PHARMACOLOGY AND TOXICOLOGY

Effects of 1,2,3,4-Tetrahydroimidazo[4,5-c]-Pyridine Derivatives on Ethanol Oxidation by Alcohol Dehydrogenase Isoforms from Human Liver

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We studied *in vitro* effects of four 1,2,3,4-tetrahydroimidazo[4,5-c]-pyridine derivatives formed in the reaction of the corresponding aldehydes with histidine on the rate of ethanol oxidation by alcohol dehydrogenase isoforms from human liver. None of test compounds inhibited ethanol oxidation by these enzymes. Some of them increased alcohol dehydrogenase activity to 220-240% of the initial level. Only one test compound accelerated ethanol oxidation by $\beta_1\beta_2$-alcohol dehydrogenase (150% of the control). The molecular mechanism underlying these effects of 1,2,3,4-tetrahydroimidazo[4,5-c]-pyridine derivatives on ethanol oxidation by alcohol dehydrogenase isoforms from human liver is discussed.

Key Words: *1,2,3,4-tetrahydroimidazo[4,5-c]-pyridine; derivatives; alcohol dehydrogenase; isoforms; ethanol*

Alcohol dehydrogenase (ADH, EC 1.1.1.1) is the main enzyme involved in primary alcohol biotransformation in the liver. This enzyme is a dimer presented by various isoforms [2,6], which are divided into 3 classes depending on their structural and functional properties [5]. Class I isoenzymes contain α-, β, and γ-subunits [6].

Typical ADH displays maximum activity at pH 10.5 and contains $\beta_1\beta_2$-subunits, while atypical ADH possesses maximum activity at pH 8.8 and consists of $\beta_2\beta_2$-subunits [1].

ADH isoforms have different catalytic activities [3]. The regulation of enzyme activities modulates alcohol metabolism in various ethnic groups. Thiourea specifically affects ADH isoforms from human liver. This compound activates ethanol oxidation by $\beta_1\beta_2$-ADH, but inhibits $\beta_2\beta_2$-ADH [9]. However, this effect is observed only at millimolar concentrations of the modulator. The search for new modulators of ethanol metabolism by liver ADH isoforms is of considerable importance for scientific purposes and medical practice.

We studied the effects of 1,2,3,4-tetrahydroimidazo[4,5-c]-pyridine derivatives formed in the reaction of aldehydes (products of alcohol biotransformation) with histidine on catalytic activity of ADH isoforms.

MATERIALS AND METHODS

1,2,3,4-Tetrahydroimidazo[4,5-c]-pyridine-3-carbonic acid hydrochlorides (S1-H) and 1-methyl-1,2,3,4-tetrahydroimidazo[4,5-c]-pyridine-3-carbonic acid (S1-M) were synthesized from L-histidine [10]. High-performance liquid chromatography showed that the purity of preparations was 99%. Physicochemical characteristics of S1-H corresponded to published data [10]. S1-M was a mixture of cis- and trans-isomers,

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which cannot be fractionated by extraction and crystallization. Cis- and trans-1-phenyl-1,2,3,4-tetrahydroimidazo[4,5-c]-pyridine-3-carbonic acids (S1-PC and S1-PT) were synthesized by the following method. L-Histidine hydrochloride monohydrate (5 g, 0.12 M) was dissolved in 60 ml distilled water. KOH (13.4 g) in 60 ml distilled water and benzaldehyde (19.1 g) in 90 ml ethanol were added. The reaction mixture was boiled under vigorous stirring for 2 h, cooled, and vacuum evaporated to a final volume of 120 ml. Benzaldehyde excess was extracted with ether (2×30 ml). The aqueous fraction was adjusted to pH 6.0 with 0.2 M H_2SO_4 and cooled. Crystals were filtered, washed, and vacuum dried. The procedure yielded 12.38 g (42%) S1-PT trans-isomer with a melting point of 259°C (255-257°C as reported previously [10]). Its chromatographic mobility on Silufol plates in an ethanol:acetic acid: water system (6.5:1:3.5) was 0.28 (yellow spot after development with ninhydrin). ^{1}H-Nuclear magnetic resonance (^{1}H-NMR, D_2O, Δ ppm): 3.06-3.16 (1H, 2d, CH_2), 3.32-3.42 (1H, 2d, CH_2), 4.06-4.12 (1H, 2d, CH), 5.79 (1H, s, CH), 7.3-7.5 (5H, m, Ph), and 7.79 (1H, s, CHim). Chemical shifts were measured in relation to the residual water signal at 40°C (4.68 ppm).

After separation of the trans-isomer, the stock solution was cooled to 0°C. White crystals were filtered, washed, and vacuum dried. The procedure yielded 7.59 g (26%) S1-PC cis-isomer with a melting point of 208°C (212-213°C as reported previously [10]). ^{1}H-NMR (D_2O, Δ ppm): 3.04-3.16 (1H, 2d, CH_2), 3.30-3.38 (1H, 2d, CH_2), 4.16-4.22 (1H, 2d, CH), 5.57 (1H, s, CH), 7.37-7.50 (5H, m, Ph), and 7.71 (1H, s, CHim).

ADH isoforms were isolated from human liver. The liver (300 g) was minced and homogenized in distilled water (1:3 w/v). The homogenate was centrifuged at 30,000*g* for 10 min on a 12-21 Beckman centrifuge and recentrifuged at 105,000*g* for 60 min on a Beckman 18-80M centrifuge. The supernatant (~1 liter) was concentrated to 100 ml, and its volume was adjusted to 200 ml with 20 mM Tris-HCl buffer (pH 8.2). The column packed with DEAE-52 (5.3×50 cm) was balanced with the same buffer. ADH was present in a free volume. Optical density was measured at 280 nm. Fractions with ADH activity were collected and concentrated to a final volume of 100-200 ml (protein content not less than 1-2 mg/ml). ADH phenotype was determined by optimum pH for ethanol oxidation reaction and thiourea inhibition [9].

For evaluation of ADH activity, the test compounds in 20 μl distilled water were added to 3 ml reaction mixture containing ADH (0.5 mg protein/ml) and 10 mM $NADH^+$ in 50 mM pyrophosphate buffer (pH 9.6). The reaction was initiated by adding ethanol (final concentration 40 mM) at 37°C. The reaction rate was estimated by changes in optical density at 340 nm (NADH reduction accompanying ADH-catalyzed ethanol oxidation). Optical density was measured on a Hitachi-557 spectrophotometer in a single-beam regimen. NADH molar extinction coefficient is 0.0062 $M^{-1}\times cm^{-1}$. The reaction rate (V) was calculated by tangent of the linear segment of the kinetic curve. The percent of activation was calculated by the formula: (V experiment/V control)×100%.

RESULTS

Preliminary experiments showed that ADH does not cause biotransformation of 1,2,3,4-tetrahydroimidazo[4,5-c]-pyridine derivatives. S1-M dose-dependently accelerated ethanol oxidation by $\beta_1\beta_2$-ADH, which

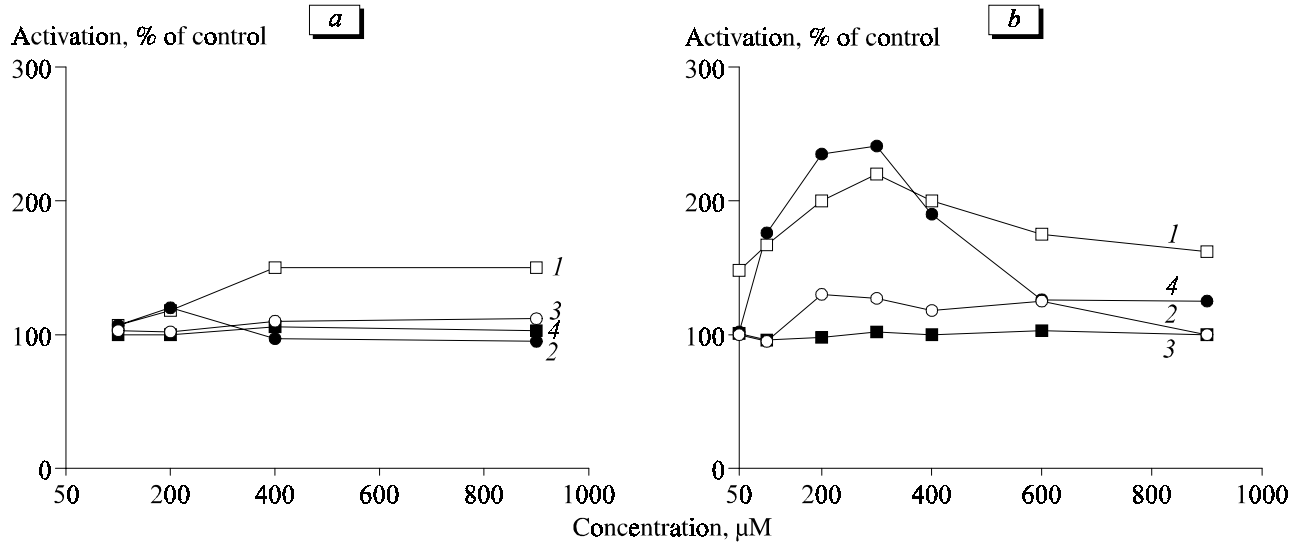

Fig. 1. Ethanol oxidation by $\beta_1\beta_2$- (*a*) and $\beta_2\beta_2$-ADH from human liver (*b*) in the presence of various concentrations of 1-methyl-3-carboxy- (*1*), trans-1-phenyl-3-carboxy- (*2*), 3-carboxy- (*3*), and cis-1-phenyl-3-carboxy-1,2,3,4-tetrahydroimidazo[4,5-c]-pyridine (*4*).

was maximum (150%) at 400 μM and then reached the plateau. S1-PT insignificantly activated ethanol oxidation (120% of the control) only in a concentration of 200 μM. S1-PC and S1-H had no effect on the reaction rate (Fig. 1, *a*).

The effects of 1,2,3,4-tetrahydroimidazo[4,5-c]-pyridine derivatives on $\beta_2\beta_2$-ADH were more pronounced (Fig. 1, *b*). The dependence of the activating effects of S1-PT on its concentration was described by a bell-shaped curve with a maximum (240%) at 200-300 μM. S1-M caused maximum activation (220%) in a concentration of 300 μM. S1-PC in a concentration of 20 μM insignificantly activated the reaction (150% of the control). S1-H was ineffective (similarly to experiments with $\beta_1\beta_2$-ADH).

Thus, S1-M and S1-PT 2-fold accelerated ethanol oxidation by human liver $\beta_2\beta_2$-ADH.

It should be emphasized that the presence of substituents in position 1 and their trans-orientation in relation to the carboxylic group play the major role in imidazopyridine-induced activation of ADH. The volume of this substituent can also be important, since S1-M produces activating effects in lower concentrations compared to S1-PT. The less pronounced activating effect of S1-M on ethanol oxidation and its dependce on activator concentration probably attested to the presence of both cis- and trans-isomers in the test sample. It can be assumed that only trans-isomers are active (similarly to 1-phenyl derivatives).

Atypical ADH differs from typical ADH by substitution of arginine in position 47 to histidine [7]. Previous studies showed that this substitution drastically changes catalytic properties of ADH [4], since the amino acid residue in position 47 is involved in $NADH^+$ coordination in the active center of ADH. Degradation of the ADH-NADH-product complex and the release of aldehyde and NADH is the rate-limiting stage in oxidation of primary alcohols by ADH [3].

These data and stereospecific effects of compounds suggest that S1-M and S1-PT bind to the active center of ADH and change $NADH^+$ coordination. The molecular mechanisms of this process require further investigations. Our findings indicate that new selective ADH activators belonging to 1,2,3,4-tetrahydroimidazo[4,5-c]-pyridine derivatives hold much promise.

REFERENCES

1. A. S. Kutsenko, D. A. Kuznetsov, V. V. Poroiko, and V. G. Tumanyan, *Bioorgan. Khimiya*, **26**, 179-189 (2000).
2. W. F. Bosron and T.-K. Li, *Semin. Liver Dis*., **1**, 179-183 (1981).
3. C.-I. Branden, H. Jornvall, H. Eklund, and B. Furugren, *The Enzymes*, Ed. P. D. Boyer, New York (1975), Vol. 11, pp. 103-190.
4. R. Buhler, J. Hempel, J.-P. von Warburg, and H. Jornvall, *FEBS Lett*., **173**, 360-365 (1984).
5. Y. Mezoy, *Alcohol Alcohol*, **29**, 707-710 (1994).
6. M. Smith, D. A. Hopkinson, and H. Harris, *Ann. Hum. Genet. (Lond.)*, **34**, 251-256 (1971).
7. G. Stamatoyannopoulos, S.-H. Chen, and M. Fukui, *Am. J. Hum. Genet*., **27**, 789-796 (1975).
8. B. L. Vallee and T. J. Bazzone, *Isoenzymes: Current Topics in Biological and Medical Research*, Eds. J. G. Scadalios, *et al*. (1983), Vol. 8, pp. 219-225.
9. J.-P. Warburg, J. Papenberg, and H. Aebi, *Can. J. Biochem*., **43**, 889-898 (1964).
10. S.-J. Yin, W. F. Bosron, L. J. Magnes, and T.-K. Li, *Biochemistry*, **23**, 5847-5853 (1984).