GENOTOXIC EFFECTS OF SOMAN AND OXIME HI-6: THE INDUCTION OF DNA BREAKS AND MICRONUCLEI IN MICE AND CHINESE HAMSTERS

[1]Rudolf ŠTĚTINA, [1]Radka NEČASOVÁ, [2]Petr ŠMERÁK, [2]Ivo BÁRTA
[1]Department of Toxicology, Purkynje Military Medical Academy, Hradec Králové
[2]Charles University, Prague, 3rd Medical Faculty, Dept. General Biology and Genetics

Summary

Soman applied i.m. at doses corresponding to 20% or 60% of LD_{50} did not induce DNA breaks, but only endonuclease III sensitive sites (representing oxidised pyrimidines or abasic sites) in the DNA of lymphocytes and liver cells of male MNRI mice and Chinese hamsters. Significant amount of micronuclei was induced also in polychromatophilic erythrocytes in bone marrow. Neither of these effects was found after application of same doses of sarin related to its toxicity in vivo. Intramuscular application of 2% or 20% LD_{50} HI-6 oxime induced single strand DNA breaks and endonuclease III sensitive sites. Micronuclei were induced as well. Results suggest that HI-6 oxime might be mildly genotoxic. Oxidative DNA damage might be induced by soman.

Introduction

Some published results (3) suggest, that sarin and soman could inhibit the DNA repair. During the "Gulf War" the former Czechoslovak troops detected measurable concentrations of nerve agents released into the air probably consequently to the bombing of Iraqi stores of chemical munition. An attention was focussed onto the mechanisms of action of these agents in connection with their possible role in so-called gulf war syndrome, a complex of health problems of gulf war veterans (4). The interest has increased also in antidotes against nerve chemical weapons. The aim of this study was to evaluate the possible genotoxicity of sarin, soman and HI-6 oxime. The genotoxicity was measured *in vivo* using males of NMRI mice and Chinese hamsters. The following parameters were folowed: 1) induction of DNA single strand breaks (SSB) and of the oxidative damage to DNA (oxidised DNA bases) in peripheral lymphocytes and in liver cells isolated from animals treated with HI-6, sarin, or soman, 2) induction of micronuclei in polychromatofilic erythrocytes in bone marrow with different doses of HI-6, sarin or soman.

Methods

Six weeks old males of NMRI mice or Chinese hamsters used in this study were obtained from Konárovice farm (Léčiva). HI-6 was dissolved and diluted in distilled water and applied i.m. in the dose corresponding to 2 % (13.8 mg/kg), or 20 % (138 mg/kg) LD_{50} for mice.

Sarin and soman were diluted in propylenglykol and applied i.m. in the dose corresponding to 20 % (sarin 0.0578 mg/kg, soman 0.024 mg/kg), or 60 % (sarin 0.113 mg/kg, soman 0.072 mg/kg) LD_{50} for mice. In the case of Chinese hamsters we have used doses of sarin 0.06 mg / kg and 0.02 mg/kg, in case of soman 0.024 mg/kg and 0.008mg/kg).

Animals were killed 2, 24, or 48 h after the application of the tested compound and SSB were measured using comet assay in lymphocytes and in liver cells (2). Micronuclei were estimated in polychromatophilic erythrocytes in bone marrow (1).

The statistical significance of micronuclei- induction was tested using analysis of variance (ANOVA) and t-test. The significance of DNA breaks induction was evaluated by Kruskal-Wallis test and Man-Whitney test.

Results

Genotoxicity of soman

Induction of DNA breaks. Soman increased the amount of endonuclease III -sensitive sites in the DNA significantly (endo III sites, which should represent oxidised pyrimidines, or abasic sites) from 0.2 to 0.62 in NMRI mice 2 h after application (See fig. 1). No remarkable increase was observed in liver cells (not shown). The significant increase of endo III sites in DNA of lymphocytes 24 h after the application of soman was found also in Chinese

hamsters, while no DNA damage was seen in liver cells of these animals (results not shown).

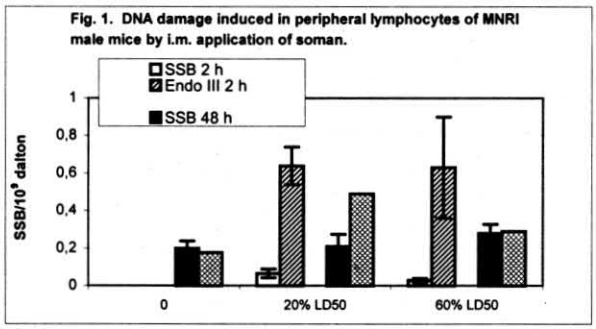

Fig. 1

Micronuclei. Soman induced significant increase in the amount of micronuclei in bone marrow cells of MNRI mice 48 h after the application from 1.98 to 5. (fig. 2). No such induction of micronuclei was observed in Chinese hamsters.

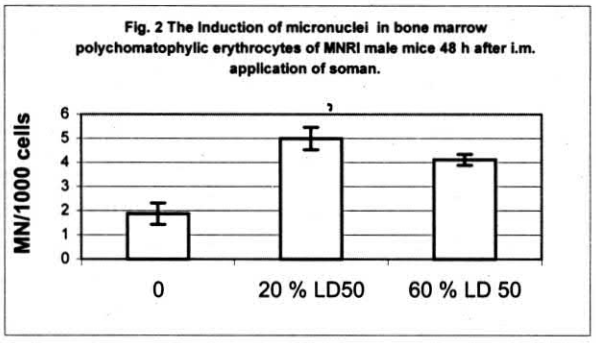

Fig. 2

Genotoxicity of sarin

Induction of DNA breaks. Sarin applied at doses corresponding to 20 % a 60 % LD_{50} did not induce significant amount neither SSB nor endo III sites in mouse or Chinese hamster lymphocytes. In liver cells we observed 2-fold increase of SSB and endo III sites 48 h after application, however, this increase was at the edge of statistical significance (results not shown).

Micronuclei Sarin did not induce micronuclei neither in mice nor in Chinese hamsters.

Genotoxicity of HI-6

Induction of DNA breaks. It is evident from results shown on fig. 3 that HI-6 enhanced SSB in mouse lymphocytes 3-5 times. This increase from 0.02 to 0.1 SSB/10^9 daltons is very low and lies within the range of variability of values among experiments. However, 24 hours after the application of the dose 2 % LD_{50} HI-6 we have observed statistically significant increase of endonuclease III- sensitive sites. An increase of endo III sites was observed also in liver cells after 24 h, but only after higher dose (20 % LD_{50}). Similar, but insignificant increase of SSB and endo III sites was found in Chinese hamster lymphocytes (data not shown).

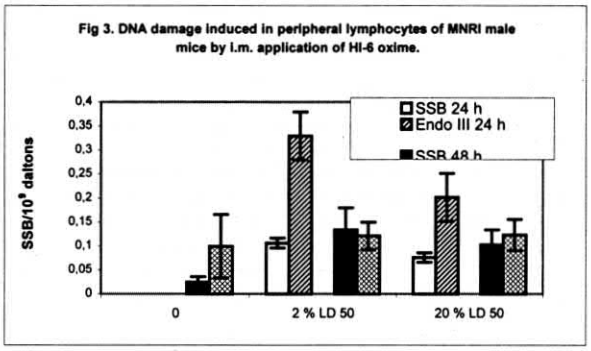

Fig. 3

Micronuclei. The possible low genotoxicity of HI-6 is also suggested by the results showing the induction of micronuclei (see fig. 4) at 48 h after the application of HI-6 to NMRI male mice. The increase of micronuclei from 3.1 in controls to 7 after the dose 2 % LD_{50} was significant. No such increase was observed in Chinese hamsters (results not shown).

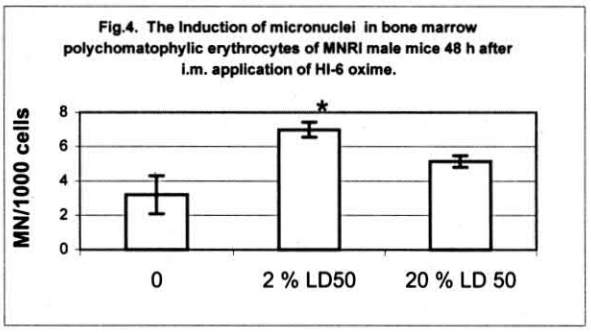

Fig. 4

Discussion

The genotoxicity of organophosphates and of oxime HI-6 is regarded as low and questionable (5). Present experiments showed a significant induction of DNA damage after the application of HI-6. This induction of SSB was found both in

lymphocytes and in liver cells in mice and Chinese hamsters. It is quite interesting, that the level of endonuclease III sensitive sites rather than the number of SSB was increased. Endonuclease III is known to cleave oxidised pyrimidines or abasic sites. What is the nature of the DNA lesions detected in our experiments can be hardly estimated on the basis of our results. However, it is possible, that HI-6 can react directly with DNA and so to form DNA breaks, or, that radicals are formed during its metabolism, which oxidise DNA (induction of endo-III sites). The induction of micronuclei can be supported by both these mechanisms.

Neither of two doses of soman did induce SSB, however, 2 h after the application a significant increase of endo III sites was found in lymphocytes and liver cells in MNRI mice. Similar results were obtained in Chinese hamsters, but 24 h after application. This result suggests, that soman does not react with DNA directly, however, it may induce the damage via forming some radicals damaging DNA. This radical- induced lesion of the DNA can be detected with endonuclease III. In mice this damage was found already 2 h after application of soman, i.e. during the probable hypoxic stage. It cannot be excluded, that the radical induced- damage appears by the mechanism hypoxia-reoxygenation. Soman also induced significant amount of micronuclei in bone marrow. All these results indicate, that some form of genotoxic or radical-induced damage of cells (nerve or other cells) caused by soman intoxication can play a role in the damage to the organism. This could be one of reasons of substantially higher toxicity of soman compared to sarin, which induced no such damage in our experiments at comparable doses regarding the general toxicity (same % of LD_{50}).

References

1. BÁRTA, I., et al.: Dose and time dependence of chromosomal aberration yields of bone marrow cells in male Chinese hamsters after a single i.p. injection of aflatoxin B_1. Mutation Res., 1990, vol. 244, p. 189-195.
2. COLLINS, AR., et al.: The comet assay: what can it really tell us? Mutation res., 1997, vol. 373, p. 183-193.
3. KLEIN, AK., et al.: The effect of in vitro exposure to the neurotoxins sarin (GB) and soman (GD) on unscheduled DNA synthesis by rat hepatocytes. Toxiclol. Lett., 1987, vol. 38, p. 239-249.
4. KURT, T.: Epidemiologic association in US veterans between gulf war illness and exposure to anticholinesterases. Toxicol. Lett., 1998, Supl 1/95, p. 25.
5. PUTMAN, D., et al.: Genetic toxicology assessment of HI-6 dichloride. Environ. Mol. Mutagen., 1996, vol. 27, p. 152-161.

Correspondence: Rudolf Štětina
Department of Toxicology
Purkyně Military Medical Academy
Třebešská 1575
500 01 Hradec Králové
Czech Republic

Received: 11. 10. 2001