CHANGES IN LIPID PEROXIDATION INDUCED BY CHLODITANE IN ADRENALS OF DOGS AND GUINEA PIGS

I. S. Chelnakova, N. D. Tron'ko, and A. S. Mikosha

UDC 616.45-008.939.15-39-02:615.252.45

KEY WORDS: lipid peroxidation; adrenals; chloditane

High activity of electron-transport systems in the adrenal cortex and the presence of large amounts of lipids, especially unsaturated fatty acids, provide the opportunity for intensive lipid peroxidation (LPO). The writers previously found that electron transport in the adrenals of dogs is retarded by the adrenocorticolytic agent chloditane (o, p'-dichlorodiphenyldichloroethane, DDD) [4], and this may be accompanied by the formation of toxic superoxide radicals, which damage cells. Chloditane is known to be a fat-soluble compound, and accordingly it can accumulate in cell membrane lipids, disturbing the normal architectonics of the membranes and leading to changes in the velocity of LPO.

It was accordingly decided to study whether changes in the concentration and rate of formation of peroxides take place in the adrenals of dogs and guinea pigs under the influence of chloditane. In dogs chloditane causes destruction of adrenocortical tissue; accumulation of the compound in the adrenals of guinea pigs does not cause damage to these glands [2, 5].

EXPERIMENTAL METHOD

Experiments were carried out on dogs and guinea pigs. Chloditane was given to dogs per os in powder form with the food at the rate of 50 mg/kg body weight, and to guinea pigs in a dose of 150 mg/kg in the form of a 20% solution in corn oil daily for 20 days. Control guinea pigs received corn oil alone.

LPO was studied in the postnuclear fraction of adrenal homogenates. The concentration of LPO products in the postnuclear homogenate was determined by measuring the formation of one of its end products, namely malonic dialdehyde (MDA) [11]. The reaction was monitored for 60 min at 37°C in an atmosphere of air. The rate of accumulation of MDA was determined under conditions of spontaneous LPO and also after addition of NADPH and ascorbate. The homogenates were incubated in 50 mM Tris-HCl buffer, containing 1.15% KCl. The incubation mixture for determination of NADPH-dependent LPO contains: 1 mM NADPH, 6 μM Mohr's salt, and 4 mM ADP. In the case of ascorbate-dependent LPO the homogenate was incubated in medium containing: buffer, 0.5 mM ascorbate, and 6 μM Mohr's salt [6]. The protein concentration in the incubation mixtures was 0.3-0.8 mg/ml. To determine the intensity of LPO in vivo, the initial MDA level was measured in homogenates before the beginning of incubation (0 min). The measurements were made at 532 nm and the MDA level was calculated with the aid of a molar extinction coefficient of $1.56 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$. Protein was determined by the method in [10]. For statistical analysis of the data Student's t test and the Wilcoxon–Mann–Whitney nonparametric tests were used [1].

EXPERIMENTAL RESULTS

The initial MDA concentration in the adrenal homogenate (zero time) reflects the state of LPO in vivo. Administration of DDD per os led to a significant rise of the MDA level in the adrenal tissues, more marked in dogs (Tables 1 and 2).

Acceleration of LPO is usually regarded as a sign of cell damage. In guinea pigs, however, unlike in dogs, feeding with DDD did not cause destruction of adrenocortical tissue,

---

Kiev Research Institute of Endocrinology and Metabolism, Ministry of Health of the Ukrainian SSR. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 2, pp. 171-174, February, 1989. Original article submitted March 1, 1988.

 0007-4888/89/0002-0180$12.50

TABLE 1. MDA Concentration (in nmoles/mg protein) in Adrenal Homogenate of Intact Dogs and Dogs Receiving DDD (M ± m)

Experimental conditions	Incubation time, min			
	0	15	30	60
Spontaneous LPO				
C	0,57±0,04 (14)	0,68±0,06 (13)	0,74±0,06 (14)	0,99±0,11 (13)
E	1,43±0,15 (7)	1,65±0,22 (7)	1,85±0,29 (7)	1,96±0,34 (7)
Ascorbate-dependent LPO				
C	0,78±0,08 (7)	1,01±0,20 (7)	0,96±0,25 (7)	1,04±0,06 (7)
E	1,59±0,16* (6)	2,08±0,40 (6)	2,30±0,43 (6)	2,45±0,41 (6)
NADPH-dependent LPO				
C	0,92±0,16 (7)	0,68±0,22 (7)	0,77±0,24 (6)	0,81±0,27 (6)
E	2,34±0,30** (7)	2,50±0,17 (7)	2,19±0,36 (7)	2,16±0,39 (7)

Legend. Here and in Table 2: *$p < 0.001$, **$p < 0.01$, ***$p < 0.05$ compared with control; number of observations given in parentheses. C) Control, E) DDD.

despite a raised LPO level. It can therefore be postulated that guinea pigs possess protective mechanisms which render peroxides harmless. The writers showed previously that feeding with DDD inhibits glutathione-S-transferase activity in the adrenals of dogs and activates it in guinea pigs [8]. Different glutathione-S-transferases strongly inhibited MDA formation induced by ascorbate + ADP + Fe^{2+} in rat liver microsomes [13]. MDA formation by trout liver microsomes in a system containing ADP + Fe^{3+} + NADPH was significantly inhibited by glutathione-S-transferase in the presence of GSH [9]. Thus in guinea pigs an increase in glutathione-S-transferase activity may exert a protective function. Because of reduction of activity of this enzyme in dogs receiving DDD, the adrenal tissue is destroyed.

Incubation of adrenal homogenates from control dogs and guinea pigs caused a spontaneous increase in the MDA concentration (Tables 1 and 2).

In the course of 60 min the MDA concentration rose on average by 80% in adrenal homogenates of dogs and by more than 2.5 times in that of guinea pigs.

Spontaneous LPO was retarded in dogs receiving DDD to such a degree that the MDA concentration in the homogenate after incubation for 1 h did not differ from the initial concentration. The MDA level in guinea pigs rose on average in the course of 1 h by 214.2% in the experimental group and by 175.8% in the control animals, but differences between the groups were not significant ($p > 0.1$).

LPO products are known to reduce the enzymic activity of cytochrome P-450 [12]. LPO is accompanied in vitro by strong inactivation of microsomal steroid-producing enzymes [14]. Slowing of LPO which we observed in dog adrenal homogenate in vitro was probably connected with inactivation of cytochrome P-450 as a result of DDD administration. Weakening of the cytochrome P-450 signal in the adrenals of dogs has been demonstrated by the EPR method even after only a single peroral dose of DDD [7]. Ascorbate-dependent LPO does not involve cytochrome P-450, and for that reason the rate of MDA accumulation in vitro in samples containing ascorbate is the same whether the adrenal homogenate incubated is obtained from control dogs or dogs receiving DDD (Table 1). In the course of 60 min the MDA level rose on average by 44% in the control and by 48% in the experimental dogs.

The effect of NADPH on the MDA concentration in the samples is very interesting. In the course of incubation for 15 min the quantity of MDA in NADPH-containing samples fell to 72.4 ± 14.6% compared with the initial level (see Table 1, control). Without NADPH the MDA level rose on average, during the same time, to 114.9 ± 3.7% (spontaneous LPO, control). This difference in the intensity of the LPO process due to the composition of the medium is highly significant ($p < 0.02$). On continued incubation the MDA concentration rose a little in the control samples, but the change was not statistically significant. Addition of NADPH to samples with guinea pig adrenal homogenate reduced the rate of MDA accumulation. The increase in the MDA concentration in the course of 1 h was 175.8% without NADPH and 108.6% in its presence. The mechanism of inhibition of MDA production in the presence of NADPH may perhaps be connected with the participation of NADPH in the glutathione reductase reaction. An adrenocortical cytosol contains active glutathione reductase [3], which maintains the reduced state

TABLE 2. MDA Concentration (in nmoles/mg protein) in Adrenal Homogenate of Intact Guinea Pigs and Guinea Pigs Receiving DDD (M ± m)

Experimental conditions	Incubation time, min			
	0	15	30	60
Spontaneous LPO				
C	0,82±0,08 (9)	1,03±0,05 (9)	1,26±0,05 (9)	2,07±0,32 (9)
E	1,14±0,06** (9)	1,58±0,18 (9)	1,96±0,20 (9)	3,58±0,71 (9)
Ascorbate-dependent LPO				
C	1,40±0,11 (9)	1,77±0,16 (9)	2,05±0,18 (9)	2,17±0,18 (9)
E	2,13±0,32*** (9)	3,07±0,46 (8)	3,33±0,53 (9)	3,57±0,59 (9)
NADPH-dependent LPO				
C	0,94±0,19 (9)	0,85±0,13 (9)	1,34±0,15 (9)	1,52±0,12 (9)
E	1,82±0,39 (9)	2,37±0,40 (9)	2,37±0,21 (8)	2,81±0,37 (9)

of glutathione. Reduced glutathione in glutathione-peroxidase and glutathione-S-transferase reactions, and also perhaps in nonenzymatic reactions, is utilized to remove peroxides. Feeding dogs with DDD induced glutathione reductase in the adrenals of dogs [3] but did not affect activity of the enzyme in adrenals of guinea pigs (unpublished details). Evidently activated glutathione reductase in dog adrenal homogenate, in the presence of NADPH, maintains an adequate level of reduced glutathione to destroy peroxides present in the tissue at the time of sacrifice of the animals, and it inhibits their further accumulation.

LITERATURE CITED

1. E. V. Gubler and A. A. Genkin, The Use of Nonparametric Statistical Tests to Evaluate Differences between Two Groups of Observations in Medico-Biological Research [in Russian], Moscow (1969).
2. V. P. Komissarenko and A. G. Reznikov, Inhibitors of Adrenocortical Function [in Russian], Kiev (1972).
3. V. P. Komissarenko, I. S. Chelnakova, and A. S. Mikosha, Probl. Éndokrinol., No. 1, 95 (1978).
4. V. P. Komissarenko, A. S. Mikosha, and I. S. Chelnakova, Ukr. Biokhim. Zh., No. 6, 74 (1981).
5. V. V. Korpachev, Fiziol. Zh. (Kiev), No. 5, 585 (1972).
6. V. V. Lemeshko, P. A. Kaliman, and Yu. V. Nikitchenko, Biokhimiya, No. 4, 620 (1981).
7. A. S. Mikosha and N. N. Beregovskaya, Probl. Éndokrinol., No. 5, 62 (1983).
8. I. S. Chelnakova, A. S. Mikosha, and I. N. Todor, Farmakol. Toksikol., No. 6, 104 (1985).
9. G. J. Bell, C. B. Cowey, and A. Youngson, Biochim. Biophys. Acta, 795, 91 (1984).
10. S. Bramhall, N. Noak, A. Wu, et al., Anal. Biochem., 31, 146 (1969).
11. W. C. Brogan, P. R. Miles, and H. D. Colby, Biochim. Biophys. Acta, 663, 230 (1981).
12. J. Klimek, A. P. Shaap, and T. Kimura, Biochem. Biophys. Res., Commun., 110, 559 (1983).
13. R. Morgenstern, J. W. De Pierre, C. Lind, et al., Biochem. Biophys. Res. Commun., 99, 682 (1981).
14. R. Takayanagi, K.-I. Kato, and H. Ibayashi, Endocrinology, 119, 464 (1986).