Pharmacokinetics of Ethanol in Mice with Different Alcohol Motivation

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We studied the pharmacokinetics of ^{14}C-ethanol administered in various doses and via different routes to CBA, C57Bl/6, and (CBA×C57Bl/6)F_1 mice. Kinetic scheme of ethanol distribution included its elimination by enzymatic (80-90% C_0) and exponential (10-20% C_0) mechanisms. Ethanol pharmacokinetics did not depend on the administration route and mouse strain. The kinetic scheme of ethanol distribution in mice was characterized by a dose-dependent linear increase in alcohol concentration in the plasma and brain and nonlinear (parabolic) increase in the area under its pharmacokinetic curve in the test tissue.

Key Words: *ethanol; kinetic scheme of distribution; modifying factors*

Ethanol is a widely known psychoactive compound [12,14]. Ethanol preference in experimental animals can be used to study differences in alcohol addiction in humans [4,5,12]. Experimental animals with different sensitivities to ethanol are characterized by different activities of enzymes metabolizing aliphatic alcohols and rates of alcohol metabolism and elimination [1,2,6,15], which probably determines biotransformation and pharmacokinetics of ethanol.

Here we studied the pharmacokinetics of ethanol administered in different doses and via different routes to alcohol-preferring C57Bl/6 mice, alcohol-nonpreferring CBA mice, and (CBA×C57Bl/6)F_1 mice.

MATERIALS AND METHODS

Experiments were performed on female CBA, C57Bl/6, and (CBA×C57Bl/6)F_1 mice weighing 18-22 g. The animals were kept under natural light/dark conditions, fed a standard diet, and had free access to water. ^{14}C-Ethanol (1.3 Ci/mol, Izotop) was administered intragastrically and intravenously in doses of 2 and 4 g/kg. The mice were decapitated 10, 20, and 30 min or 1, 2, 4, 6, 8 and 26 h after treatment. The content of ^{14}C-products in the plasma and brain was estimated as described previously [3]. Radioactivity was measured on a Rackbeta liquid scintillation counter (LKB). The results were analyzed by Statistica 5.0 software. Pharmacokinetic parameters were calculated using WinNonlin Pro Software.

RESULTS

Intravenous and intragastric administration of 2 g/kg ^{14}C-ethanol to CBA and C57Bl/6 mice was followed by its appearance in the circulation (T_{max}=0.17-0.5, Fig. 1). In both CBA and C57Bl/6 mice ^{14}C-ethanol rapidly entered the circulation and was eliminated from the plasma over 4-6 h and after 8 h the total radioactivity was low. The rates of ethanol elimination in the rapid phase (0.5-6 h) were similar in CBA (k_{el} 0.22± 0.01/h and 0.190±0.005/h after intravenous and intragastric treatment, respectively) and C57Bl/6 mice (k_{el} 0.25±0.01/h after intravenous treatment). In C57Bl/6 mice receiving ethanol intragastrically the rate of its elimination was higher than in CBA mice (k_{el} 0.297± 0.016/h). The absolute biological availability of ethanol was high in C57Bl/6 and CBA mice (0.94 and 0.91, respectively).

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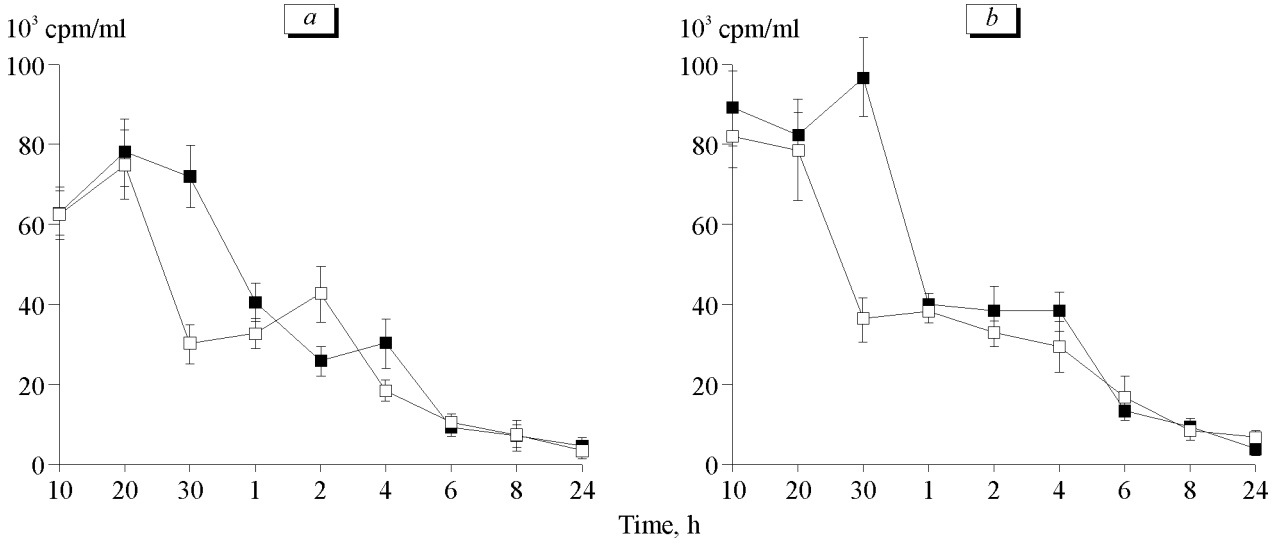

Fig. 1. Blood contents of ^{14}C-ethanol and its metabolites in C57Bl/6 (*a*) and CBA mice (*b*) after intravenous (*1*) and intragastric administration (*2*) in a dose of 2 g/kg.

^{14}C-ethanol administered intragastrically or intravenously to C57Bl/6, CBA (2 g/kg), and (CBA×C57Bl/6)F_1 mice (4 g/kg) rapidly entered the brain (Fig. 2). Rapid accumulation of ethanol over 10 min after treatment (biological phase of alcohol-induced effects) gives no way for estimating parameters of these processes. Ethanol underwent biphasic distribution and elimination in mice (rapid phase, 10 min-4 h; slow phase, 4-24 h).

The content of ^{14}C-ethanol in the plasma and brain changed in parallel; the ratio between ethanol concentrations in the plasma and brain remained unchanged for 10 min-24 h after treatment (~l, Figs. 1, 2). Therefore, the kinetic scheme of ethanol distribution can be derived from changes in radioactivity either in the plasma or in the brain.

For elaboration of the kinetic scheme of ethanol distribution in the body, one can assume that ethanol accumulation is a complete process and its rate far surpasses the rate ^{14}C-product elimination. This implies rapid distribution of ethanol between 2 regions determining the slow (exponential, 4-24 h) and rapid (enzymatic, 10 min-4 h) phases of its elimination described by formulas (**1**) and (**2**), respectively:

$$C_{1,t}=C_{1,0}e^{-kt}, \quad \textbf{(1)}$$

$$C_{2,t}=C_{2,0}[V_m t-K_m \ln(C_{2,0}/C_{2,t})], \quad \textbf{(2)}$$

where $C_{1,0}$, $C_{2,0}$, $C_{1,t}$, and $C_{2,t}$ are initial and actual ethanol concentrations in the brain in compartments **(1)** and **(2)**; k is the elimination constant; V_m is the maximum reaction rate; and K_m is the Michaelis constant.

We measured the total content of ethanol ($C_{1,t}$+ $C_{2,t}$). $C_{1,t}$ and k were evaluated by the ($\ln C_t$; t) curve. $C_{2,t}$ was calculated as $C_{exp,\,t}$-$C_{1,t}e^{-kt}$. $C_{2,0}$ and V_m were estimated by the (C_t; t) curve. V_m/K_m was measured by the ($\ln C_t$; t) curve. The calculation of theoretical C_t values is difficult, therefore we proposed the following

TABLE 1. Pharmacokinetics of ^{14}C-Ethanol and Its Metabolites (*M*±*m*)

Parameters	C57Bl/6, 2 g/kg		CBA, 2 g/kg		(CBA×C57Bl/6)F_1	
	intragastrically	intravenously	intragastrically	intravenously	intragastrically	
					2 g/kg	4 g/kg
k, h^{-1}	0.020	0.012	0.0167	0.03	0.023	0.027
V_m, 10^3 dpm/min/h	40.0	32.2	34	48.5	45.0	30.27
K_m, 10^3 dpm/min	27.00	31.6	26.30	49.0	44.0	32.2
AUC_t, 10^3 dpm/min×h	360.63±67.50	417.27±33.1	450.5±23.2	527.83±58.2	426.112±36.700	1414.8±112.5
AUC_e, 10^3 dpm/min×h	405.6±78.3	437.48±43.80	450.06±34.40	533.68±65.40	440.31±54.10	1403.0±127.8

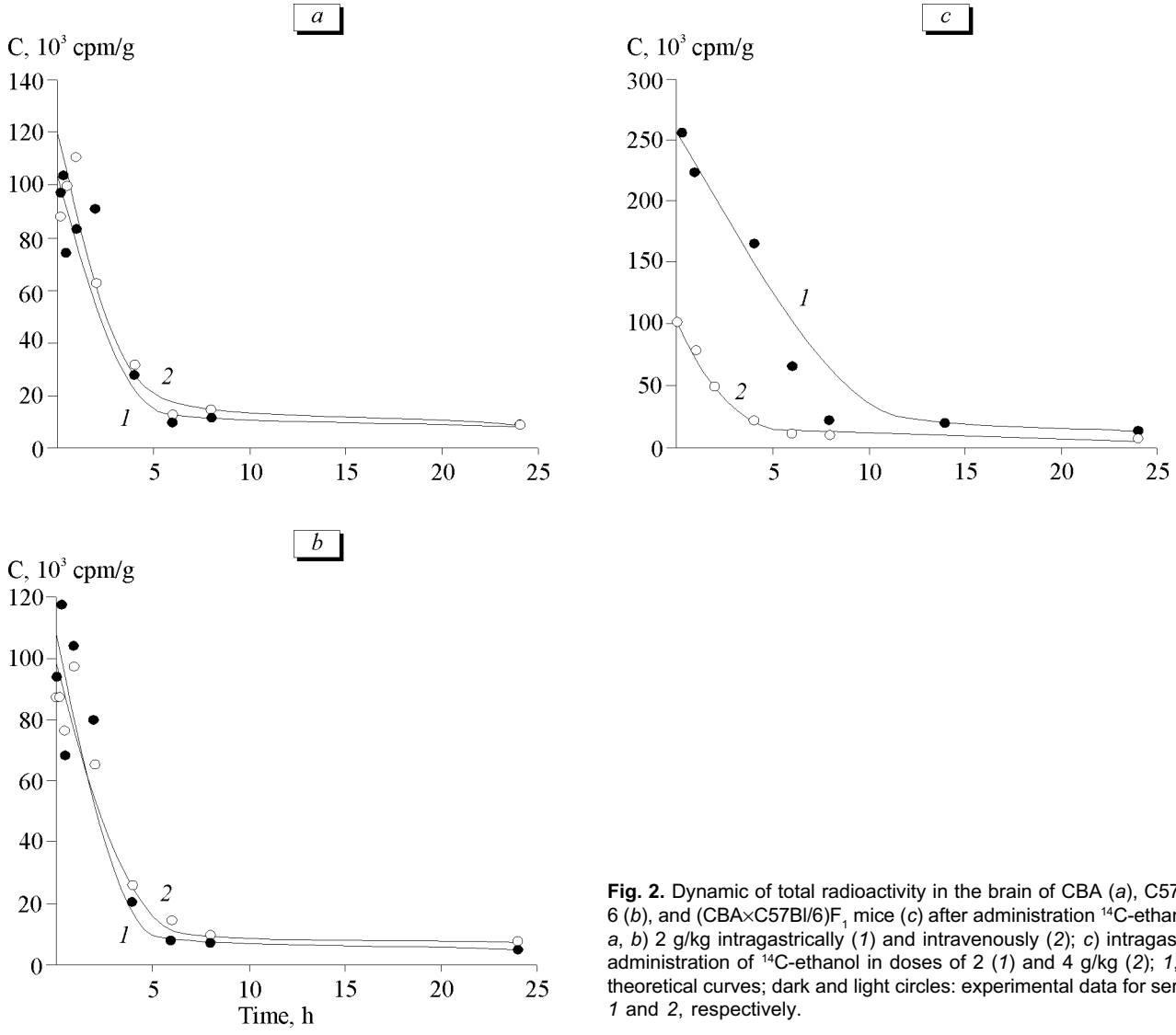

Fig. 2. Dynamic of total radioactivity in the brain of CBA (*a*), C57Bl/6 (*b*), and (CBA×C57Bl/6)F_1 mice (*c*) after administration ^{14}C-ethanol: *a*, *b*) 2 g/kg intragastrically (*1*) and intravenously (*2*); *c*) intragastric administration of ^{14}C-ethanol in doses of 2 (*1*) and 4 g/kg (*2*); *1*, *2*) theoretical curves; dark and light circles: experimental data for series *1* and *2*, respectively.

calculation scheme: $C_{2,t}$ values are specified with certain limits and the time to reach these concentrations can be calculated by the formula:

$$t = \frac{C_{2,0}-C_{2,t}}{V_m} - \frac{K_m}{V_m} \ln\left(\frac{C_{2,0}}{C_{2,t}}\right).$$

$C_{1,t}$ values for certain *t* are calculated:

$$C_{1,t}=C_{1,0}e^{-kt}.$$

$C_{1,t}$ and $C_{2,t}$ for the corresponding *t* are summed. For integral evaluation of the ethanol pharmacokinetics, the area under the curve (AUC) of ^{14}C-product content in the brain was determined. AUC can be estimated by the method of trapezoids (AUC_t) or from equations **(1)** and **(2)**, AUC_e. If the elimination process is linear, AUC depends on the dose of ethanol:

$$AUC_1=CL^{-1}D_1 \text{ at } D_m=mD_1$$
$$\text{and } AUC_m=mCL^{-1}D_1=mAUC_1,$$

where CL is ethanol clearance, D_1 is the administered dose of ethanol, and D_m is a proportionally increasing dose.

If the rate of ethanol elimination is constant (V_m, boundary enzymatic process), these relationships appear as:

$$AUC_1=\left(\frac{D1}{V_{SS}}\right)^2 \frac{1}{2V_m},$$

where V_{SS} is the volume of distribution. $AUC_m=m^2D_1(V^2_{ss}2V_m)^{-1}=mAUC_1$ at $D_m=mD_1$. Thus, for nonlinear elimination processes $mAUC_1<AUC_m<m^2AUC_1$. If ethanol is administered in increasing doses (m>1), the degree of nonlinearity increases and the dependence of

AUC on ethanol dose, which is linear at low concentrations, approaches a parabola.

Our results (Fig. 2) suggest that elimination of ^{14}C-ethanol and its metabolites in mice proceeds as two parallel processes: nonlinear (80-90% C_0) and exponential (10-20% C_0). The rate of the decrease of ^{14}C-product content in the specified interval does not depend on ethanol dose and concentration of products in the biological substrate. In the initial period the rate of this process is constant, which corresponds to the integral pattern of enzymatic process **(2)**. V_m and K_m values for organs and tissues functioning as a single compartment of the kinetic scheme change proportionally to the initial content ($C_{2,0}$) of ^{14}C-products in the compartment **(2)**. In the slow phase elimination of ^{14}C-product from the brain is the first-order process: increasing the dose of ethanol leads to a proportional increase in its concentration and acceleration of the process **(1)**.

The increase in ethanol dose caused a right shift (by 4.5 h) of the concentration curve (lnC) in the rapid phase (Fig. 2, *c*). Similar parameters of ethanol pharmacokinetics in rats were reported by D. V. Gauvin *et al*. [7]. The comparability of ethanol pharmacokinetics in various animal species indicates its determinacy and similarity. After intragastric administration of 4 g/kg ethanol (m=2) to (CBA×C57Bl/6)F_1 mice AUC of ^{14}C-product content in the brain 3.2-3.3-fold surpassed that after administration of ethanol in a dose of 2 g/kg, which implies nonlinearity of ethanol elimination in mice (Table 1). Ethanol elimination did not depend on the administration route and mouse strain. These findings and published data [3,9,10] do not confirm the hypothesis that ethanol pharmacokinetics plays the major role in determining alcohol preference [1]. Our previous studies showed that transient effects of ethanol linearly depend on alcohol content in the brain [3]. However, the cytotoxic and other effects of ethanol strongly correlate with AUC of physiologically active substances. These results indicate that the distribution of ethanol and its metabolites in experimental animals is characterized by a dose-dependent linear increase in alcohol concentration in the plasma and brain and nonlinear (parabolic) increase in their AUC in the test tissue. This probably determines the ratio between the pharmacological and toxic effects of ethanol.

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