

Influence of diethylenetriamine (DETA) and sodium nitroprusside (NaNP) on sister chromatid exchange frequency and cell kinetics in cultured human lymphocytes

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Abstract. Diethylenetriamine (DETA) and Sodium Nitroprusside (NaNP), the exogenous NO-generating compounds, 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 concentrations from 0.3 to 30 pM. However both compounds displayed an inhibiting effect on cell kinetics.

Key words: cell cycle kinetics, diethylenetriamine, myocardial ischemia, restenosis, sister chromatid exchange, sodium nitroprusside.

Both DETA and NaNP belong to the group of exogenous NO-generating compounds, which seem to have therapeutic advantages in providing restenosis after angioplasty in patients with myocardial ischemia. Diethylenetriamine is a novel agent, but sodium nitroprusside is a well known clinical drug usually used to obtain rapid relaxant effect due to vascular crisis and dissecting aortic aneurysm. (BREDT, SNYDER 1994, WILLIAMSON et al. 1996). There are no published data on cytogenetic tests of human cells exposed *in vitro* to DETA and/or NaNP.

The aim of this work was to ascertain whether both compounds have any mutagenic effect on chromosomes of human lymphocytes *in vitro* and whether they have any influence on their proliferation kinetics.

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The tested compounds were dissolved in *aqua pro iniectio* and the working concentrations were: 0.3 pM, 3 pM, 30 pM, 300 pM, as both compounds are known to elicit biological effect at these concentrations (personal information of Professor A. DEMBIŃSKA-KIEĆ). Diethylenetriamine was obtained from GibcoBRL, and sodium nitroprusside from SIGMA. Cultures of human lymphocytes were prepared from fresh, heparinized, peripheral blood (0.5 mL) suspended in a standard medium containing 3.5 mL Eagle's medium (Gibco,BRL), 1 mL inactivated calf serum (Wytwórnia Surówic i Szczepionek, Lublin, Poland), 300 μ L PHA (Biomed, Kraków, Poland), and 15 μ M bromodeoxyuridine (BrdU) (SERVA). During the manipulations, culture flasks were protected from light. After 24 hrs of incubation (37°C) 10 μ L of solutions of the tested compound were added. Every compound was tested twice in similarly done set cultures; as the negative control water was used. 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 μ L of colcemid were added. The cultures were continued until 72 hrs, and after that time cultures were harvested according to standard protocols. Chromosomes were stained by the method described by WOLFF and PERRY (1974). SCE were counted

Table. The frequency of sister chromatid exchange (SCE) and proliferation index (PI) for all DETA and NaNP concentrations and controls

Concentrations [pM]	DETA		NaNP	
	average SCE per metaphase	proliferation index (PI)	average SCE per metaphase	proliferation index (PI)
0.3	4.45	1.15	4.70	1.29
3	5.65	1.20	5.45	1.11
30	7.00	1.08	6.60	1.10
positive control	8.75	1.34	8.30	1.36
negative control	4.67	1.39	4.90	1.51

To obtain these results 2500 metaphases were analysed.

in metaphases after the second and the third cell division. To determine cell kinetics, the proliferation index (PI) was calculated with the formula (PRESTON et al. 1987): $PI = (M_1 + 2M_2 + 3M_3)/100$ metaphases, where M_1 , M_2 , M_3 are the numbers of metaphases after the first, second and third cell cycle, respectively. The results of PI were analysed by the χ^2 test. The frequency of SCE in control cultures and for testing compounds are shown in the Table. Both tested compounds did not induce SCE in any of the tested concentrations. In addition, for the highest concentration of 300 pM a strong inhibitory effect was observed – only a few metaphases were found. However, both of them caused strong cell proliferation inhibition

which was statistically significant ($p < 0.001$). For all cultures treated with DETA or NaNP a decrease in M_2 and especially M_3 metaphases in comparison with the control cultures was observed.

Although nitric oxide and its donors are known for their relaxant effect on the smooth muscle, especially on myocardium, they may be metabolised to ONOO – which is responsible for defects of lipids, proteins and DNA damages (CULOTTA, KOSHLAND 1992).

Our results show that both compounds: diethylenetriamine (DETA) and sodium nitroprusside (NaNP) did not any display mutagenic effect on chromosomes of human lymphocytes at concentrations: 0.3 pM, 3 pM and 30 pM. For all tested concentrations cell proliferation was strongly inhibited. The significance of this phenomenon in providing restenosis through limiting lymphocyte proliferation during inflammatory reaction in angioplasty remains to be elucidated.

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