Comparative Analysis of Esterase Activities of Human, Mouse, and Rat Blood

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Acetylcholinesterase, butyrylcholinesterase, carboxylesterase, and paraoxonase activities in human, mouse, and rat blood were measured. The proportions of these enzymes activities differed significantly. In humans, the most significant were cholinesterase activities, while in rats and mice the contribution of carboxylesterase activity was the greatest. High arylesterase activity of paraoxonase was observed in all cases. Species-specific differences should be taken into consideration when carrying out preclinical trials on rodents for optimization of the pharmacokinetic characteristics of drugs containing complex ester groups.

Key Words: *acetylcholinesterase; butyrylcholinesterase; carboxylesterase; paraoxonase; blood*

Preclinical pharmacokinetic and toxicological studies of new drugs are carried out on animal experimental models. As the isoform composition, levels of expression and activities of the enzymes involved in the pro-drug and drug metabolism are different in humans and laboratory animal species, one has to face the problem of the most correct extrapolation of experimental data, obtained in animal models, to humans. Species-specific differences should be taken into consideration when evaluating the efficiency and safety of a new drug, for prediction of the optimal pharmacokinetic and toxicological characteristics of new compounds intended for clinical use, and in creation of new drugs with special characteristics.

We evaluated the similarity and differences between humans and rodents (mice and rats) in the levels of blood esterases: acetylcholinesterase (ACE, EC 3.1.1.7), butyrylcholinesterase (BCE, EC 3.1.1.8), carboxylesterase (CE, EC 3.1.1.1), and paraoxonase (PON1, EC 3.1.8.1). Butyrylcholinesterase, CE, and PON1 play an important role in hydrolytic metabolism of drugs containing a complex ester (and lactone PON1) group, by participating in both activation of the drug from the pro-drug and inactivation of the active therapeutic agent; many new drugs are created with consideration for their metabolic activation/inactivation by hydrolysis [8]. In addition, these blood enzymes are involved in the defense function under the effects of anticholinesterase compounds by binding (ACE, BCE, CE) or hydrolyzing (CE, PON1) the potential toxicants. We defined summary activities of these esterases as the esterase status of the body, a marker of some diseases and indicator of patient metabolic status [1]. The data on activities of these esterases in humans and experimental animals are contradictory [6,13,14]. It was therefore interesting to carry out a comparative study under identical conditions using standard methods.

Individual activities of ACE, BCE, CE, and PON1 were measured in the blood of humans, rats, and mice and the differences in the esterase status of humans and typical experimental animals (rodents) were evaluated.

MATERIALS AND METHODS

Experiments were carried out on outbred male albino mice (18-25 g) and male Wistar rats (160-180 g).

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The animals were decapitated under CO_2 anesthesia, the blood was collected in small glass flasks washed with 3.8% sodium citrate. Heparin (20 μl of 500 U/ml solution) served as the anticoagulant. Specimens of human whole blood were collected from 5 healthy volunteers (women aged 35-48 years) taking no anticholinesterase substances. The blood was collected in an inpatient setting using a Vacuet vacuum system into tubes with 3.8% sodium citrate as the anticoagulant. The blood was divided into aliquots, frozen in liquid nitrogen, and stored at -70°C until measurements. The plasma was separated by centrifugation at 2500*g* for 15 min, frozen in liquid nitrogen, and stored at -70° until measurements. Frozen blood was defrosted in water bath with ice, after which 1:100 hemolysate specimens were obtained by rapid dilution of one blood volume in 100 volumes of cold (in water bath with ice) buffer. After thorough mixing, the aliquots of hymolysates were directly frozen in liquid nitrogen for stimulation of complete hemolysis and stored at -20°C until analysis. Before analysis the samples were defrosted and kept on ice. The volume of blood for the analysis of each esterase was chosen by the linear site at the curve presenting the relationship between this esterase activity and blood concentration (evaluated in a special experiment).

Activity of ACE in the whole blood was evaluated by the velocity of acetylthiocholine (1 μM) hydrolysis by Ellman's colorimetric method in 0.1 M K,Na-phosphate buffer (pH 7.5) at 25°C in the presence of ACE inhibitor ethopropazine (0.02 mM) [11,13,15]. Activity of BCE in the whole blood was measured under the same conditions with butyrylthiocholine (1 mM) as the substrate. In order to minimize the impact of hemoglobin absorption, the measurements were carried out at λ=436 nm (ε_{436}=10,600 $M^{-1}cm^{-1}$ [15]). Spectrophotometry of CE in whole blood was carried out with 1 mM 1-naphthylacetate as the standard substrate in 0.1 M K,Na-phosphate buffer (pH 8.0) at 25°C. The measurements were carried out at λ=322 nm (ε_{322}=2200 $M^{-1}cm^{-1}$ [5,6]). Differential inhibitory analysis with specific inhibitors of PON1/arylesterases (2 mM EDTA) and cholinesterases (40 μM eserin) [3] was carried out for elimination of PON1 and cholinesterase hydrolysis. Activity of PON1 was evaluated by the paraoxon hydrolysis velocity at λ=405 nm (paraoxonase activity ε_{405}=18,050 $M^{-1}cm^{-1}$) and phenylacetate at λ=270 nm (arylesterase activity ε_{270}=1310 $M^{-1}cm^{-1}$). Hydrolytic activity was evaluated by spectrophotometry at 25°C in 0.1 M Tris-HCl buffer (pH 8.0) with $CaCl_2$ and eserin (specific cholinesterase inhibitor). All measurements were carried out on a Gilford-250 spectrophotometer.

The results were presented as the means with errors in the means and calculated using GraphPad Prism 3.0 software.

TABLE 1. Plasma Activities of Esterase Status Enzymes in Humans and Rats (*M*±*m*)

Enzyme	Esterase activities, μmol substrate/min/ml plasma	
	human (*n*=5)	rat (*n*=6)
ACE	0.035±0.003	0.216±0.004
BCE	2.21±0.11	0.073±0.003
CE	0.046±0.002	3.69±0.15
PON1 (phenylacetate)	63.5±9.7	83.0±12.9
PON1 (paraoxon)	0.024±0.003	0.085±0.015

RESULTS

In the blood of the examined species, arylesterase activity of PON1 (with phenylacetate as the substrate) was the highest, while paraoxonase activity of PON1 (substrate paraoxon) was significantly lower (Table 1). Both PON1 activities (arylesterase and paraoxonase) in rat plasma were higher than in human plasma. Virtually complete absence of ACE activity in human plasma and its appreciable level in rat plasma was worthy of note. This fact was explained by the location of ACE protein in human blood in erythrocytes only, while in rats the ACE protein was present in both erythrocytes and plasma [3].

Activity of BCE in human plasma was 30 times higher than in rats. On the contrary, activity of CE was extremely low in human plasma and high in rats. This was in good agreement with the data on negligible level of CE protein and its activity in human plasma [9,12].

Activities of "esterase status" enzymes in the whole blood of humans, mice, and rats are presented in Table 2. The proportions of these enzymes activities were similar to the picture in the plasma. Arylesterase activity of PON1 predominated in the blood of all studied species.

Cholinesterase activities in human blood were higher in humans than in rodents (Table 2). ACE in human blood was 4- and 6-fold more active than in mice and rats, while BCE activity was 2- and 11-fold higher, respectively. Experiments showed that CE activity in human blood was somewhat higher than in the plasma, presumably because of CE presence in monocytes [5] and leukocytes [10]. However, CE activity in the blood of humans was 16-fold lower than in rats and 30-fold lower than in mice.

Hence, analysis of the esterase status of humans and rodents showed high levels of arylesterase ac-

TABLE 2. Activity of Esterase Status Enzymes in the Whole Blood of Humans, Mice, and Rats (*M*±*m*)

Enzyme	Esterase activities, μmol substrate/min/ml blood		
	human (*n*=5)	mouse (*n*=24)	rat (*n*=6)
ACE	4.16±0.14	1.02±0.15	0.723±0.060
BCE	1.40±0.08	0.710±0.061	0.128±0.010
CE	0.23±0.11	6.80±0.40	3.59±0.43
PON1 (phenylacetate)	34.6±4.1	23.2±8.6	37.3±3.2

tivity of PON1 in all the studied species. In human blood, esterase activity was presented (in addition to PON1) mainly by the erythrocyte ACE and plasma BCE, while CE activity was negligible. Rodents exhibited high activity of plasma CE, its activity being the maximum in mice, while cholinesterase activities were low. Importantly, esterase activities in mice are higher than in rats, which should be taken into consideration when selecting the experimental model for studies of compounds containing complex ester groups. Our data were in good agreement with recent data on ACE, BCE, CE, and PON1 proteins distribution in human, mouse, and rat plasma and erythrocytes, obtained by PAAG native electrophoresis [2].

The results indicate significant differences in the esterase status of humans, rats, and mice: in humans cholinesterase activities are significant, while in rats and mice the CE activity is the highest. Very high PON1 arylesterase activity has been found in all cases.

Comparative data on the basal esterase activities in humans and laboratory animals (mice and rats) used as experimental models are essential for experimental studies in medicine, toxicology, medical chemistry, particularly in creation of drugs with consideration for metabolic transformations. These data are useful for more correct extrapolation of experimental results from animals (rodents in our case) to humans and for predicting the probable specific features of compound drug metabolism in different species.

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