 μM) and it was not dose-dependent. The decrease of PI caused by ASA was shown for concentrations (1-1000 μM) similar to those presented in other reports (MAUER et al. 1970, WATANABE 1982). It was observed that ASA depress lymphocytes transformation by inhibition of DNA synthesis in mitogen-stimulated lymphocytes (PANUSH, ANTHONY 1976, DEWSE 1977). In vivo experiments with lymphocytes obtained from ASA-treated mice, further cultured in the presence of ASA, either showed an inhibition of mitogen-induces proliferation (BARASOAIN et al. 1980). On the other hand different results were found by other authors ASA in various concentrations had no influence on lymphocytes proliferation, stimulated with PHA (UHLENBRUCK et al. 1993) or even stimulates the proliferative responses of T-lymphocytes (HASIA et al. 1989). In the face of such contradictory results the phenomenon of lymphocytes proliferation inhibition by ASA and *PD-101* requires further experiments.

Conclusions

Acetylsalicylic acid (ASA) and the new pyrazine derivative (*PD-101*) did not induce SCE in vitro in chromosomes obtained from human lymphocytes. Both substances showed an inhibitory effect on the proliferation index. This effect occurred even at the lowest concentration and was not dose-dependent.

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