LITERATURE CITED

1. S. N. Lyzlova, Vopr. Med. Khim., 33, No. 5, 43 (1987).
2. A. M. Mayanskii and D. M. Mayanskii, Essays on the Neutrophil and Macrophage [in Russian], Novosibirsk (1983).
3. Z. I. Sukhova, V. V. Ivanitskaya, and Yu. P. Sergeev, Abstracts of Proceedings of the 3rd All-Russian Congress on Remedial Gymnastics and Sport Medicine [in Russian], Sverdlovsk (1986), p. 193.
4. B. I. Fel'dkoren and T. P. Kotsegub, Uch. Zap. Tartu. Gos. Univ., No. 10, 114 (1981).
5. P. V. Tsyplenkov, V. I. Morozov, V. N. Kokryakov, and V. A. Rogozkin, Ukr. Biokhim. Zh., 60, No. 6, 72 (1988).
6. V. S. Chaikovskii, O. B. Basharina, I. V. Shalyapina, and V. A. Rogozkin, Vopr. Med. Khim., 33, No. 4, 79 (1987).
7. O. Yu. Yankovskii, T. E. Dovnar, and A. A. Tkachenko, Zh. Mikrobiol., No. 6, 58 (1981).
8. A. Böyum, Scand. J. Clin. Lab. Invest., 21, Suppl. 97, 77 (1968).
9. V. B. Fiedler and E. Bischoff, J. Mol. Cell. Cardiol., 19, Suppl. 3, S22 (1987).
10. J. Friden, U. Kjorell, and L. E. Thonell, Int. J. Sports Med., 5, 15 (1984).
11. A. Liboshy, S. Tocuda, T. Nichimura, et al., J. Sports Med., 22, 284 (1982).
12. S. J. Klebanoff and R. A. Clark, The Neutrophil: Function and Clinical Disorders, New York (1978).
13. H. Kutchai, Y. Barenholz, T. F. Ross, and D. E. Wermer, Biochim. Biophys. Acta, 436, 101 (1976).
14. J. Palmblad, Scand. J. Rheum., 13, No. 2, 163 (1984).

DETERMINATION OF OXIDATION PHENOTYPE IN INBRED $C_{57}Bl/_6$ AND $BALB/_C$ MICE

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UDC 615.2/.3.015.4.076.9

KEY WORDS: oxidative processes, inbred mice, antipyrin

An important achievement in pharmacogenetics has been the obtaining of evidence of inherited differences in ability to oxidize drugs, showing that the human population can be divided into strong and weak oxidizers, the ratio between which varies in different countries and in different racial and ethnic groups between quite wide limits [7, 10]. Genetic heterogeneity of oxidation in some cases may be the cause of the ineffectiveness of pharmacotherapy and the development of side effects [9]. It therefore was considered worthwhile to create an experimental model for use in studying dependence of the pharmacologic effect on the character of oxidation. The theoretical grounds for undertaking such a task, in our point of view, was N. I. Vavilov's general biological law of homologous series of inherited variation [1]. The experimental basis for the investigation consisted of interlinear differences established previously between the conversion of phenazepam into trihydroxyphenazepam and of synocarb into β-hydroxysydnocarb [5, 7].

The aim of this investigation was to study the oxidation phenotype of mice of strains $C_{57}Bl/_6(B_6)$ and $BALB/_C(C)$, used in previous investigations, in relation to the model drug antipyrin (AP).

EXPERIMENTAL METHOD

Experiments were carried out on male B_6 and C mice and on their F_1-hybrids (B × C), weighing 20-25 g.

AP and its metabolites were determined by gas chromatography on the basis of methods described in [8, 12]. The measurements were made on an "Varian Aerograph 2800" gas chromatograph with "Shimadzu" CR-1a integrator ("Chromatopal"). A spiral glass column 1.5 m long was used. The adsorbent was "Chromosorb G 100" (120 mesh) with OV phase of 17-3%. The

Research Laboratory of Pharmacogenetics, aand Research Laboratory of Pharmacokinetics, Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 11, pp. 491-493, November, 1990. Original article submitted November 22, 1989.

0007-4888/90/0011-1505$12.50

operating conditions of the chromatograph were: column temperature 230°C, injector temperature 275°C, detector temperature 300°C. The air flow to the column was applied at the rate of 300 ml/min, the hydrogen flow 30 ml/min, and the nitrogen flow 40 ml/min.

To study AP metabolism in vivo it was injected intraperitoneally in a dose of 100 mg/kg in the form of an aqueous solution. The animals were decapitated 2, 5, 15, 30, 60, and 150 min after injection of the drug. To measure concentration of AP and its derivatives blood and urine were collected, and samples obtained from six mice were pooled at each time point. Blood was centrifuged with heparin at 1500 g and the plasma separated. AP was extracted from the blood plasma after alkaline hydrolysis and norantipyrin (NAP), 4-hydroxyantipyrin (OHA), and AP were extracted from the urine after acid hydrolysis, with chloroform, followed by evaporation and dissolving of the residue in methanol. A sample of 2 μl was introduced into the chromatograph. Concentrations of AP and its metabolites in the biological substrate were calculated against an absolute calibration scale within limits of 5 to 50 μg/ml for AP, 10-20 μg/ml for NAP, and 50-500 μg/ml for OHA. To characterize differences in the velocity of AP oxidation in B_6 and C mice and their F_1-hybrids, values of the metabolic ratio (NAP/AP and OHA/AP) were estimated.

The numerical results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Table 1 shows that during the first 30 min after injection of AP its concentration in the blood plasma was similar in mice of the two lines. From 60 to 150 min more rapid clearance of the plasma from AP was found in C mice: after 1 h its concentration in C mice was only half of that in B_6 mice, after 1.5 h it was 4 times less, and after 2.5 h it was 5.5 times less than in the B_6 mice. As early as 2 min after injection of AP its concentration in the urine of C mice was 9 times higher than in the case of B_6 mice, and after 5 min these differences were reduced to a difference of twice. Later the concentration of the unchanged AP was higher in B_6 mice. Significant interlinear differences also were found for the OHA level, for after 30 and 60 min this metabolite was present in higher concentrations in C mice. A recordable level of NAP was shown to appear in the urine somewhat later than in the case of OHA. For the latter product, interlinear differences in the rate of rise of concentrations also were observed. The NAP level reached maximal values in the urine of the C mice 1 h after injection of AP, and it was more than twice as high as in the B_6 mice, in which the highest concentration of this metabolite was observed after 90 min (Table 1). On the basis of these data on concentrations of AP, OHA, and NAP, values of the metabolic ratio were calclated (Table 2). From the first stages of the investigation this parameter was higher in C mice for both AP derivatives, both in the blood plasma and in the urine. Thus taken as a whole, the results demonstrate that AP is metabolized more rapidly in C mice than in B_6 mice.

To determine the character of inheritance of ability to oxidize AP, its concentration and the level of its metabolites were measured in hybrid $(B_6 \times C)F_1$ animals at times at which marked differences were established between the metabolic behavior of the parental lines. It was shown that after 90 min the concentration of the original compound in plasma of the hybrids and C line was about equal, and almost 5 times lower than in the B_6 line; after 150 min its level was lower than in C and B_6 by about 3 and 14 times, respectively.

The ratio of NAP and OHA to AP in the urine and plasma was higher than the corresponding values in mice of the parental lines (Table 2).

It can accordingly be concluded that the high ability of the $(B_6 \times C)F_1$ hybrids to oxidize AP is inherited through animals of the C line.

The experiment showed that B_6 mice have a weak, and C mice a strong phenotype for AP oxidation. This trait is inherited through the parental line C, in agreement with the data on dominance of the strong oxidation phenotype in the human population [6]. Mendelian inheritance of ability to oxidize sydnocarb was demonstrated previously on the same models [2], and this also agrees with data obtained in man relating to monogenic control of oxidation processes [11].

The results of these experiments thus lead to the conclusion that homology exists between phenotypes of AP oxidation described in the human population and those discovered in C and B_6 C and B_6 mice discovered in the present study. It will be evident that the lines studied cannot be recommended for the analysis of absolutely every drug, for their selection

TABLE 1. Concentrations (in μg/ml) of AP, OHA, and NAP in Blood Plasma and Urine of B_6, C, and (B_6 × C) F_1 Mice after Injection of Preparation in a Dose of 100 μg/kg (M ± m, n = 4)

Line	Preparation	Time in min						
		2	5	15	30	60	90	150
B_6	Blood plasma	49,5±11,1	86,9±18,4	91,4±5,0	94,3±21,9*	48,6±1,3**	26,6±5,6*	12,0±1,6**
	AP	2,8***	10,8±2,6*	59,0±5,9**	100,6±21,9*	76,3±25,7	93,5±12,5	78,5±25,6
	Urine							
	OHA	—	18,1±3,3	51,34	95,4±8,5	411,2±100,2*	1173,2±303,1	1185,5±134,3
	NAP	—	—	—	527,7±139,4	540,4±161,0**	1432,3±242,2	703,2±148,6
C	Blood plasma	43,1±10,3	67,5±5,6	89,9±13,2	63,09±3,39*	22,6±3,7**	6,0±1,2*	2,3±0,7
	AP	25,2±3,69***	18,0±2,5*	20,0±7,5***	50,3±6,3*	95,5±11,6	70,35±16,5	67,5±19,5
	Urine	—	10,82	174,8±30,01	489,3±126,1*	1033,8±236,1*	1211,0±233,1	969,7±178,9
	OHA	—	—	464,47	519,8±71,7	1209,3±340,8**	988,3±113,1	589,8±125,0
	NAP	—	—	—	—	—	5,2±0,6*	0,8±0,3***
F_1	Blood plasma							
(B_6×C)	AP	—	—	—	—	—	86,8±6,9	9,0±6,7
	Urine	—	—	—	—	—	1893,8±743,8	1254,7±236,2
	OHA	—	—	—	—	—	2394,8±1296,7*	1385,5±544,6
	NAP							

Note. Significance of differences by Student's tests in B_6 and C mice: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; F_1 and B_6: *$p < 0.05$, **$p < 0.001$, F_1 and C: *$p < 0.01$.

TABLE 2. Metabolic Ratio of OHA and NAP to AP in Blood Plasma and Urine of B_6, C, and F_1 (B_6 × C) Mice after Injection of Drug in a Dose of 100 mg/kg (M ± m, n = 4)

Time, min	OHA/AP plasma			NAP/AP plasma			OHA/AP urine			NAP/AP urine		
	B_6	C	F_1(B_6×C)	B_6	C	F_1(B_6×C)	B_6	C	F_1(B_6×C)	B_6	C	F_1(B_6×C)
5	0,18	0,08	—	—	—	—	1,84	0,65	—	—	—	—
15	1,11	1,83	—	—	4,85	—	1,41	1,43	—	—	2,49	—
30	1,70	5,63	—	10,04	6,93	—	6,83	9,52	—	3,98	10,96	—
60	18,35	54,28	—	18,0	55,07	—	7,46	7,83	—	4,45	10,93	—
90	47,67	231,8	348,3	51,4	167,1	450,33	10,86	25,49	20,92	13,47	18,68	26,86
150	112,46	620,4	1724,4	64,02	450,05	2070,0	27,35	23,56	25,93	16,26	15,64	30,44

lead to significant differences in the organization of the neurotransmitter and neurohormonal systems [3, 4]. However, for drugs whose pharmacodynamics is similar in these animals, it is highly likely that these general principles will be established.

LITERATURE CITED

1. N. I. Vavilov, Proceedings of the 3rd All-Russian Plant Breeding Congress [in Russian], Saratov (1920), pp. 5 and 6.
2. I. V. Rybina, S. B. Seredenin, and L. B. Pirogova, Experimental and Clinical Pharmacokinetics [in Russian], Moscow (1988), pp. 39-48.
3. S. B. Seredenin, V. G. Zin'kovskii, and B. A. Badyshtov, Byull. Éksp. Biol. Med., No. 10, 450 (1981).
4. S. B. Seredenin, Yu. A. Blednov, and B. A. Badyshtov, Progress in Science and Technology. Series: Human Genetics [in Russian], Vol. 6, Moscow (1982), p. 90-143.
5. S. B. Seredenin and I. V. Rybina, Farmakol. Toksikol., No. 4, 79 (1985).
6. D. H. Huffman, D. M. Shoeman, and D. G. Azarnoff, Biochem. Pharmacol., 23, No. 2, 197 (1974).
7. J. R. Idle and R. L. Smith, Drug Metab. Rev., 9, 301 (1979).
8. T. Inaba and N. E. Fisher, Canad. J. Physiol. Pharmacol., 58, No. 1, 17 (1980).
9. T. Inaba, S. V. Otton, and W. Kalov, Clin. Pharmacol. Ther., 27, No. 4, 547 (1980).
10. B. Testa and P. Jenner, Drug Metab. Rev., 12, 1 (1981).
11. E. S. Vessel, Clin. Pharmacol. Ther., 16, No. 1, 135 (1974).
12. E. S. Vessel and M. B. Penno, Fed. Proc., 43, No. 8, 2342 (1984).