Acta Veterinaria (Beograd), Vol. 52. No. 5-6, 329-336, 2002.

UDK 619:612.015.11:636.91

THE EFFECT OF DIETHYLDITHIOCARBAMATE ON ANTIOXIDANT ENZYME ACTIVITIES IN THE BLOOD OF RATS

RADOJIČIĆ R*, BORKOVIĆ SLAVICA**, PAVLOVIĆ S**, NIKOLIĆ ALEKSANDRA**, BLAGOJEVIĆ D**, SAIČIĆ ZORICA** and SPASIĆ M **

** Institute of Physiology and Biochemistry, Faculty of Biology, University of Belgrade, ** "Siniša Stanković", Institute for Biological Research Department of Physiology, Belgrade, Serbia, Yugoslavia*

(Received 12. September 2002)

Diethyldithiocarbamate (DDC) exhibits a variety of effects including neurotoxic, radioprotective and sensitizing activity. It is a potent copper chelating agent used for the treatment of oxygen toxicity, as an imunomodulator in cancer therapy, as well as in HIV infected patients. In this study we examined the effect of DDC, a superoxide dismutase (SOD) inhibitor, on the activities of copper-zinc containing superoxide dismutase (CuZn SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GR) and glutathione-S-transferase (GST). Three hours after diethyldithiocarbamate treatment (1 g/kg b.m, i.p.) a significant decrease of SOD and increase of GR and GST activities were found in the blood of rats. A negative correlation between SOD and GR and a positive correlation between GR and GST activities were also obtained. DDC induced a concentration dependent increase of GR activity and NADPH consumption in an enzymatic assay in vitro.

The obtained results may be interpreted to indicate that a decrease in SOD activity in the blood of rats, after DDC administration, may be compensated for by changes in the activity of some other compounds suggesting that regulation of antioxidative defence is very complex.

Key words : diethyldithiocarbamate, rats, blood, antioxidant enzymes.

INTRODUCTION

Recent results have revealed that targeting superoxide dismutase (SOD) may be a promising approach to the selective killing of cancer cells and that a combination of SOD inhibitors with free radical producing agents may have clinical applications (Huang *et al.*, 2000). Diethyldithiocarbamate (DDC) is a potent copper chelating agent and one of the most widely used SOD inhibitors both *in vivo* and *in vitro* (Iciek and Wlodek, 2001). It is well known, that DDC can inhibit the tumorigenic effect of chemical carcinogens, due to induction of glutat-

hione-S-transferase (GST) activity (Krebs, 1968). It is evident that DDC changes thiol/disulfide balance acting as a donor of -SH groups, as well as changing the activity of enzymes of the glutathione redox cycle. Reduced glutathione (GSH) is considered to be the major thiol-disulfide redox buffer of the cell (Gilbert, 1990) and the redox environment might determine if a cell will proliferate, differentiate or die (Burdon, 1995, Hutter *et al.*, 1998).

As the recent results indicate SOD as a target for selective killing of cancer cells, the reexamination of acute DDC treatment seems to be reasonable. Since DDC affects antioxidant levels, the following antioxidant enzyme activities were studied: copper-zinc containing superoxide dismutase (CuZn SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), glutathione peroxidase (GSH-Px, EC 1.11.1.9) and glutathione reductase (GR, EC 1.6.4.2.) in red blood cells (RBCs), as well as glutathione-S-transferase (GST, EC 2.5.1.18) in the blood plasma of rats. The influence of DDC on GR activity was also studied using an *in vitro* assay.

MATERIALS AND METHODS

The experiments were carried out with female, 60 day old *Wistar albino* rats, weighing 207.5 ± 3.56 g. The animals were housed in individual cages under standard laboratory conditions. The rats were divided into two experimental groups: the first group was a control (C) and the second group was treated with DDC (1 g/kg b.m, i.p). After 3 hours the animals were sacrificed by decapitation between 8 and 10 A.M. Each experimental group consisted of 7 animals.

RBCs was separated from plasma and washed three times with 3 vol. of ice cold 155 mmol/L NaCl. Haemolysates containing about 50 g Hb/L were prepared according to McCord and Fridovich (1969) and used for the determination of CAT, GSH-Px and GR activities. CuZn SOD activity was measured in haemolysates according to the epinephrine method of Misra and Fridovich (1972). Hemoglobin was previously removed by the method of Tsuchihashi (1923). CAT activity was assayed as suggested by Beutler (1982) and the activity of GSH-Px was determined by the method of Maral *et al.* (1977). The activity of GR was assayed according to Glatzle *et al.* (1974) and this method was also employed in order to determine the effect of DDC on GR activity and NADPH consumption *in vitro*. GR (5 IU) was incubated for 10 min at 37°C, and calculated as standard activity. The DDC effects were measured by addition of increased amounts of DDC (1, 2, 5, 10, 15 and 20 μmol to the reaction mixture of 3 mL, respectively).

For the measurement of GST activity in the plasma, 1-chloro-2,4-dinitrobenzene was used as the substrate (Habig *et al.*, 1974).

The statistical significance of differences between the control and DDC treated group was tested by one way analysis of variance (ANOVA). Pearson's correlation coefficient between antioxidant enzyme activities was also estimated and $p<0.05$ was considered significant.

RESULTS

The results presented in Fig. 1 show that after (3 hours) treatment of rats with DDC (1 g/kg b.m, i.p) there was a significant inhibition of CuZn SOD activity (which

dismutate superoxide anion radicals to hydrogen peroxide) in the RBCs ($p<0.05$). At the same time, the activities of the hydrogen peroxide eliminating enzymes CAT and GSH-Px in the RBCs of rats treated with DDC were not significantly changed.

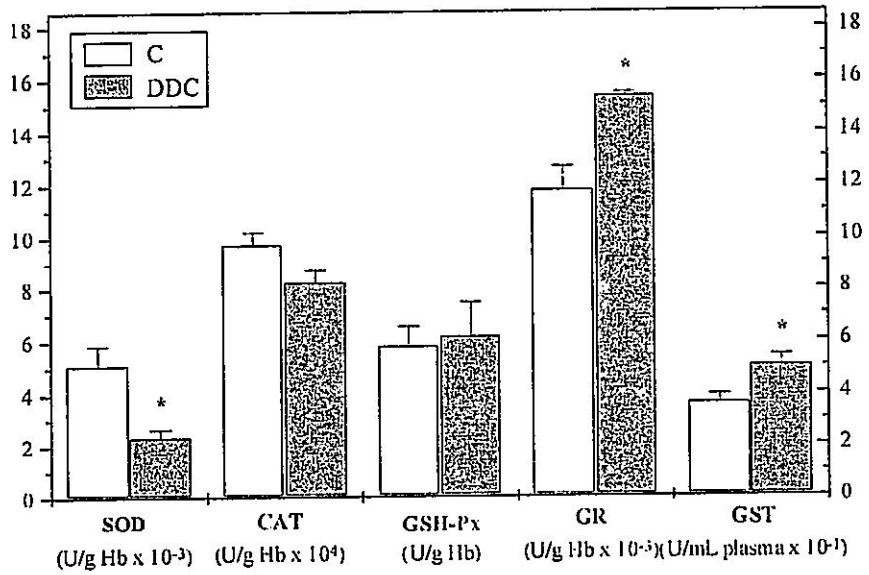

Figure 1. The activities of copper zinc containing superoxide dismutase (CuZn SOD, U/g Hb x 10^{-3}), catalase (CAT, U/g Hb x 10^{-4}), glutathione peroxidase (GSH-Px, nmol NADPH/min/g Hb) and glutathione reductase (GR, nmol NADPH/min/g Hb x 10^{-3}) in red blood cells, as well as glutathione-S-transferase (GST, nmol GSH/min/mL plasma x 10^{-1}) in the plasma of control rats and rats treated with 1 g DDC/kg b.m., i.p. during 3 hours. Means ± SE from 7 animals in each group. Significantly different from (C): *$p<0.05$.

The data obtained in our experiments show that the activity of GR was significantly increased (Fig. 1) in RBCs of rats treated with DDC ($p<0.05$). Pearson's analysis gave a significant negative correlation between SOD and GR activities as presented in Table 1 (-0.83, $p<0.05$).

Table 1. Pearson's correlation coefficients between antioxidant enzyme activities (under and above the diagonal, respectively).

	SOD	CAT	GSH–Px	GR	GST
SOD		0.38	-0.33	**-0.83** *	-0.79
CAT			0.64	-0.16	-0.08
GSH–Px				0.40	0.52
GR					**0.92** **
GST					

* $p<0.05$ ** $p<0.01$

The activity of GST in the plasma of rats treated with DDC was significantly higher ($p<0.05$) in respect to the controls (Fig. 1). At the same time, Pearson's analysis showed a significant positive correlation between GR and GST activities (0.92, $p<0.01$) which is presented in Table 1.

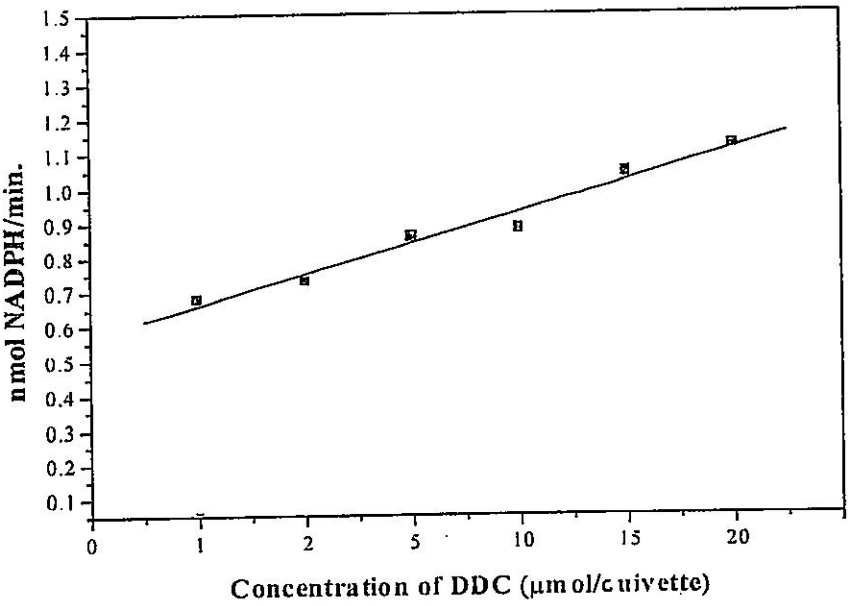

Figure 2. DDC effect on NADPH consumption in a GR enzymatic assay *in vitro*.

In the *in vitro* system for the determination of GR activity (Fig. 2) the addition of 1, 2, 5, 10, 15 and 20 μmol/cuivette of DDC gave a linear increase of NADPH consumption (0.68, 0.73, 0.86, 0.88, 1.04 and 1.12 nmol NADPH/min., respectively).

DISCUSSION

DDC is a powerful copper chelator and this is the proposed mechanism for CuZn SOD inactivation (Kelner *et al.*, 1989). The overproduction of superoxide anion radicals due to inhibition of CuZn SOD activity is considered to be the most important factor in the development of DDC induced pathogenesis (Wilson and Trambetta, 1999; Chen and Pan, 1998). The degree of CuZn SOD inhibition is also dose-dependent (Radojičić *et al.*, 1987). Various concentrations of DDC can induce inhibition of CuZn SOD from a very low level to levels over 95%.

It is well known, that GST is a multifunctional enzyme which represents the first line of defense in detoxification of many toxic compounds, such as carbon tetrachloride (Olatunde, 2000), heavy metals (Štajn et al., 1997) and some others.

Some authors have also shown that DDC and disulfiram (DS) induce an increase of GST activity in various organs of mice (Benson and Barreto, 1985). At the same time under *in vitro* conditions DS can reversibly inhibit GST activity *(alpha-, mu- and pi-* class), (Ploemen *et al.,* 1996)..

In our experiments, we observed a significant increase of GR activity in rat erythrocytes after i.p. injection of DDC. A direct effect of DDC on GR activity was also noted *in vitro.* Addition of increasing concentrations of DDC to the medium for the determination of GR activity led to a linear increase of NADPH consumption (Fig. 2). The fact that DDC may influence GSH metabolism and the activities of GSH-dependent enzymes was used for the explanation of its various pharmacological effects (Kumar et al., 1986). Intensive studies show that GSH brings about non-enzymatic reduction of DS via sulphydryl group exchange (Nagendra *et al.,* 1991), while GR *per se* does not reduce DS. The results of our study *in vitro* show that DDC brings about non-enzymatic reduction of GSSG to produce the mixed disulphide (DDC-SG) which is a substrate for GR. Our data indicate, that other mixed disulfides can also be substrates for GR. This quite opposite effect of DDC and DS on GSH metabolism and the activities of glutathione-dependent enzymes may be the explanation for the complex pharmacological activity of these substances. Also, a metabolic balance between DDC and its oxidized form DS may be the most importance for cytotoxic or cytoprotective effects of these compounds.

Acknowledgements: This research was supported by the Ministry of Science, Technologies and Development of Serbia, Yugoslavia, Grant No. 1669.

Address for correspondence:
Prof. Ratko M. Radojičić, Ph.D.
Institute of Physiology and Biochemistry
Faculty of Biology, University of Belgrade
Studentski Trg 16, 11000 Belgrade,
Serbia, Yugoslavia
E-mail: ratko@bf.bio.bg.ac.yu

REFERENCES

1. *Benson AM , Barreto PB,* 1985, Effects of disulfiram, diethyldithiocarbamate, bisethylxanthogen, and benzyl isothiocyanate on glutathione transferase activities in mouse organs. *Cancer Res,* 45, 4219-23.
2. *Beutler E,* 1982 , Catalase. In: Beutler E. editor , *Red Cell Metabolism, a Manual of Biochemical Methods,* New York: Grune and Stratton, 105-6.
3. *Burdon RH,* 1995, Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. *Free Radic Biol Med* 18, 775-94.
4. *Chen SH, Pan S,* 1998, Sources of superoxide radicals involved in the pathogenesis of diethyldithiocarbamate-induced gastric antral ulcer in rats. *J Formos Med Assoc* 97,131-4.
5. *Gilbert HF,* 1990, Molecular and cellular aspects of thiol-disulfide exchange. In: Meister A, editor, *Advances in Enzymology,* New York: Wiley Interscience; 69-73. .
6. *Glatzle D, Vulliemuier JP, Weber F, Decker K,* 1974, Glutathione reductase test with whole blood, a convenient procedure for the assessment of the riboflavin status in humans. *Experientia,* 30,665-8.
7. *Habig WH, Pubst MJ, Jakoby WB,* 1974, Glutathione S-transferase. *J Biol Chem,* 249, 7130-39.
8. *Huang P, Feng Li, Oldham E, Keating MJ, Plunkett W,* 2000, Superoxide dismutase as a target for the selective killing of cancer cells. *Nature,* 407, 390-95.
9. *Hutter DE, Till BG, Greene JJ,* 1998, Redox state changes in density-dependent regulation of proliferation. *Exp Cell Res,* 232, 435-38.

10. *Iciek M, Wlodek L,* 2001, Biosynthesis and biological properties of compounds containing highly reactive, reduced sulfane sulfur. *Polish J Pharmacol,* 53, 215-25.
11. *Kelner MJ, Bagnell R, Hale B, Alexander NM,* 1989, Inactivation of intracellular copper-zinc superoxide dismutase by copper chelating agents without glutathione depletion methemoglobin formation. *Free Radic Biol Med,* 6,355-60.
12. *Krebs HA,* 1968, The redox state of the nicotineamide adenine dinucleotides in rat liver homogenates. *Biochem J,* 108, 513-20.
13. *Kumar KS, Sancho AM, Weiss JF,* 1986, A novel interaction of diethyldithiocarbamate with the glutathione/glutathione peroxidase system. *Int J Radiat Oncol Biol Phys,* 12, 1463-67.
14. *Maral J, Puget K, Michelson AM,* 1977, Comparative study of superoxide dismutase, catalase and glutathione peroxidase levels in erythrocytes of different animals. *Biochem Biophys Res Commun,* 77,1525-35.
15. *McCord JMC, Fridovich I,* 1969, Superoxide dismutase. An enzymatic function for erythrocuprein (haemocuprein). *J Biol Chem,* 244, 6049-56.
16. *Misra HP, Fridovich I,* 1972, The role of the superoxide anion in the autooxidation of epinephrine and simple assay for superoxide dismutase. *J Biol Chem,* 247, 3170-75.
17. *Nagendra SN, Shetty KT, Subhash MN, Guru SC,* 1991, Role of glutathione reductase in disulfiram conversion to diethyldithiocarbamate. *Life Sci,* 49, 23-28.
18. *Olatunde FE,* 2000, Mechanisms for the hepatoprotective action of kolaviron: studies on hepatic enzymes, microsomal lipids and lipid peroxidation in carbon tetrachloride-treated rats. *Pharmacol Res,* 42, 75-80.
19. *Ploemen JP, Van Iersel ML, Wormhoudt LW, Commandeur JN, Vermeulen NP, Van Bladeren PJ,* 1996, In vitro inhibition of rat and human glutathione-S-transferase isoenzymes by disulfiram and diethyldithiocarbamate. *Biochem Pharmacol,* 52,197-204.
20. *Radojičić R, Spasić M, Milić B, Saičić ZS, Petrović VM,* 1987, Age-related differences in the effect of diethyldithiocarbamate and cyclohexamide on the liver copper, zinc-containing superoxide dismutase in the rat. Iugoslav *Physiol Pharmacol Acta,* 23, 227-33.
21. *Štajn A, Žikić RV, Ognjanović BI, Saičić ZS, Pavlović SZ, Kostić MM, Petrović VM,* 1997, Effect of cadmium and selenium on the antioxidant defense system in rat kidneys. *Comp Biochem Physiol,* 117C,167-72.
22. *Tsuchihashi M,* 1923, Zür Kenntnis der Blutkatalase. *Biochem Z,* 140, 65-74.
23. *Wilson A, Trambetta LD,* 1999, The protective effect of zinc on diethyldithiocarbamate toxicity on rat astrocytes *in vitro. Toxicol Lett,* 105,129-40.

UTICAJ DIETILDITIOKARBAMATA NA AKTIVNOST ANTIOKSIDACIONIH ENZIMA U KRVI PACOVA

RADOJIČIĆ R, BORKOVIĆ SLAVICA, PAVLOVIĆ S, NIKOLIĆ ALEKSANDRA, BLAGOJEVIĆ D, SAIČIĆ ZORICA i SPASIĆ M

SADRŽAJ

Dietilditiokarbamat (DDC) ispoljava različite uticaje kao što su neurotoksičnost, radioprotekcija i senzibilizacja. On je snažan helirajući agens za bakar i koristi se u tretiranju kiseonične toksičnosti, imunomodulaciji kao i kod pacijenata zaraženih HIV virusom.

U ovom radu smo proučavali uticaj DDC, inhibitora superoksid dismutaze (SOD), na aktivnost bakar cink sadržavajuće superoksid dismutaze (CuZn SOD), katalaze (CAT), glutation peroksidaze (GSH-Px), glutation reduktaze (GR) i glutation-S-transferaze (GST).Tretiranje pacova dietilditiokarbamatom u trajanju od tri časa (1g/kg t.m, i.p.) dovodi do značajnog smanjenja aktivnosti CuZn SOD i povećanja aktivnosti GR i GST u krvi pacova. Takodje je dobijena negativna korelacija izmedju SOD i GR aktivnosti. DDC indukuje povećanje GR aktivnosti

koje je koncentraciono zavisno kao i NADPH potrošnju u *in vitro* enzimatskom eseju.

Dobijeni rezultati ukazuju na činjunicu da smanjivanje aktivnosti SOD u krvi pacova posle tretiranja DDCom može biti kompenzovano promenom aktivnosti drugih komponenti što dalje sugeriše da je regulacija antioksidacione zaštite veoma kompleksna.