

## Studies on the nature of genotoxic and cytotoxic effects induced by hydralazine

Jadwiga Marczevska<sup>✉</sup> and Jadwiga H. Kozirowska

Drug Institute, Chelmska 30/34, 00-725 Warsaw, Poland

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**The nature of genotoxic and cytotoxic effects induced by hydralazine was analyzed taking into account possible protection of cells by catalase, superoxide dismutase and dimethyl sulfoxide. For the experiments designed to evaluate the influence of scavengers on the genotoxicity expressed as the SOS induction factor the *E. coli* PQ37 strain was used. The cytotoxic effects were investigated in V3 cells cultured *in vitro*. The genotoxicity and cytotoxicity of hydralazine were suppressed by catalase in a dose-dependent manner but they were enhanced by superoxide dismutase. No protective effect of dimethyl sulfoxide was observed. Our results indicate that H<sub>2</sub>O<sub>2</sub> plays an essential role in the genotoxicity and cytotoxicity of hydralazine.**

Hydrazine and its derivatives are used in industry, agriculture and medicine. Large amounts of carcinogenic hydrazines are present in edible mushrooms. Most hydrazines that have been tested are carcinogens [1].

Hydralazine (1-hydrazinophthalazine), an aromatic hydrazine derivative, is a hypotensive drug. Induction of a disease resembling systemic lupus erythematosus [2-4] is an undesirable side effect of hydralazine treatment. Hydralazine was shown to be mutagenic and genotoxic in bacterial test systems [5-9]. An increased incidence of lung tumors in mice treated with hydralazine has been reported [10, 11]. Other studies suggested an association between exposure to hydralazine and human cancer [12, 13]. The drug was found to generate oxygen free radicals [14-16] and to alter the activity of antioxidant enzymes in fibroblasts cultured *in vitro* [17]. These findings suggest that reactive oxygen species may be relevant to the expression of deleterious effects produced by

the drug. However, the exact role of these species remains to be clarified. In this study we attempted to elucidate the molecular basis of genotoxicity and cytotoxicity of hydralazine. In order to find whether reactive oxygen species are involved in these processes we investigated the modifying effects of scavengers on the genotoxicity of the drug (expressed as SOS induction factor) in bacteria as well as on the hydralazine induced cytotoxicity in mammalian cells.

### MATERIALS and METHODS

**Chemicals.** Hydralazine (1-hydrazinophthalazine), catalase (EC 1.11.1.6, bovine liver, CAT), superoxide dismutase (EC 1.15.1.1, SOD) *o*-nitrophenyl- $\beta$ -D-galactopyranoside, the substrate for galactosidase, and *p*-nitrophenyl phosphate disodium salt, the substrate for alkaline phosphatase were obtained from Sigma; 199 medium, mini-

<sup>✉</sup>Corresponding author. Tel. (022) 41-39-91; Fax. (022) 41-06-52

mum Eagle's medium 1959 (MEM) and phosphate buffered saline (PBS), pH 7.2, from Manufacture of Sera and Vaccines (Lublin, Poland), fetal calf serum from Bioproduct (Budapest, Hungary), dimethyl sulfoxide (DMSO), gas chromatographic grade, was from Merck. All substances tested were dissolved in water.

**SOS chromotest.** In the SOS chromotest the *Escherichia coli* PQ37 strain was used. This strain carries a *sfiA::lacZ* fusion and has a deletion in normal *lac* region, so that  $\beta$ -galactosidase level is strictly dependent on the *sfiA* expression. The following genetic markers of this strain are important to the test: *uvrA*, *Pho<sup>c</sup>*, *rfa*. A *uvrA* mutation renders the strain deficient in excision repair and results in the increased response to certain DNA-damaging agents. A *rfa* mutation renders the cell envelope more permeable to a number of compounds. The level of alkaline phosphatase (*Pho<sup>c</sup>*), the synthesis of which is independent of the SOS control, served as control for toxicity. A detailed description of the tester strain and its construction has been given by Quillardet & Hofnung [18]. Overnight cultures of *E. coli* PQ37 were diluted 1:10 into fresh medium and grown for 2 h at 37°C in a shaking incubator. SOD, catalase and DMSO were added to the tester strain together with hydralazine and incubated for 2 h at 37°C in a shaking incubator. The measurements of  $\beta$ -galactosidase and alkaline phosphatase activities and determination of the SOS induction factor (IF) were performed according to Quillardet & Hofnung [18]. The scavengers at concentrations tested (indicated in Figs. 2 and 3) did not influence the viability of bacterial cells, as measured by the activity of  $\beta$ -galactosidase.

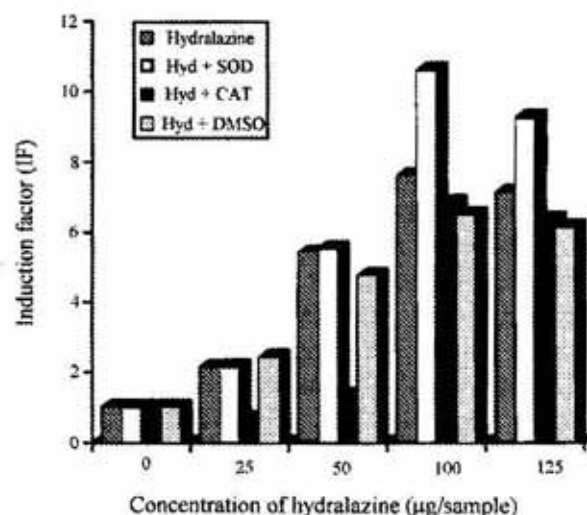
**Cell cultures.** V<sub>3</sub> cells (an African Green monkey kidney cell line) were routinely grown in monolayer culture in 199 medium with Earle's salts, containing 8% fetal calf serum, penicillin 100 U/ml and streptomycin 100  $\mu$ g/ml.

**Assays for cytotoxicity.** The cultures were refed with appropriate media (control cultures) or with media containing hydralazine at different concentrations, 24 h after seeding of  $1 \times 10^5$  cells in 25 cm<sup>2</sup> plastic flasks. After 24 h sets of cultures were washed with

PBS. Cell number and viability were determined in a haemocytometer (Coulter Electronics Inc., U.S.A.) by trypan blue exclusion using a 0.4% solution of trypan blue in PBS. Cell suspensions (0.5 ml) were exposed to trypan blue solution (0.1 ml) for 5 min. Series of experiments were carried out that involved the preincubation of cells with scavengers for different periods of time (2 h with catalase or DMSO and 30 min with SOD). This was followed by washing the cells with PBS and replacing them in the medium containing hydralazine. In another series of experiments cultures of V<sub>3</sub> cells (24 h old) were washed twice with PBS and treated with hydralazine plus scavengers. After 24 h cytotoxicity assays were performed as above. The applied doses of scavengers (shown in Table 1) did not influence either the viability or the capacity of proliferation of V<sub>3</sub> cells.

## RESULTS

In order to study the effects of scavengers on the hydralazine induced SOS response, *E. coli* PQ37 bacteria were incubated in the absence or presence of scavengers. Figure 1 presents the average data from six experi-



**Figure 1. Genotoxicity of hydralazine in the absence and presence of scavengers of active oxygen species.**

The effects of SOD, CAT and DMSO at the concentration of 300 U/sample, 889 U/sample, 70  $\mu$ mol/sample, respectively, on induction factor (IF) of hydralazine.

ments that show the effects of catalase, SOD and DMSO on DNA damaging activity of hydralazine at different concentrations. The data show clearly that catalase suppressed by 69% or 76% the SOS response induced by hydralazine at the concentration of 25 or 50  $\mu\text{g}/\text{ml}$ , respectively. The effect was absent at higher concentrations of hydralazine. On the other hand, superoxide dismutase enhanced by 38% and 29%, respectively, the SOS response induced by 100 or 125  $\mu\text{g}/\text{sample}$  of hydralazine. The presence of DMSO did not influence the SOS response induced by the drug.

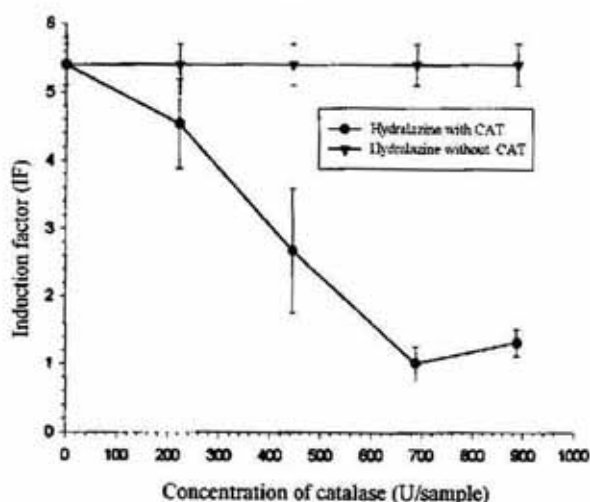
In a further study the dependence of the effects of different concentrations of catalase or superoxide dismutase on the SOS response induced by a set dose of hydralazine was evaluated. As can be seen from Fig. 2, catalase at a concentration range from 222 to 889 U/sample diminished the genotoxic effects of hydralazine. By contrast (Fig. 3) superoxide dismutase at a concentration range from 300 to 900 U/sample enhanced the SOS response of bacteria. Both kinds of effects were dose dependent.

To study the cytotoxic effect of hydralazine experiments were carried out on  $V_3$  cells exposed to hydralazine at different concentrations for 2 h. Viable cells were counted 24 h thereafter. The  $\text{EC}_{50}$  value (concentration

of the drug required to diminish viable cells density to 50%) was 427  $\mu\text{g}/\text{ml}$ . This concentration was applied in the experiments designed to evaluate the possible influence of scavengers. As presented in Table 1 the co-administration of catalase at concentration of 500 U/ml or 750 U/ml reduced significantly the cytotoxicity of the drug. Preincubation of cells with catalase followed by placing them in the hydralazine containing medium reduced the drug cytotoxicity in a similar manner. Under the experimental conditions applied no protective effect of DMSO was observed (Table 1). The results presented in Table 1 show also that superoxide dismutase at a concentration of 200 or 300 U/ml enhanced significantly the cytotoxicity of hydralazine in both kinds of experiments.

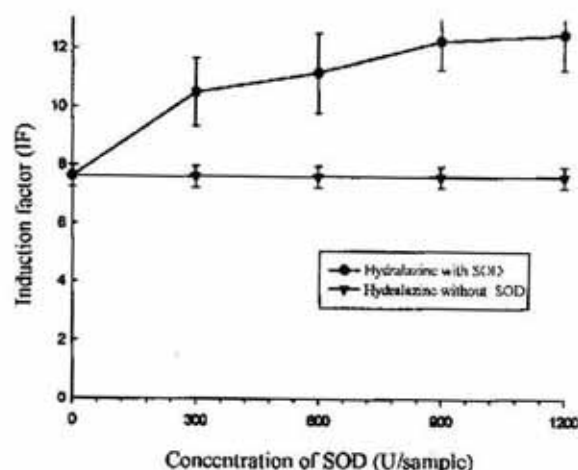
## DISCUSSION

The production of oxygen free radicals by hydralazine in the presence of metal ions were studied by Sinha & Patterson [14]. According to these authors,  $\text{H}_2\text{O}_2$  or  $\cdot\text{OH}$  radicals generated by hydralazine induce the aldehyde groups formation in DNA, presumably as a result of disruption of deoxyribose moieties, which in turn may lead to DNA degradation [19]. Formation of aldehyde was



**Figure 2.** Effect of catalase on the induction by hydralazine of the SOS response.

Hydralazine was applied at the concentration of 50  $\mu\text{g}/\text{sample}$  in the absence or presence of CAT. Each point represents the mean value  $\pm$ S.E. of six duplicate experiments.



**Figure 3.** Effect of superoxide dismutase on the induction by hydralazine of the SOS response.

Hydralazine was applied at the concentration of 100  $\mu\text{g}/\text{sample}$  in the absence or presence of SOD. Each point represents the mean value  $\pm$ S.E. of six duplicate experiments.

Table 1. Effects of scavengers on cytotoxicity of hydralazine towards V<sub>3</sub> cells

Compounds		Relative cell survival (Percent of control)	
Hydralazine (427 µg/ml)		50 ± 5	
Hydralazine + CAT (U/ml)			
125	Exposure	48 ± 4.1	Pretreatment
250		55 ± 4.6	55 ± 4.8
500		81 ± 7.9	63 ± 5.2
750		93 ± 8.4	72 ± 6.9
Hydralazine + DMSO (M)			
0.25		51 ± 4.5	98 ± 8.1
0.5		55 ± 4.8	52 ± 3.8
1		52 ± 7.5	49 ± 4.1
Hydralazine + SOD (U/ml)			
50		49 ± 4.3	54 ± 6.0
100		48 ± 7.0	51 ± 4.7
200		21 ± 4.9	48 ± 3.9
300		18 ± 3.7	32 ± 2.8
			15 ± 1.8

The cells were either exposed simultaneously with hydralazine and the indicated scavengers, or preincubated with the scavenger prior to treatment with the drug.

The time of exposure or pretreatment with CAT or DMSO was 2 h. The time of exposure or pretreatment with SOD was 30 min.

stimulated by SOD and inhibited by catalase. We have found that addition of SOD can enhance, while the addition of catalase can minimize, the genotoxicity of the drug. On the basis of the parallelism of our results with those of Sinha & Patterson [14] it is tempting to speculate that the formation of aldehyde in DNA contributes to damaging effects induced by hydralazine, described in our study. Since the addition of DMSO had no influence on hydralazine genotoxicity it may be assumed that, in our experiments, neither the extracellular nor the intracellular milieus were favorable for the generation of ·OH radicals. This situation may be explained by metal-coupling properties of hydralazine [20]. If so, the principal mechanism of hydralazine genotoxicity at the concentrations used in this study seems to involve the generation and the action of H<sub>2</sub>O<sub>2</sub>. A high

concentration of hydralazine was reported to bind native DNA and to alter its tertiary structure [21]. Our studies do not exclude the possibility that, at higher concentration of the drug, the lethal activity is due not only to the action of H<sub>2</sub>O<sub>2</sub> but also to its direct action on DNA, in a manner similar to the action of other intercalating agents [22, 23].

As demonstrated in this study, the cytotoxic action of hydralazine was suppressed in a dose-dependent manner by catalase and enhanced by superoxide dismutase. Addition of DMSO did not influence the SOS response. A comparison of the results obtained in the two assays shows that there is a parallelism between hydralazine toxicity in mammalian cells and genotoxicity in bacteria, and a parallelism between the effects of scavengers on hydralazine cytotoxicity and SOS induction. A plethora of studies indicate that in mam-

malian cells as well as in bacteria, oxidative stresses due to exposure to exogenous hydrogen peroxide can be lethal and genotoxic.

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