CORRELATION BETWEEN THE TOXICITY OF CHEMICAL AGENTS AND THEIR INHIBITORY ACTIVITY ON ISOLATED MITOCHONDRIA

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UDC 612.26.014.46-085.1

Correlation was found between the concentrations of compounds inhibiting respiration of isolated mitochondria and the toxicity of the same substances for the whole organism. This correlation was observed for different intensities of action in vivo and for different pathways of entry of the toxic agent into the body. The relationship discovered applies to all inhibitors of tissue respiration irrespective of their point of application and of the molecular mechanism of their action.

KEY WORDS: toxic agents; respiration of mitochondria; inhibitors of tissue respiration.

The question of to what extent the results obtained in experiments in vitro can be extrapolated to the intact organism is still debated. This is especially true of the quantitative aspect of the problem.

The quest for correlations of this sort as applied to inhibitors of tissue respiration is very promising, for besides some agents that are highly specific but are used chiefly in research there is a whole host of compounds, of widely different nature, belonging to this category of inhibitors and used extensively in industry, agriculture, and medicine [4, 7, 8-10].

EXPERIMENTAL METHOD

The concentrations of a compound inhibiting mitochondrial respiration by 50% (CI_{50}) were compared with the parameters of its toxicity (median lethal dose, LD_{50}; median lethal concentration, CL_{50}; maximal allowable concentrations in the air of the work and in the atmospheric air, MAC_w and MAC_a) by the method of regression analysis [6] using personal observations and data in the literature. Personal data on the values of CI_{50} were obtained by titration of the ADP-stimulated respiration of the liver mitochondria of albino rats in an incubation medium containing (in mM): sucrose 200, KH_2PO_4 10, $MgCl_2$ 10, KCl 15, glutamate 5, and malate 5, followed by analysis of the results by the method of Lineweaver and Burke. The acute toxicity parameters were determined by the method adopted in experimental toxicology [3]. The values of MAC_w and MAC_a were taken from the official standard SN-245-71. When the data in the literature were analyzed, only statistically defined parameters of toxicity and CI_{50} were taken into account. All parameters of toxicity were converted into molar units.

Since the toxicity parameters and CI_{50} values for different compounds differ by several orders of magnitude, logarithms of their reciprocals [$\log(1/CI_{50})$, $\log(1/LD_{50})$, and so on] were used in the work.

EXPERIMENTAL RESULTS AND DISCUSSION

Values of the inhibitory concentrations and toxicity parameters for some of the compounds tested are given in Table 1. Analysis showed that a direct relationship exists between CI_{50}, on the one hand, and the values of LD_{50} and CL_{50}, on the other, expressed by the equations

Toxicological Laboratory, Public Health Station, Moscow. (Presented by Academician S. E. Severin.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 78, No. 7, pp. 65-68, July, 1974. Original article submitted May 25, 1973.

TABLE 1. Inhibitory Concentrations and Toxicity Parameters

Compound	$\lg \frac{1}{CI_{50}}$	$\lg \frac{1}{LD_{50}}$	$\lg \frac{1}{CL_{50}}$	$\lg \frac{1}{MAC_W}$	$\lg \frac{1}{MAC_a}$
Phenol	2,00	2,27	4,37	7,28	9,98
4-Chlorophenol	2,85	2,75	4,60	8,11	—
2,4-Dichlorophenol	3,65	3,29	4,72	—	—
2,4,6-Trichlorophenol	3,40	3,29	—	8,29	—
Pentachlorophenol	4,40	3,68	—	9,42	—
Aniline	1,72	2,67	4,76	8,97	9,5
4-Methylaniline	2,2	—	—	7,55	—
N,N-Dimethylaniline	2,82	—	6,02	8,70	10,35
Methanol	—0,3	1,96	3,50	6,81	—
Ethanol	0,3	0,76	2,60	4,66	6,97
Butanol	0,65	1,41	3,57	6,87	—
2,4-DNP	4,2	3,64	5,66	9,57	—
Butophen (2,4-dinitro-6-fluorobutylphenol)	4,75	3,86	—	9,68	—
Ethyl-β,β,β-trichloropropionate	3,15	2,75	—	—	—
Monochloroacetate	2,30	2,75	—	—	—
Monobromoacetate	3,3	3,14	—	—	—
Monoiodoacetate	4,30	3,44	—	—	—
Cyanides	5,70	4,51	6,43	7,94	9,43
Azides	4,26	3,81	6,34	—	—
Hydrogen sulfide	4,00	—	4,83	7,11	9,63
Sulfurous anhydride and sulfites	1,62	2,00	—	6,83	9,1
Formaldehyde	2,58	—	4,84	7,78	—
Divalent mercury	5,00	4,25	—	9,43	11,82
Arsenites	2,92	3,07	—	8,61	10,61
Lead	4,3	—	—	10,3	11,47
Isopropylbenzene hydroperoxide	3,00	—	—	8,18	—
Cadmium	4,75	—	6,45	9,11	—
Ethylbenzene	2,80	—	—	—	9,72
Acetone	0,3	1,08	2,60	5,46	8,22

$$\lg \frac{1}{LD_{50}} = 1.39 + 0.54 \lg \frac{1}{CI_{50}} \quad (1)$$

$$\lg \frac{1}{CL_{50}} = 3.05 + 0.60 \lg \frac{1}{CI_{50}} \quad (2)$$

with coefficients of r = 0.92 and 0.81 ($P < 0.001$ and $P < 0.01$), respectively. The mean error of the calculated acute toxicity parameters was 103% for LD_{50} and 140% for CL_{50}, and according to current toxicological opinion [5] this is assessed as very satisfactory agreement.

In both cases methanol, ethanol, and aniline and also dimethylaniline remained outside the confidence limits. The experimentally determined value of LD_{50} for ethanol was 5.8 times greater, and for methanol and aniline 6.7 and 2.6 times smaller, respectively, than the calculated values. This divergence can evidently be explained by the rapid oxidation of ethanol in the body to nontoxic products, whereas the oxidation of methanol leads to the formation of the highly toxic products formaldehyde and formic acid. The comparatively wide divergence of the calculated toxicity parameters of aniline and dimethylaniline from those determined experimentally can be attributed to the methemoglobin-forming action of these compounds. In fact LD_{50} for aniline in rabbits, in which no methemoglobin is formed [2], is 2.5-3 times greater than for rats [1].

Reasonably close correlation also is found between CI_{50} and the MAC values, as expressed by the equations

$$\lg \frac{1}{MAC_W} = 6.00 + 0.73 \lg \frac{1}{CI_{50}} \quad (3)$$

$$\lg \frac{1}{MAC_a} = 7.70 + 0.95 \lg \frac{1}{CI_{50}} \quad (4)$$

with coefficients of correlation of 0.92 ($P < 0.001$) and 0.90 ($P = 0.01$), respectively. The mean error of the calculated MAC values was 109% for MAC_W and 129% for MAC_a (disregarding the cyanides and H_2S). Besides methanol, ethanol, and aniline, the cyanides and hydrogen sulfide also were outside the confidence limits. The calculated MAC_W values for these compounds differed from their actual values by 164 and 144 times respectively, although the acute toxicity parameters correlate well with CI_{50}. The evident reason for this divergence is the high risk of acute poisoning by these compounds, together with their comparatively low powers of cumulation, as reflected in their MAC levels.

These findings suggest that the results of a study of inhibitors of respiration in isolated mitochondria can be transferred both qualitatively and quantitatively to the whole organism, a valid argument for the introduction of this express method of assay of new toxic (and perhaps pharmacological also) agents with the aid of an experimental model into experimental toxicology. The considerable divergence between the calculated and experimentally obtained toxicity parameters suggests that the compound concerned may have a different mechanism of action or that metabolites play an important role in the mechanism of poisoning.

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