[Chem. Pharm. Bull. 18(1) 143—146 (1970)]

UDC 615.225.2.033.034 : 547.856.1.09

Metabolic Fate of 2-Methyl-3-*o*-tolyl-4(3*H*)-quinazolinone. III.[1,2] Metabolism of 2-Methyl-3-*o*-tolyl-4(3*H*)-quinazolinone-N-oxide *in Vivo* and *in Vitro*

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(Received July 12, 1969)

The metabolism of 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone-N-oxide in rabbits was investigated *in vivo* and *in vitro*.

In vivo metabolism, 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone, 2-methyl-3-*o*-hydroxymethylphenyl-4(3*H*)-quinazolinone and 2-nitrobenzo-*o*-toluidide were found in the urine receiving 20 mg/kg of 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone-N-oxide, which have been considered to be a possible intermediate of 2-nitrobenzo-*o*-toluidide.

In vitro metabolism, 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone-N-oxide was reduced preferentially to 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone (about 50—60%), while 2-nitrobenzo-*o*-toluidide was not detected.

When the incubation of 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone-N-oxide with the liver preparation was carried out in oxygen, the formation of 2-nitrobenzo-*o*-toluidide was confirmed by thin-layer chromatography.

In the preceding paper,[1] it was reported that 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone-N-oxide (MTQNO), a metabolite of 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone (MTQ), was found in the incubation mixture of MTQ with rabbit liver preparation, but 2-nitrobenzo-*o*-toluidide (NBT) which has been isolated from the urine of human,[4] was not detected. MTQNO was suggested to be an intermediate in the oxidation of MTQ to NBT. However, further oxidation of MTQNO to NBT is not clarified yet.

In this paper, the metabolism of MTQNO in rabbit was investigated both *in vivo* and *in vitro*. Three kinds of metabolites of MTQNO, such as MTQ, 2-methyl-3-*o*-hydroxymethylphenyl-4(3*H*)-quinazolinone(MHQ) and NBT, were found in the urine of rabbits receiving MTQNO orally.

Materials and Methods

Materials

Materials (MTQ, MHQ, MTQNO, and NBT) used were the same products as reported in the preceding paper.[1]

Animals——Male albino rabbits weighing about 3 kg were kept on the solid food and water in individual metabolic cages.

Paper and Thin-Layer Chromatography——Toyo Roshi No. 51 chromatography paper (2×40 cm) was employed for ascending development by using the following solvent systems: (A) BuOH saturated with 28% NH_4OH (B) BuOH–AcOH–H_2O (4:1:5). The following solvent systems for thin-layer chromatography were used: (C) Hexane–$CHCl_3$–EtOH (6:3:1) (D) $CHCl_3$–acetone (9:1) (E) benzene–ethyl acetate (9:1) (F) $CHCl_3$–pyridine (3:1) (G) ethyl acetate–EtOH (3:1) (H) CH_2Cl_2–MeOH (9:1). For the detection of spots on both chromatograms, the following reagents were used: (1) Dragendorff reagent (2) I_2 vapor.

Administration of MTQNO and Collection of Urine——Twenty mg/kg of MTQNO was administered orally by using stomach tube in the form of emulsion in 10 ml of 1.5% CMC solution and the urine was

1) Part II; T. Murata and I. Yamamoto, *Chem. Pharm. Bull.* (Tokyo), **18**, 138 (1970).
2) This was reported in part at the 88th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April 1968.
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4) T. Murata and I. Yamamoto, *Chem. Pharm. Bull.* (Tokyo), **18**, 133 (1970).

collected in the flask which contained toluene for preventing putrefaction. The urine sample was extracted as reported in the preceding paper.[4]

Preparation of Rabbit Liver 9000×*g* Supernatant and Incubation System——A 9000×*g* supernatant fraction of a rabbit liver homogenate was prepared as reported before.[1] The incubation mixture consisted of 3 ml supernatant fraction (equivalent to 1 g of liver), 10 μmoles of substrate, 100 μmoles of nicotinamide, 100 μmoles of Mg_2Cl_2 and 7.5 ml of 0.2M phosphate buffer (pH 7.4). The incubation mixture was incubated in air or oxygen gas by using Thunberg's tube at 37° for 2 hr.

Estimation of MTQ and MTQNO——After incubation was over, 1 ml of 40% TCA solution was added to the mixture. The precipitates thus formed were removed and the TCA solution was neutralized to pH 9.0 with 2N-NH_4OH. The neutral solution was extracted twice with each 10 ml of $CHCl_3$. The $CHCl_3$ extract was combined and evaporated to dryness under reduced pressure.

MTQ was separated from the $CHCl_3$ extract by thin-layer chromatogram, and the estimation of MTQ was carried out according to the method reported by Akagi, *et al.*[5]

For the estimation of MTQNO, the extract was dissolved in 10 ml EtOH, and the solution was used for spectrophotometric estimation at 315 mμ. In this method, the recoveries of MTQNO are shown in Table I.

Table I. Recoveries of MTQNO from Aqueous Solution, Urine and Incubation Mixture

	Aqueous solution					Urine					Incubation mixture				
Concentration μg/ml	50	100	250	375	500	50	100	250	375	500	50	100	250	375	500
No. of Samples	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Recovered μg/ml	49.9	100.1	250.3	374.6	499.2	49.2	100.3	250.1	372.8	496.3	48.3	96.0	250.5	375.0	496.3
Recovery %	99.8	100.1	100.0	99.8	99.8	98.4	101.3	100.4	99.2	99.3	96.6	96.0	102.0	100.0	98.7
Average %			99.8					99.7					98.6		

Results

In Vivo Metabolism of MTQNO

(1) Detection of MTQ

Table II. *Rf* Values of Authentic Samples and Metabolites of MTQNO in Urine of Rabbits

Solvent systems	*Rf* values							
	PPC		TLC					
	A	B	C	D	E	F	G	H
Authentic samples								
MTQ	0.88	0.92	0.85	0.86	0.65	0.98	—	0.90
NBT	0.92	0.96	—	0.92	0.44	—	0.93	—
MTQNO*	0.02	0.72	0.67	0.22	0.09	0.50	0.57	—
MHQ	0.76	0.90	—	0.36	0.18	—	—	0.87
Metabolites								
I	0.88	0.92	0.84	0.87	0.66	0.98	—	0.90
II	0.92	0.95	—	0.90	0.45	—	0.93	—
III	0.78	0.90	—	0.36	0.18	—	—	0.87
IV				0.00	0.00			
V unknown				0.45	0.23			

* Unchanged MTQNO was not detected.

5) M. Akagi, Y. Oketani and M. Takada, *Chem. Pharm. Bull.* (Tokyo), **11**, 62 (1963).

A 24 hr urine specimen after administrating MTQNO (20 mg/kg) was acidified with 6N HCl to pH 3.0, and was extracted with CH_2Cl_2 or $CHCl_3$ for 18 hr. Total dose of MTQNO administered was 5.0 g.

MTQ was detected on the thin-layer chromatograms as an orange yellow spot of *Rf* 0.87 in solvent system (D) and *Rf* 0.66 in (E) by Dragendorff reagent. These *Rf* values were coincident with those of authentic MTQ as shown Table II.

The spot was extracted from thin-layer chromatogram with EtOH and measured ultraviolet absorption spectrum in various kinds of solvent shown in Fig. 1.

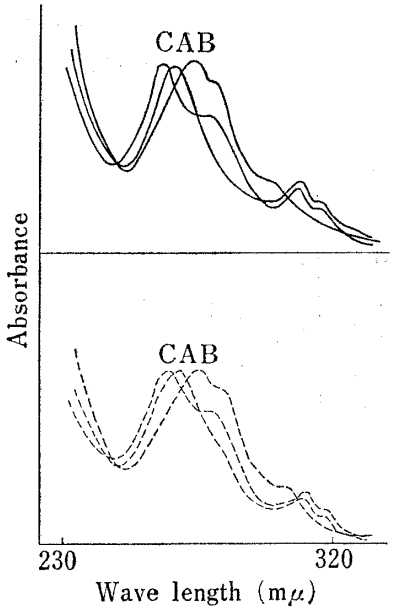

Fig. 1. Ultraviolet Absorption Spectra of Authentic MTQ (solid line) and Extract (broken line) of *Rf* 0.66 in Solvent System E

A: EtOH B: 0.1N-HCl C: 0.1N-NaOH

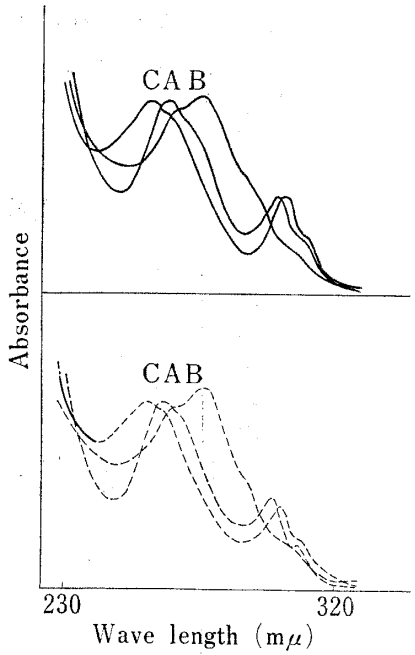

Fig. 2. Ultraviolet Absorption Spectra of Authentic MHQ (solid line) and Extract (broken line) of *Rf* 0.36 in Solvent System D

A: EtOH B: 0.1N-HCl C: 0.1N-NaOH

(2) Detection of MHQ

By using the same method mentioned above, MHQ was confirmed by ultraviolet absorption spectra as shown in Fig. 2.

(3) Detection of NBT

It was observed that the CH_2Cl_2 extract contained NBT by thin-layer chromatography and ultraviolet absorption spectra.

(4) Isolation of NBT

The isolation of NBT from urine specimen was carried out according to the same method reported before by the authors[4]. The extract was evaporated to dryness under reduced pressure and the residue was dissolved in a small amount of CH_2Cl_2, and then passed through alumina column. The effluents contained NBT were collected and were evaporated to dryness under reduced pressure to give crystallines. The crystallines was recrystallized from EtOH to colorless needles, mp 175° (about 40 mg). The compound was confirmed to be identical with authentic NBT by admixture and comparing the infrared absorption spectra.

In Vitro Metabolism of MTQNO

(1) Detection of MTQNO and MTQ

The incubation mixture contained $9000\times g$ supernatant fraction and MTQNO was extracted with $CHCl_3$ at pH 9.0. The $CHCl_3$ extract was evaporated to dryness. Thin-layer chromatogram of the extract showed two orange spots by Dragendorff reagent, and *Rf* values of the spots were the same as those of authentic MTQ and MTQNO, respectively. Those spots were scraped off and eluated with solvent, and were measured spectrophotomerically. The ultraviolet absorption spectra showed that those were well coincident with the spectra of the authentic samples.

(2) Estimation of MTQ and MTQNO in Incubation Mixture

When MTQ was metabolized with liver preparation, MTQ was oxidized to MTQNO (about 5—8%). While, MTQNO was reduced to MTQ (about 50—60%) *in vitro*. These results were shown in Fig. 3.

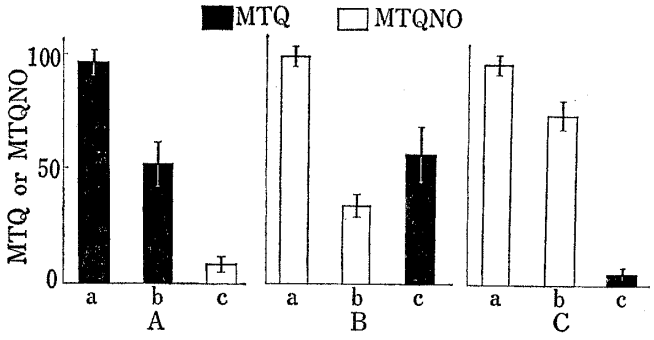

Fig. 3. Estimation of MTQand MTQNO *iu Vitro*

A: MTQ as substrate
B: MTQNO as substrate (in Air)
C: MTQNO as substrate (in Oxygen)
a: Control used heated enzyme system.
b: MTQ or MTQNO remained c: MTQ or MTQNO formed
Each bar represents the average obtained from four experiments.

In Vitro Metabolism of MTQNO in Oxygen

As mentioned above, MTQNO was not oxidized further in this experimental condition, but was reduced to MTQ *in vitro*. In order to confirm that if the oxidation system was absent or not in the $9000\times g$ supernatant, the incubation in oxygen was attempted. The results thus obtained was shown in Fig. 3. Three or four spots were found and one of them (*Rf* 0.45) in solvent system (E) was identical with NBT. On the contrary, MTQ was not detected in this experiment.

Discussion

In this experiment *in vivo*, MTQNO was changed to urinary NBT, but MTQ and MHQ were also found in the urine of rabbits receiving MTQNO. Therefore, it is probable that MTQNO was reduced to MTQ at first, and then the MTQ was metabolized to NBT and MHQ.

In vitro experiment showed that MTQNO was metabolized preferentially to MTQ. In other words, MTQNO was not oxidized further to NBT with liver $9000\times g$ supernatant.

Those results obtained in this experiment suggested that MTQNO was not seemed to be an intermediary metabolite of MTQ. However, when MTQNO was incubated with the liver preparation in oxygen, NBT was detected in the incubation mixture by thin-layer chromatography. And this observation does not exclude the intermediary formation of MTQNO during the course of metabolism of MTQ *in vivo*.

Further studies on these mechanism of the formation of NBT are now in progress.