There are indications in the literature that trialkyltin derivatives are capable in extremely low concentrations of inhibiting ATP-dependent transport of NAD-dependent substrates, in particular, malate, pyruvate, citrate, and β-hydroxybutyrate [8]. It is quite possible that the OTC investigated in the present work also, possessing the trialkyltin group, give the same effect, lowering the activity of the dicarboxylate transporter, like mersalyl [9].

It is known that the inhibiting effect of OTC is removed by thiol compounds, for example, reducing glutathione, dihydrolipoate, 2,3-dimercaptopropanol, and dihydrolipoamide [7]. Therefore it is most probable that the action of OTC is directed toward the SH groups of the enzyme systems enumerated above, and differences in the inhibition constants are explained by the different accessibility of these groups to the inhibitors.

On the whole, it can be concluded on the basis of the experimental data presented and the information from the literature that OTC can inhibit respiration of the mitochondria not only on account of inhibition of H^+-ATPase but also as a result of a disruption of the oxidation of NAD-dependent substrates and succinate. The most probable mechanism of this disruption is a hydrophobic binding of OTC to NADH dehydrogenase and components of the mitochondrial membranes, responsible for the transport of substrates.

The results obtained in this work can be used for the directed synthesis of new OTC with set inhibiting characteristics.

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INVESTIGATION OF THE METABOLISM OF THE o-TOLYL ANALOG OF FENCAROL

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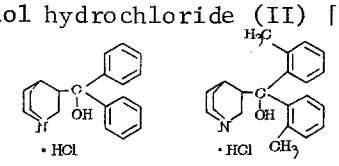
UDC 615.218.2.015.4

Earlier we reported on an investigation of the pharmacokinetics [1] and metabolism [2] of an original antiallergic preparation fencarol — (quinuclidyl-3)-diphenylcarbinol hydrochloride (I) — which belongs to a new chemical class of compounds for antihistamines [3] and is now being produced by the chemicopharmaceutical industry.

In the synthesis and pharmacological investigation of fencarol analogs — derivatives of (quinuclidyl-3-diaryl(heteryl)carbinols — substances were obtained that are also characterized by antihistamine properties but differ from fencarol by greater duration of action and by the presence of high antiserotonin activity [4]. One of these substances is o-tolyl I — (quinuclidyl-3)-di-(o-tolyl)carbinol hydrochloride (II) [4].



I II

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Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 16, No. 11, pp. 1300-1304, November, 1982. Original article submitted March 16, 1982.

0091-150X/82/1611-0796$07.50

To expand or deepen our concepts of the new class of antihistamines, we investigated the pharmacokinetics of compound II [5]. The present work is devoted to a study of its metabolism.

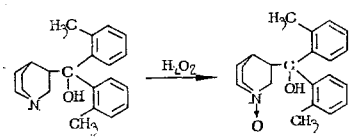
EXPERIMENTAL PHARMACOLOGICAL SECTION

The investigation was conducted on male white rats weighing 120-180 g. Compound II was introduced into the stomach in the form of a 2% suspension in a 1% carboxymethylcellulose solution; water loading was given simultaneously with a 0.45% sodium chloride solution in an amount of 5 ml to 100 g of body weight of the animals. The metabolites of compound II were determined in the urine collected in 96 h, since, according to the pharmacokinetic data, during this period after the administration of tritium-labeled compound II to rats, the bulk of the radioactive products were excreted with the urine. The urine (150-200 ml) was filtered to remove mechanical impurities; the products of metabolism were extracted with chloroform, the extract evaporated under vacuum, and the residue chromatographed on plates with a fixed layer of silica gel (mobile phase methanol). In this case three substances with different chromatographic mobilities were obtained: R_f 0.08, 0.46, and ∿1.0.

In elution of the substance with R_f 0.08 from the plate with chloroform and its recrystallization from ethanol, pure (quinoclidyl-3)-di(o-tolyl)carbinol with mp 242-243°C was obtained. The base of the substance II, isolated from the urine, was identified according to the absence of depression during melting of a mixed sample and complete coincidence of the IR spectra with a known sample. The mass spectra of the base of substance II isolated from rat urine and of a known sample also coincided. In both mass spectra, peaks of a molecular ion with mass number m/e 321 was observed. The main decomposition of the molecular ion under electron impact occurred in both cases with the formation of fragments with m/e 306 $[M-CH_3]^+$; 304 $[M-OH]^+$; 303 $[M-H_2O]^+$; 211 $[HO^+-C(C_6H_4CH_3)_2]$; 202 $[M-CH_3C_6H_4C=O]^+$; 119 $[CH_3C_6H_4C=O]^+$; 97 $[C_6H_{11}N]^+$; 96 $[C_6H_{10}N]^+$; 91 $[C_6H_4CH]^+$.

The metabolite with R_f 0.46, like the first substance, was eluted from the plates with chloroform. After the chloroform was distilled off, the substance was recrystallized from 50% methanol. In this case colorless crystals with mp 259-260°C were obtained.

Since the main product of fencarol (I) metabolism in the rat organism is the corresponding N-oxide [2], we suggested that another of the pathways of biotransformation of compound II is its N-oxidation. Elementary analysis of the metabolite isolated from rat urine, with mp 259-260°C, was in good agreement with this assumption. To confirm the structure of the metabolite, the N-oxide of (quinuclidyl-3) di(o-tolyl)carbinol (III) was produced by countersynthesis by oxidation of (quinuclidyl-3-)-di(o-tolyl)carbinol.



III

The synthesized N-oxide III gave no depression when a sample was melted together with the product isolated from rat urine, possessing R_f 0.46, and proved identical with it according to chromatographic mobility and IR spectrum. The mass spectra of these compounds also proved identical. Molecular peaks with m/e 337 and fragments with m/e 321, 306, 304, 303, 211, 202, 119, 91 were observed in them. The indicated breakdown under electron impact is due to elimination of the oxygen atom from the molecular ion, which is characteristic of N-oxides. The further decomposition of the ion with m/e 321 virtually coincides with the decomposition of the base of substance II. The most intense fragments belong to the ions with m/e 119, 91, and 211, to which the following structures correspond: $[CH_3C_6H_4C=O]^+$; $[C_6H_4CH]^+$; $[HO^+=C(C_6H_4CH_3)_2]$, respectively.

Thus, the main pathway of metabolism of compound II, like that of I, is enzymatic oxidation at the nitrogen atom of the heterocycle with the formation of the corresponding N-oxide.

The spot on the chromatogram with $R_f \approx 1.0$ proved in a more detailed study not to correspond to one substance. Chromatography of the sample eluted from silica gel in a different system (benzene–isopropanol, 1:2) showed that it contains at least five different compounds of increased polarity. However, the five indicated metabolites are minor products of biotransformation of substance II, since their total amount in the urine (according to the data

of thin-layer chromatography and preliminary isolation) is related to the amounts of the base (quinuclidyl-3)-di-(o-tolyl)carbinol and its N-oxide (substance III) as 0.3:1.15. The isolation of these minor products in an individual state and the establishment of their structure are being studied.

Considering the possibility of various pathways of metabolism of drug preparations in the bodies of animals and humans, it was of interest to study the products of biotransformation of substance II in the human organism. For this purpose urine was collected from four persons who had taken substance II orally in a dose of 100 mg. The isolation of metabolites from the urine of humans was performed by the method of ion exchange chromatography. After passage of the urine through a column with the cation exchange resin KU-2 in the H^+-form, the products of metabolism were eluted with a mixture of ethanol and 0.1 N hydrochloric acid (9:1), and 10 ml fractions of the eluate were collected (fractions 1-10). After chromatographic monitoring on silica gel plates according to the method described above and combining of the fractions containing substances with the same chromatographic mobility, they were evaporated under vacuum and the residues recrystallized from methanol. A substance with R_f 0.07 was isolated from fraction 1. According to the data of mass and IR spectroscopy, as well as according to the chromatographic mobility, this substance proved to be (quinuclidyl-3)-di-(o-tolyl)carbinol (the base of substance II). The mass and IR spectra of the substance with R_f 0.49 (from fractions 7, 8, and 9) entirely coincide with the mass and IR spectra of samples of the N-oxide of III, isolated from rat urine and produced by countersynthesis. A study of the remaining products of metabolism of substance II in human urine, isolated from other fractions of the eluate, is continuing.

The investigation conducted permits us to conclude that the base of substance II and N-oxide of (quinuclidyl-3)-di(o-tolyl)carbinol (III) as the main product of biotransformation of this quinuclidine compound can be isolated from the urine both of rats and of humans that took substance II.

The pharmacological activity of the metabolite III was compared in parallel experiments with the activity of compound II according to the main indices characteristic of this preparation: Antihistamine activity was determined on unanesthetized guinea pigs after inhalation of an aerosol of a 1% histamine solution; the action of the preparations (1 h after their internal administration) was judged according to the increase in the latent period of the intoxication reaction (t is the time in seconds from the beginning of inhalation of histamine until the appearance of clonic-tonic convulsions) in comparison with the latent period of the reaction in the control group (t_{contr}).

The antiserotonin activity was determined on guinea pigs after inhalation of a 1.5% serotonin solution; it was estimated according to the increase in the latent period of intoxication in seconds, i.e., just as in the determination of antihistamine action.

Antiedematous properties were studied on Wistar rats weighing 110-120 g; edema of the hind foot was induced by subplantar injection of dextran (0.05 ml of a 6% solution); the increase in the cross-section of the foot was measured after 30 min with calipers and expressed in millimeters. The preparations were introduced into the stomach with a probe 1 h before the injection of dextran; the antiedematous action was judged according to the reduction of edema in the experimental animals in comparison with the control.

The influence of compound II and its metabolite III on the functional state of the central nervous system was judged according to the change in the spontaneous motor activity in mice [motor activity was recorded with an Animex instrument (Sweden)], according to the change in the duration of sleep induced in mice by intraperitoneal injection of hexenal (100 mg/kg), and according to the influence of the preparations on the effects of reserpine — hypothermia and ptosis [6].

The toxicity (value of LD_{50}) was determined on mice after a single internal administration of the preparations. The results were treated statistically.

RESULTS AND DISCUSSION

The results of a comparative pharmacological investigation of compound II and its metabolite III are presented in Table 1. From the table it is evident that metabolite III possesses the basic pharmacological properties of compound II: it increases the latent period of the intoxication reaction after the inhalation of aerosols of histamine or serotonin by guinea pigs, and it exerts antiedematous effects in rats in the case of disruption of the vascular

TABLE 1. Comparative Pharmacological Activity of Compound II and Its Metabolite III

Index	Dose, mg/kg (internally)	Compound II	Compound III
Antihistamine activity: increase in the latent period of the intoxication reaction (in sec) after inhalation of a histamine aerosol*	50	226 (148—304)	36 (17—55)
	150	—	141 (44—238)
Antiserotonin activity: increase in the latent period of the intoxication reaction (in sec) after inhalation of serotonin aerosol*	10	189 (101—277)	17
	30	—	144 (61—227)
Antiedematous activity: reduction of edema of the foot of rats (in mm) after subplantar injection of dextran*	50	3,60 (2,03—5,17)	1,4†
Toxicity: LD_{50} (in mg/kg) in mice after a single internal administration of the preparations		530 (548—512)	1500

*Difference of the arithmetic means and its confidence interval at P = 0.05.
†The difference is statistically insignificant.

permeability, induced by dextran. According to all these indices, the activity of the metabolite III is at least three times lower than the activity of compound II.

Compound II and its N-oxide III did not affect the functional state of the central nervous system. At doses of 50 and 100 mg/kg internally, both preparations did not produce any statistically significant changes in the motor activity of the mice; they did not change the duration of sleep induced by hexenal, and they did not affect hypothermia and blepharoptosis induced by reserpine. Dimedrol and especially promethazine hydrochloride (pipolphen), at doses of 25-50 mg/kg internally, had an effect characteristic of sedatives according to these indices: they reduced the motor activity, prolonged the action of soporifics, and enhanced the hypothermia and blepharoptosis induced by reserpine.

Thus, compound II and its metabolite III, just like I and its metabolite (quinuclidyl-3)-diphenylcarbinol N-oxide [2, 7], have practically no effect on the functional state of the central nervous system. Considering the data on the pharmacokinetics of I and II, as well as data on the absence of any pronounced influence of these compounds and their metabolites on the functional state of the central nervous system, we can assume that the metabolites, like the compounds themselves, penetrate little into the tissues of the central nervous system.

The toxicity of the metabolite III is also significantly below the toxicity of the main compound II — when administered to mice in a dose three times the value of LD_{50} of compound II, the metabolite III gives practically no toxic effects.

Summarizing the experimental data obtained and comparing them with the data on the metabolism of I, we can conclude that the biotransformation of the investigated derivatives of quinuclidyl carbinols does not lead to the formation of compounds pharmacologically more active or more toxic — the metabolites are less active and less toxic than the original compound. Evidently, N-oxidation at the nodal nitrogen atom is one of the ways of biological inactivation and detoxification of the investigated derivatives of quinuclidyl-diaryl(heteryl)carbinols.

EXPERIMENTAL CHEMICAL SECTION

The IR spectra were recorded on a Perkin-Elmer 599 spectrophotometer (Sweden) in the form of suspensions in liquid petrolatum. The mass spectra were obtained on a Varian MAT-112 mass spectrometer (United States) with direct introduction into the source. The energy of ionizing electrons was 70 eV, temperature of the ionization chamber 180°C.

(Quinuclidyl-3)-di(o-tolyl)carbinol N-Oxide (III). A mixture of 10 g (quinuclidyl-3)-di(o-tolyl)carbinol, 100 ml methanol, and 35 ml 33% hydrogen peroxide is boiled for 5 h. The reaction mass is evaporated to one third of its volume; the precipitate formed is filtered off, washed with water, and shaken for 10 min with 40 ml of a 20% sodium hydroxide solution. The residue is filtered off, washed with water, and recrystallized from 40% aqueous methanol. Yield 2.8 g III with mp 259-260°C, R_f 0.46. Found, %: N 3.88%. $C_{22}H_{27}NO$. Calculated, %: N 4.15.

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INTERACTION OF CHICK ERYTHROCYTES *IN VITRO* WITH 4-AMINOSUBSTITUTED BENZO[g]QUINOLINES

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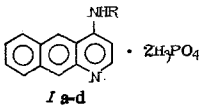
UDC 615.283.926:547.831].015.4:612.111.1.015.348:547.963:32

A study of the interaction of chemical compounds with nucleic acids is of vital significance for an understanding of the mechanisms of the action of drugs. Many heteroaromatic compounds form complexes with DNA, which is determined by various physicochemical methods, spectrophotometrically, fluorometrically, viscosimetrically, etc. It has been established [1-10] that antimalarial preparations — quinine, chloroquine, acriquine, and certain others — are capable of reacting with DNA. These compounds have in common the presence of planar heterocycles and side chains bonded to them in their structure.

We studied the formation of complexes of DNA with derivatives of 4-aminobenzo[g]quinoline, first synthesized by A. F. Bekhli and N. P. Kozyreva [11]. The general formula of these compounds is presented below.



I a-d

Ia: $R = (CH_2)_2N(C_2H_5)_2$; Ib: $R = (CH_2)_3N(C_2H_5)_2$; (1)
Ic: $R = (CH_2)_4N(C_2H_5)_2$; Id: $R = CH(CH_3)(CH_2)_3N(C_2H_5)_2$.

Among these compounds highly active antimalarial preparations Ia and Ib, possessing advantages with respect to lower toxicity and longer duration of action over the main antimalarial preparation, chloroquine, have been found [12]. Special attention is merited by preparation Ia, named dabequine.

EXPERIMENTAL

A study of the formation of complexes of DNA with derivatives of 4-aminobenzo[g]quinoline was conducted in Tris-buffer ($5 \cdot 10^{-3}$ M; pH 7.5). The thermal stability of the complexes was studied in solutions of EDTA ($2.5 \cdot 10^{-4}$ M; pH 7.0). We used a commercial preparation of DNA from chick erythrocytes, produced by Reanal (Hungary). The DNA preparations were subjected to supplementary purification; deproteinization and reprecipitation (two to three times)

E. I. Martsinovskii Institute of Medical Parasitology and Tropical Medicine. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 16, No. 11, pp. 1304-1306, November, 1982. Original article submitted May 25, 1981.

 0091-150X/82/1611-0800$07.50