Structural and Functional Characteristics for the Antiinflammatory Effect of New Water-Soluble Sulfur-Containing Phenol Antioxidants

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Structurally related phenols containing the *p*-alkylthiosulfonate substituent and partially hindered by tert-butyl groups were synthesized for the search and development of new synthetic antioxidant, which would be effective *in vivo* in preventing free radical-induced pathological processes. Sodium 3-(3'-tert-butyl-4'-hydroxyphenyl)propylthiosulfonate had high antiinflammatory activity and produced a dose-dependent effect (IC_{50}=36 mg/kg). Changes in the length of the carbohydrate chain in the *p*-alkyl substituent had no effect on *in vitro* antiradical activity of the test compounds. However, the decrease in the length of this chain by one methylene unit was accompanied by reduction of antiinflammatory activity of the end product. Lengthening of the chain did not modulate these properties.

Key Words: *synthetic antioxidants; inflammation; QSAR analysis*

Reactive species of oxygen and nitrogen play a role in the pathogenesis of various diseases and aging. Much attention is paid to the mechanisms of action and therapeutic efficacy of antioxidants (AO). Despite the absence of strong prophylactic effect of AO vitamins in large-scale trials in the 1990s, studying the natural and synthetic AO is still of considerable importance. There are several directions of these studies. Some attempts were made to obtain AO with new properties. Water-soluble forms of vitamin E and coenzyme Q_{10} were developed [10]. New preparations of combined action contain not only AO, but also a specific drug [1]. Good results were obtained in the development of targeted AO (*e.g.*, products with primary accumulation in mitochondria) [7,10].

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We synthesized structurally related monophenols, which differ by the number of tert-butyl substituents in the *o*-position (relative to the OH group) and presence of the sulfonate or thiosulfonate group in the *p*-propyl substituent. Hindered phenols exhibit high AO activity in *in vitro* model systems. They inhibit peroxyl radicals due to the presence of functional OH group in the benzol ring. This reaction leads to the formation of a stable phenoxyl radical. Hindered phenols reduce hydroperoxides into nonreactive alcohols, which is related to the thiosulfonate group [2]. Animal experiments showed that low toxicity (toxicity class 3-4) and high antiinflammatory activity (AIA) of these compounds are associated with the ability to induce the AO-responsive element [3]. Partially hindered thiosulfonate TS-13 (sodium 3-(3'-tert-butyl-4'-hydroxyphenyl)propylthiosulfonate) was most effective in this respect.

Here we performed a quantitative analysis of TS-13. Phenol compounds with varying length of

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the carbohydrate chain in the *p*-position were synthesized for the search and development of structurally optimal products. A quantitative structure-activity relationship analysis (QSAR analysis) was performed under *in vitro* and *in vivo* conditions to evaluate the AO and antiinflammatory properties of these compounds.

MATERIALS AND METHODS

S-Alkylthiosulfonates TS-12 (sodium 3-(3'-tert-butyl-4'-hydroxyphenyl)ethyllthiosulfonate), TS-13 (sodium 3-(3'-tert-butyl-4'-hydroxyphenyl)propylthiosulfonate), and TS-14 (sodium 3-(3'-tert-butyl-4'-hydroxyphenyl)butylthiosulfonate) were obtained during the interaction of halogen alkanes with sodium thiosulfate in an aqueous alcohol solution [4].

AO activity of synthetic compounds was evaluated under *in vitro* conditions by oxidation of methyl oleate (133 mM) in aqueous solution of sodium dodecyl sulfate (SDS, 250 mM) in the presence of inducing agent 2,2'-azo-bis-(2-methylpropionamidine)-dihydrochloride (APH, 6.15 mM) [4, 6]. The test compounds were used in a dose of 45-230 μM. Potassium β-(3,5-di-tert-butyl-2-hydroxyphenyl)propionate (potassium phenosan) and 2,6-di-tert-butyl-4-methylphenol (ionol) were used as the reference preparations. The inhibition rate (W_i, 7×10^{-8} mol/liter/sec) was estimated by the method of inhibitors. The oxidation chains consisted of at least 27 units.

AIA of phenol compounds was studied on rats with paw inflammation [9]. Experiments were performed on Wistar rats weighing 280-300 g. The animals received an intraplantar injection of 10% carrageenan (Sigma) in physiological saline into the right hindlimb. The inflammatory reaction was evaluated from paw edema. The degree of paw edema was estimated plethysmographically 5 h after carrageenan injection. It was calculated as follows: $((V_R-V_L)/V_L)\times100\%$, where V_R and V_L are volumes of the right and left paws, respectively. The test compounds were dissolved in 1 ml distilled water and administered orally through an probe 1 h before induction of inflammation. Compound TS-13 was given in doses of 5, 25, 100, 400, and 1600 mg/kg to evaluate the dose-dependence of AIA and in a dose of 100 mg/kg to study the effect of variations in the length of the carbohydrate chain of the *p*-alkyl substituent.

The half-inhibitory concentration (IC_{50}) was estimated by interpolation of the dose-response dependence (experiments with at least four concentrations of study compounds).

The results were analyzed by Student's *t* test.

RESULTS

IC_{50} was evaluated by variation in the dose of TS-13. This phenol had a strong dose-dependent effect. IC_{50} was 36 mg/kg (r=-0.65, p=0.0006; Fig. 1, *a*). Administration of TS-13 in high concentration (1.6 g/kg) was not accompanied by complete inhibition of carrageenan-induced paw edema in rats (Fig. 1, *b*). Inflammation is a multicomponent process, which involves a variety of pathogenetic mechanisms. TS-13 probably inhibits only some pathological reactions, including those mediated and regulated by the AO-responsive element. For example,

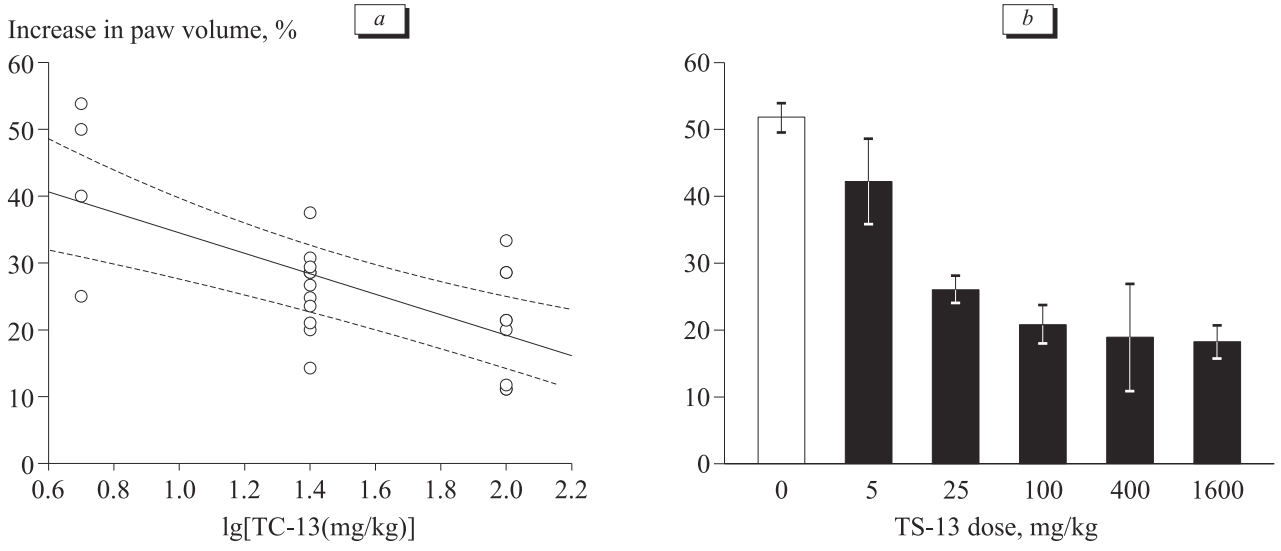

Fig. 1. Dose-dependence of AIA for compound TS-13. *a*) Linear region of the dose-effect curve, individual values (scatter diagram with the regression curve and 95% confidence interval); *b*) mean values.

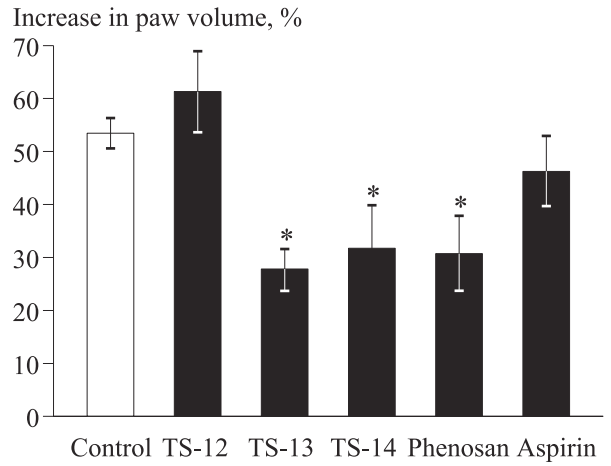

Fig. 2. Effect of TS-13 analogues with various lengths of the *p*-alkyl substituent on the severity of inflammation. **p*<0.05 compared to the control.

AIA of tert-butylhydroquinone is related to activation of Nrf2. Due to interaction with the tumor necrosis factor-α gene enhancer, Nrf2 competes with antiinflammatory transcription factor NF-κB for binding to this region of DNA. It contributes to inhibition of cytokine synthesis [8].

Oxidation of methyl oleate (RH) in aqueous solution of SDS in the presence of APH was used as a model system to study the antiradical activity of water-soluble phenol AO (TS-13 and its homologues with various lengths of the *p*-propyl substituent). This system simulates oxidation of lipids in biological membranes and proceeds as a free radical chain process, which is described by the following kinetic scheme:

$$APH \rightarrow r^{\bullet} \rightarrow R^{\bullet},$$
$$R^{\bullet} + O_2 \rightarrow RO_2^{\bullet},$$
$$RO_2^{\bullet} + RH \rightarrow ROOH + R^{\bullet}, \quad (1)$$
$$RO_2^{\bullet} \rightarrow \text{molecular products, and} \quad (2)$$
$$ArOH + RO_2^{\bullet} \rightarrow ROOH + ArO^{\bullet}, \quad (3)$$

where $r^{\cdot}$ is the radical of an inducing agent; $R^{\bullet}$ and $RO_2^{\bullet}$ are the alkyl and peroxyl radicals of methyl oleate, respectively; and ArOH and $ArO^{\bullet}$ are the molecule and radical of an inhibitor, respectively.

TABLE 1. AO Activity of Synthetic Phenol Compounds

Compound	k_3/k_1	k_3, $M^{-1}sec^{-1}$
TS-12	80	1600
TS-13	83	1700
TS-14	81	1700
Potassium phenosan	280	5800
Ionol	1400	20 700

The differential equation system for this kinetic scheme was solved with estimated rates of spontaneous and inhibited oxidation. The k_3/k_1 ratio reflected reaction ability of synthetic compounds to peroxyl radicals of methyl oleate. The k_3/k_1 ratios (mean values of several measurements, n=3-8) are shown in Table 1. The mean-square error did not exceed 25%. Values of k_1 and k_3 for study compounds in the model system are calculated from k_3 for ionol during oxidation of methyl linoleate in an aqueous solution of SDS [6]. This value depends weakly on the nature of $RO_2^{\bullet}$ [5]. Our results indicate that TS-12, TS-13, and TS-14 did not differ by antiradical activity during oxidation of methyl oleate in an aqueous solution of SDS. Antiradical activity of these compounds is slightly lower than that of the hydrophilic reference preparation phenosan. The test compound are less potent than lipophilic ionol. It is related to hydration of OH groups of mono-tert-butyl-substituted phenols in an aqueous micellar system. Moreover, these compounds are characterized by different location in an aqueous micellar solution. Lipophilic ionol is located inside the micelle, while hydrophilic antioxidants exist in an aqueous medium.

Structural analogues of TS-13 with various lengths of the carbohydrate chain in the *p*-alkyl substituent were tested for AIA. Shortening of the substituent by one methylene unit (TS-12) was followed by the loss of its ability to prevent paw edema (Fig. 2). At the same time, the preparation TS-14 with active bivalent sulfur distant from the benzene nucleus in the thiosulfonate fragment was as potent as the parent compound TS-13. Activity of TS-14 was comparable with that of phenosan, but exceeded the effect acetylsalicylic acid. Unfortunately, it is difficult to increase AIA of these compounds by further increasing the length of the carbohydrate chain in the *p*-alkyl substituent. ω-(3'-tert-Butyl-4'-hydroxyphenyl)alkylthiosulfonates with a considerable number of methylene units are characterized by high hygroscopicity, which makes it difficult to isolate, purify, and operate with these compounds.

These results and QSAR analysis show that variations in the length of the carbohydrate chain residue in the *p*-alkyl homologue TS-13 (sodium 3-(3'-tert-butyl-4'-hydroxyphenyl)propylthiosulfonate) have little effect on antiradical activity of compounds *in vitro*. However, the decrease in the length of this chain by one methylene unit in sodium 3-(3'-tert-butyl-4'-hydroxyphenyl)ethyllthiosulfonate (TS-12) is accompanied by the disappearance of AIA. Lengthening of the chain in sodium 3-(3'-tert-butyl-4'-hydroxyphenyl)butylthiosulfonate (TS-14) has no effect on AIA.

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