Does Trimetazidine Act as Antioxidant?

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Trimetazidine is 2-fold inferior to probucol in antioxidant activity measured using the model of copper-induced free radical oxidation of human plasma lipoproteins. EPR-spectroscopy shows that trimetazidine forms no free-radical intermediates in the presence of generated lipid alkoxyl or hydroxyl radicals, while probucol under these conditions forms phenoxyl radicals. Trimetazidine does not interact with superoxide radicals generated in the xanthine-xanthine oxidase system, since it does not inhibit reduction of tetrazolium nitroblue. However, indirect effect of trimetazidine on free radical oxidation cannot be excluded *in vivo*.

Key Words: *free radical oxidation; trimetazidine; probucol*

Recently, new therapeutic approaches have been developed to the treatment of ischemic heart disease, in particular, new drugs directly modulating the metabolism of ischemized cardiomyocytes [1]. Trimetazidine (1-[(2,3,4- trimethoxyphenyl)methyl] piperazine, TMZ, Servier [1,7]), a piperazine derivative, is a new cytoprotector with pronounced antiischemic and antihypoxic activities. It improves energy metabolism in cardiomyocytes, normalizes ionic homeostasis, reduces cell acidosis, decreases platelet aggregation via inhibition of thromboxane A_2 formation, and suppresses neutrophil activation in ischemized tissue [1,7]. Antioxidant activity and/or recombination of oxygen radicals have been discussed as possible components of its biological activity [1,5-7]. In particular, TMZ reduces the concentration of lipid free radical oxidation (FRO) products in perfusate during reperfusion of ischemized tissues characterized by enhanced generation of active oxygen radicals [6,7]; it also improves cell and tissue resistance to prooxidants [5,7]. However, exact mechanism of this antioxidant effect remains unclear, moreover, direct antioxidant activity of TMZ cannot be anticipated on the basis of its chemical structure. In light of this, we studied the antioxidant effect of TMZ in common model systems: FRO of human plasma low density lipoproteins (LDL) induced by transient metals and tetrazolium nitroblue reduction by superoxide anion-radicals generated in the xanthine-xanthine oxidase system. Additionally, the possibility of TMZ radical formation was explored by EPR spectroscopy.

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MATERIALS AND METHODS

LDL were isolated from venous blood of male patients with ischemic heart disease without hyperlipidemia; 1 mg/ml EDTA was added to the blood as antioxidant and anticoagulant. The plasma was centrifuged 2 times in a NaBr density gradient at 42,000 rpm for 2 h (4°C, 50 Ti angle rotor, Beckman L-8 centrifuge) [11], floating LDL were collected and dialyzed at 4°C for 16 h. The LDL fraction was not contaminated by plasma proteins or other lipoproteins and was identical in particle size and composition to the LDL fraction isolated as described elsewhere [10]. Protein content in LDL samples was measured by the method of Lowry. LDL were adjusted to a concentration of 50 μg/ml with 0.154 M NaCl and 50 mM phosphate buffer (pH 7.4), incubated at 37°C for 1 h, and LDL oxidation was induced by adding 3×10^{-5} $CuSO_4$ to the incubation medium.

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The concentration of diene conjugates in the incubation medium was measured spectrophotometrically in a Hitachi-220A spectrophotometer at 233 nm [12]. The test inhibitors TMZ and probucol in ethanol (2% final concentration) were added before FRO induction. An equivalent volume of ethanol was added to the control samples. The duration of induction and maximum oxidation rate were determined by kinetic curves.

Superoxide anion radicals were generated in the reaction of xanthine oxidation catalyzed by milk xanthine oxidase, the rate of superoxide radical production was measured in a Hitachi-557 spectrophotometer (560 nm) by reduction of tetrazolium nitroblue to diformazan [4]. The incubation medium contained 0.05 M sodium carbonate (pH 10.2), 10^{-4} M EDTA, 10^{-4} M xanthine, 10^{-7} M milk xanthine oxidase, 2.2×10^{-5} M nitroblue tetrazolium, and test inhibitors.

Arachidonic acid micelles (final concentration 40 mM) prepared an 50 mM K,Na-phosphate buffer (pH 7.4) using a Soniprer-150 ultrasonic disintegrator (MSE) [9] were oxidized for 10 min at 25°C in the presence of 100 U/ml soybean lipoxigenase, then 100 μM TMZ or probucol was added, and generation of alkoxyl radicals was induced by adding 25 μM hemin to 2 mM H_2O_2. Electron paramagnetic resonance (EPR) spectra were recorded in an X-band E-109E Varian spectrometer at room temperature and constant aeration of the sample (0.25 mT field modulation, 100 kHz MW frequency, and 100 mW MW power [3].

Trimetazidine dichlorhydrate was synthesized according to Patent No. 3262852 (USA). Probucol, xanthine, xanthine oxidase, nitroblue tetrazolium, arachidonic acid, soybean lipoxigenase, hemin, and other reagents were purchased from Sigma.

RESULTS

Probucol (4,4'-(isopropylidenedithio)bis(2,6-di-tret-butylphenol), a nontoxic antioxidant exhibiting moderate hypocholesterolemic activity, is widely used in complex therapy of ischemic heart disease and atherosclerosis [2,8]. Structurally, probucol is a hindered phenol with 2 hydroxyl groups (Fig. 1) which loose their hydrogen atoms in the reaction with active peroxide radicals, yielding low-activity phenoxyl radicals than cannot continue to chain reaction of lipid oxidation: PhOH+LOO•—PhO•+LOOH [2]. Unlike probucol, TMZ had no free hydroxyl groups in the phenol ring (Fig. 1), therefore it cannot inhibit FRO via an analogous mechanism, i.e., though the formation of intermediate phenoxyl radicals. However, generation of other TMZ radicals cannot be excluded. Indeed, probucol even in a concentration of 5 μM considerably and in concentrations of 50-100 μM completely (over 3 h) inhibited copper-induced FRE of LDL, while TMZ in a concentration of 50 μM slightly activated this process (Fig. 1). Some prolongation of the oxidation latency and reduction of the oxidation rate was noted only with extremely high TMZ concentrations (250-500 μM) one order of magnitude surpassing FRE-inhibiting concentrations of probucol.

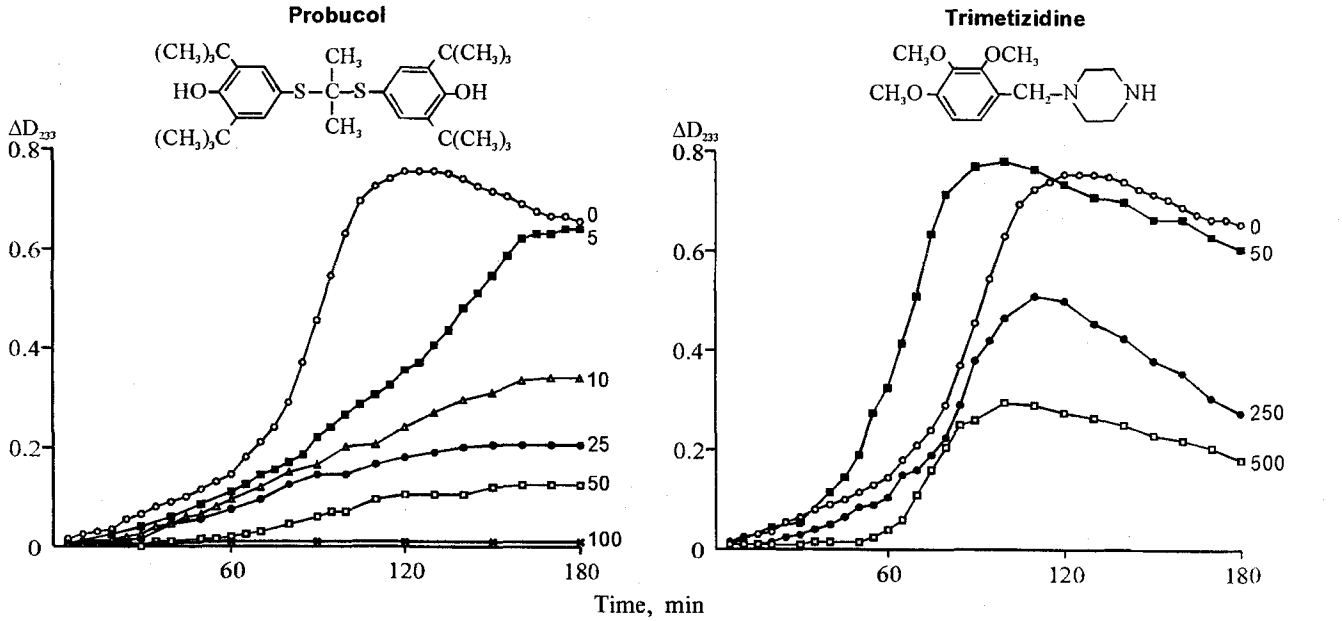

Fig. 1. Kinetic curves of copper-induced free-radical oxidation of human plasma lipoproteins in the presence of probucol and trimetazidine in the incubation medium. Numbers on the curves indicate drug concentrations, μM.

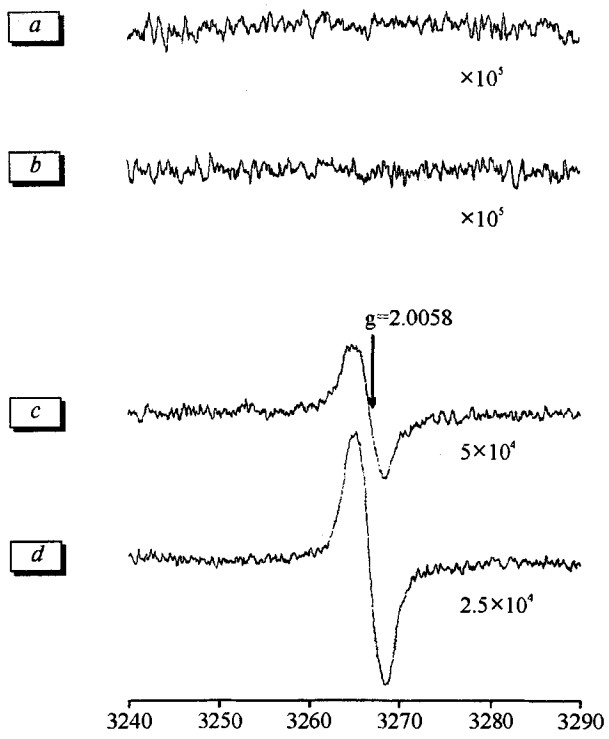

Fig. 2. Electron paramagnetic resonance spectra of incubatin media containing trimetazidine (*a, b*) and probucol (*c, d*) under conditions of generation of lipid alkoxyl (*a, c*) and hydroxyl (*b, d*) radicals. Abscissa, Gs.

EPR spectroscopy revealed the formation of long-lived probucol phenoxyl radicals in the presence of lipid alkoxyl radicals or hydroxyl radicals (Fig. 2), while TMZ under the same conditions formed no free radical intermediates. In a concentration range of 50-500 μM TMZ did not interact with superoxide anion radicals: it had no effect on the rate of tetrazolium nitroblue reduction with superoxide radicals generated by the xanthine-xanthine oxidase system.

Thus, our findings suggest that TMZ does not interact directly with active lipid ($LO^{\bullet}$) or oxygen ($OH^{\bullet}$, O_2^{-}) radicals and it is hardly possible that TMZ inhibits FRE of LDL via direct reaction with lipoperoxides (Fig. 1 and 2). Inhibition of FRE in the presence of high concentrations of TMZ (Fig. 1) is probably due to modulation of LDL structure. Since therapeutic dose of TMZ (60 mg/day) is about 20-fold lower than that of probucol (1000 mg), whereas FRO-inhibiting activity of TMZ is one order of magnitude lower than that of probucol, the possibility of in vivo inhibition of LDL FRO via the direct antioxidant mechanism is practically excluded. Despite the fact that TMZ cannot act as a radical trap, an indirect influence of this drug on the *in vivo* FRO cannot be excluded. Published data [2,8] and our findings (Fig. 1) suggest that probucol directly interacts with free radicals. We have previously shown that antioxidant activity of probucol *in vivo* are to a great extent realized through indirect modulation of free-radical reaction in tissues, in particular, due to activation of antioxidant enzymes. Now we explore the possibility of indirect TMZ-modulation of enzymatic utilization of oxygen radicals and lipoperoxides in the blood of patients with ischemic heart disease treated with TMZ (Preductal-20).

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