Effects of D-penicillamine on some oxidative enzymes of rat organs in vivo[1]

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Summary. In the liver of neonate rats and in tissues of adult rats a study was made of the effect of D-penicillamine treatment in vivo on the enzymes of peroxide metabolism, lipid peroxidation and other components. It was found that D-penicillamine primarily acts by decreasing lipid peroxidation, thus stabilizing the membrane.

Lakatos et al. have observed that D-penicillamine (D-PA) has a significant therapeutic effect in infants with neo-natal jaundice[5-8]. Also, the incidence of retrolental fibroplasia cases was lower in premature infants treated with D-PA compared with neonates receiving oxygen therapy alone after birth[9]. We began studies to clarify the mechanism of molecular action of D-PA and showed that D-PA shortens the sleeping time in neonates[10]. Our attention therefore turned primarily to changes in the oxidative enzymes. Accordingly, in liver homogenates from neonatal rats, and in organs of adult rats, we examined how in vivo D-PA treatment affects the peroxide metabolism enzymes (PME), i.e. superoxide dismutase (SOD; EC 1.15.1.1), catalase (C-ase; EC 1.11.1.6) and peroxidase (P-ase; EC 1.11.1.7), and other parameters.

Materials and methods. For neonatal rats the D-PA treatment procedure was as follows. Progeny from the same litter of rats of the CFY strain were used. After birth, the neonate rats were divided into 2 equal groups. 1 group served as the control (C), while every member of the other group was treated (T) with a single dose of 1000 mg D-PA/kg b.wt 24 h before being sacrificed. After exsanguination, the rats in both groups had their livers removed, homogenized and centrifuged, and the supernatant was separated and used for measurements. In the other series, adult female CFY rats of the same age and of about the same weight (200–250 g) were fasted for12 h, and then treated i.p. with the D-PA dose found most suitable in earlier experiments (333 mg/kg)[10]. After 24 h, the rats were decapitated and exsanguinated. Subsequently, their organs were homogenized and treated as before until the samples were used for measurements. The superoxide dismutase activities were determined by the epinephrine-adrenochrome method described by Misra et al.[11,12], peroxidase activities by the method of Chance et al.[11,13], and catalase activities from the extent of H_2O_2 decomposition occurring due to the action of the enzyme in a given time[11,14]. The lipid peroxidation (LP) in the supernatant aliquots was measured by the thiobarbituric acid spectral method of Placer et al.[15]. Protein was estimated according to the method of Lowry et al.[16]. The liver microsomes were prepared by the method of Greim[17] for cytochrome P-450 measurements (c.P-450), and determined quantitatively on the basis of the CO spectrum. The copper content of the neonatal liver was measured with Meckotest kits (No.3319, Merck Co. Darmstadt, FRG). The D-PA was used in the form of the

Table 1. SOD, P-ase, C-ase, protein, lipid peroxidase and c.P-450 values in the liver of control and D-PA treated rats

Parameters	C (4)	T (4)	C (7)	T (7)	C (10)	T (10)
SOD U/g w.l.wt	1.281±115	1.308±120	1.366±116	1.418±122	1.384±118	1.466±132
SOD U/mg protein	4.7±0.28	3.9±0.3	4.9±0.35	4.4±0.41	5.3±0.27	4.5±0.36
P-ase U/g w.l.wt	759±60	1.028±101	300±15	775±64	37±3	225±18
C-ase BU/g w.l.wt	1.02±0.10	1.07±0.10	2.1±0.2	1.7±0.1	1.14±0.11	1.86±0.15
c.P-450 nM/mg protein	0.1956±0.0012	0.2642±0.0015	0.2130±0.0018	0.2667±0.0022	0.1223±0.0011	0.2094±0.0017
Protein mg/g w.l.wt	270±22	335±30	281±26	324±29	263±23	327±30
LP nM MDA/g w.l.wt.	623±60	498±48	543±52	433±42	1.046±98	736±70
Cu microg/g w.l.wt	45.8±4.1	46.1±4.0	37±3	61±5	22.5±2.0	45±4

Numbers in parentheses denote the number of days the rats lived after birth e.g., C (4) = control animals killed 4 days after birth. Data given as mean ± SD of 10 measurements.

Table 2. SOD, P-ase, C-ase, protein, lipid peroxidase and c.P-450 values in various organs of control and D-PA treated rats

Tissues		Liver	Kidney	Lung	Spleen	Brain	Heart	Blood haemolyzate	plasma (cm³)
SOD	C	3.333±202	1.876±110	491±45	870±60	667±37	538±14.5	0	41±0.33
U/g w.wt	T	5.140±325	2.247±118	645±52	996±88	1.000±49	898±66		26±0.2
SOD	C	21.6±1.8	14.8±0.9	7.5±0.51	6.2±0.43	9.8±0.73	5.4±0.4	0	0.56±0.033
U/mg protein	T	36.5±2.9	14.9±1.2	6.8±0.37	7.6±0.55	16.9±1.2	12.8±0.98	0	0.41±0.027
P-ase	C	0	450±23	2.160±198	2.925±223	0	2.340±200	0	870±56
U/g w.wt	T	0	110±9	1.340±121	2.900±253	0	1.110±76	0	285±21
C-ase	C	6.8±0.3	4±0.28	0.52±0.03	0.45±0.015	0.06±0.002	0.4±0.013	1.6±0.05	0.08±0.0016
BU/g w.wt	T	6.53±0.42	4.13±0.33	0.57±0.04	0.27±0.020	0.213±0.018	0.35±0.022	1.35±0.1	0.02±0.001
Protein	C	154±12	127±10	65±3.5	141±11	68±2.8	100±8.7	280±16	73±4.6
mg/g w.wt	T	141±10.5	151±8	95±7.2	131±10.5	59±3.3	70±6.7	314±21	64±2.2
LP nM	C	33.2±1.6	22.4±1.8	9.3±0.5	0	43.6±2.9	0	0	0
MDA/g w.wt	T	19.9±1.3	16.0±0.8	0.1±0.3	0	42.1±3.8	0	0	0
c.P-450	C	0.3315±0.0113							
nM/mg protein	T	0.5011±0.0255							

Data given as mean ± SD of 10 measurements.

injection Metalcaptase® (Knoll AG, Ludwigshafen, FRG).

Results and discussion. Table 1 shows the enzyme activities and other parameters measured in liver homogenates from neonate rats treated with D-PA (T) as detailed in the Materials and methods section, and controls (C) of the same age.

Table 2 gives the corresponding data measured in organs (liver, kidney, lung, spleen, brain, heart and haemolyzate) of D-PA-treated and control adult rats.

The enzyme activities were calculated in all cases on 1 g wet liver weight (w.l.wt) or 1 g wet tissue weight (w.wt). The SOD activities are given in values of activity units per g w.l.wt or per g w.wt (data in the thousand range) and of U/mg protein (data in the range 3–6). As a consequence of the considerably increased protein values following D-PA treatment, the SOD activities calculated per mg protein are lower than the control values (see table 1). In contrast, the D-PA treatment leads to an increase in the liver P-ase, c. P-450, protein and Cu contents, and in the value of the C-ase activity on the 10th day. As already mentioned, the SOD activity and the LP of the treated liver are decreased.

The enzyme changes observed in the organs of the adult rats following D-PA treatment are not as uniform as those in the liver of the neonates. The SOD and the liver microsomal c.P-450 activities are higher (with the exception of the kidney and the lung), while the P-ase values and (apart from the brain homogenate) the C-ase and LP activities are lower.

We consider that our data point to important correlations in connection with the mechanism of the molecular action of D-PA, as regards the enzymes and other parameters studied here. The two tables reveal that, under the conditions employed, D-PA acts in different ways on the parameters in question in neonate and adult rats. A number of points may be emphasized:

1. In both cases D-PA was found to increase the liver microsomal c.P-450 activity and to decrease the LP. The former results in an increase of the drug metabolism via an enhancement of the mixed function oxidase activity, whereas the latter is connected with the membrane-stabilizing action of D-PA. These two findings provide a good explanation in terms of the molecular mechanism for the observations of Lakatos et al. that D-PA diminishes the hexobarbital sleeping time of rats[10], and for the important therapeutic effect observed in human neonatal jaundice[8]. The LP decrease promotes the rapid elimination of the haemoprotein decomposition products by conservation of the UDP-glucuronyl transferase activity.
2. The above statements also explain the observation that D-PA treatment prevents the development of retrolental hyperplasia, which frequently follows neonatal oxygen therapy[9].
3. The data illustrate well that D-PA does not influence uniformly the enzyme activities and other parameters examined in neonate and adult animals. More correctly, it may be assumed that the reactions of the enzymes differ as a function of the age.

1 "Properties of enzymes". Serial publication Part XVI.
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5 L. Lakatos és B. Kövér, Orv. Hetil. *115*, 307 (1974) (in Hungarian).
6 L. Lakatos, B. Kövér and F. Péter, Acta paediat. hung. *15*, 77 (1974).
7 L. Lakatos, B. Kövér, S. Vekerdy and É. Dvorcsek, Acta paediat. hung. *17*, 93 (1976).
8 L. Lakatos, B. Kövér, Gy. Oroszlán and S. Vekerdy, Eur. J. Pediat. *123*, 133 (1976).
9 L. Lakatos, I. Hatvani, L. Karmazsin, B. Albert, S. Vekerdy és A. Boros, Szemészet *117*, 9 (1980) (in Hungarian).
10 L. Lakatos, L. Karmazsin, Gy. Oroszlán, T. Tóth és S. Vekerdy, Kísérl. Orvostud. *31*, 357 (1979) (in Hungarian).
11 B. Matkovics, R. Novák, Hoang Duc Hanh, L. Szabó, Sz.I. Varga and G. Zalesna, Comp. Biochem. Physiol. *56B*, 31 (1977).
12 H.P. Misra and I. Fridovich, J. biol. Chem. *247*, 3170 (1972).
13 B. Chance and A.C. Maehly, in: Methods in enzymology vol.2. Eds S.P. Colowick and N.O. Kaplan. Academic Press, New York 1955.
14 R.F. Beers and I.W. Sizer, J. biol. Chem. *195*, 133 (1952).
15 Z.A. Placer, L. Cushman and B.C. Johnson, Analyt. Biochem. *16*, 359 (1966).
16 O.H. Lowry, N.I. Rosebrough, A.L. Farr and R.I. Randall, J. biol. Chem. *193*, 265 (1951).
17 H. Greim, Naunyn-Schmiedebergs Arch. Pharmak. *266*, 261 (1970).
18 A. Finazzi-Agró, G. Floris, M.B. Fadda and C. Crifó, Experientia *35*, 1445 (1979).
19 D.N. Bentley, G.C. Wood and A.B. Graham, Med. Biol. *57*, 274 (1979).

New non-photodynamic (type B) phototoxic molecules[1]

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Summary. This report completes the examination of the 3 butadiynes substituted with phenyl and thienyl groups, and their related thiophene molecules. With the exception of 2,5-diphenylthiophene, they are all non-photodynamic (type B) phototoxic compounds.

Photodynamic compounds[2] owe their phototoxicity to the conversion of molecular oxygen into singlet oxygen or superoxide ion which they mediate.

As can later be confirmed by observing the phototoxicity to a microorganism such as *E. coli* B or *Saccharomyces cerevisiae* under nitrogen, a simple test with *Candida utilis* accurately detects molecules which can be phototoxic in the absence of oxygen even if they are also efficient singlet oxygen sensitizers[3,4].

In response to a need for a simple nomenclature of phototoxic molecules, we designate by A and B the 2 general types of phototoxicity. Type A molecules generate an oxygen-dependent phototoxicity, whether through singlet oxygen or superoxide[5]. The molecules for which the phototoxicity in the absence of oxygen can be demonstrated belong to class B. The furocoumarins (psoralens) which have considerable importance in molecular biology and in medicine[6], were the only members of this latter category.