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Urinary Metabolism and Excretion of Carbinoxamine after Oral Administration to Man

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Abstract – The metabolism of carbinoxamine, 2-[(4-chlorophenyl)-2-pyridinyl-methoxy]-N, N-dimethylethaneamine, was studied in adult male volunteers after an oral dose of 15 mg. Solvent extracts of urine obtained with or without enzyme hydrolysis were analyzed by gas chromatography-mass spectrometry after derivatization with MSTFA/TMSCl (N-methyl-N-trimethylsilyltrifluoroacetamide/trimethyl chlorosilane). The structures of metabolites were determined based on the electron impact (EI) and chemical ionization (CI) mass spectra. Nonconjugated metabolites identified in the urine were carbinoxamine, nor-carbinoxamine, and bis-nor-carbinoxamine. Parent drug, nor-carbinoxamine, and bis-nor-carbinoxamine were also detected as conjugated forms. These metabolites observed in human urine were different from those previously reported in the rat. Urinary excretions of carbinoxamine were reached to maxima in 4 hours after drug administration with 4.9%-8.1% and 2.5-4.2% of the dose excreted during 24 h as carbinoxamine and its glucuronide, respectively.

Keywords □ carbinoxamine, human, metabolism, GC-MS (gas chromatography-mass spectrometry)

Carbinoxamine maleate, ethanolamine type H1 receptor antagonist belongs to a class of antihistamines (Drug Information 1995) that is used frequently with pseudoephedrine as a controlled release formulation for the treatment of nasal allergy (Connell et al. 1984).

Antihistamines are currently widely used in prescription and non-prescription drugs for the treatment of cold and allergy (Douglas 1985) but CNS depression, sedation ranging from mild drowsiness to deep sleep (Drug Information, 1995) and dermatitis (Coskey 1983) occur frequently with usual dosage. Research on antihistamines like carbinoxamine maleate, structural homologue of carcinogenic compound methapyrilene, has been focused on the determination of mutagenic and carcinogenic potential (Probst and Neal 1980, McQueen and Williams 1981, Andrew et al. 1984, Budroe et al. 1984).

Metabolism of carbinoxamine maleate has been studied in human urine that was treated by acid for hydrolysis of conjugates. (Maurer and Pfleger 1988) and in rat urine. (B.H.Jung et al. 1993) However no metabolic information and no urinary excretion study has been reported for untreated human urine.

We describe a urinary excretion and metabolism study using gas chromatography-mass spectrometry (GC/MS) in healthy volunteers after oral administration of carbinoxamine.

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MATERIALS AND METHODS

Materials

Carbinoxamine maleate was supplied by Choong Wae Co. (Seoul, Korea) and pyrilamine maleate, internal standard, was purchased from Sigma Co. (St Louis, MO, U.S.A). Amberlite XAD-2 resin (particle size 0.15-0.2 mm) was supplied by Serva Co. (Heidelberg, Germany) and washed with acetone, methanol and distilled water before use. β-Glucuronidase (from E-coli, activity : 200 U/ml) was purchased from Boehringer Mannheim Co. (Mannheim, Germany). Na_2HPO_4 and NaH_2PO_4 were obtained from Junsei Chemical Co. (Japan). The silylating agents, MSTFA (N-methyl-N-trimethylsilyl-trifluoroacetamide) and TMSCl (trimethyl chlorosilane) were obtained from Sigma Co. and other organic solvents were purchased from J.T. Baker (Phillsberg, NJ, U.S.A.).

Drug administration

Carbinoxamine was given to three healthy male volunteers as a single oral dose of 15 mg in the morning. The volunteers were 40, 37 and 31 years old and weigh-

ed 78, 75 and 73 kg, respectively. Urine was collected just before drug administration and at 0 -1, 1-2, 2-4, 4-6, 6-8, 8-12, 12-24 h after dosing and immediately stored at -20 ℃ until analysis.

Instruments

A Hewlett-Packard gas chromatograph/mass engine (5890A/ 5989B) was used. The mass spectrometer was operated with a filament current of 300 μA, an electron energy of 70 eV in the electron impact (EI) mode and 230 eV in the chemical ionization (CI) mode, respectively. Methane gas was introduced as a reagent gas for CI mode. The ion source was held at 200 ℃ and at 0.8 torr in the CI mode. A cross linked SE-30 capillary column (length 17 m, i.d. 0.2 mm, film thickness 0.11 μm, Hewlett-Packard Co., CA, USA) was directly connected to the ion source. Helium at a flow rate of 1 ml/min was employed as a carrier gas. Samples were injected in split (10:1) mode. Injector and transfer line were at 250 ℃ and 280 ℃, respectively. Initial oven temperature was 100 ℃ and it was subsequently increased by 10 ℃ per min to 300 ℃ and held there for 5 min.

Sample preparation

Identification of metabolites

An aqueous XAD-2 slurry was filled into a pasteur pipette (i.d. 0.5 cm) until a bed height of 3-4 cm was achieved. Urine was applied to the column.

The column was washed with an equal volume of water and adsorbed materials were eluted with methanol (3× 1 ml). The eluent was evaporated to dryness with a rotary evaporator at ambient temperature using water pump aspiration.

Phosphate buffer (1 ml, 0.2 M, pH 7.0) and 5 ml diethylether were then added to the residue. After mechanical shaking (10 min) and centrifugation (800 g, 5 min), the organic layer was transferred to another test tube, and the ether was removed with a rotary evaporator. The buffer solution was extracted nine more times with 5 ml ether to completely remove the metabolites and the resulting extract was evaporated to dryness. Before silylation, the residue was dried in a vacuum desiccator over P_2O_5/KOH for 30 min.

Derivatization

Residues were trimethylsilyated with 50 μl of the reagent mixture MSTFA/TMSCI (100:1, v/v) and heated at 60 ℃ for 15 min.

Quantitation of carbinoxamine and metabolites.

Pyrilamine standard solution (30 μl, 100 μg/ml) as an internal standard was added to a 5 ml sample of each urine collection and the samples were extracted three times as described above.

After the sample work up and derivatization, the MS was operated in the selected ion monitoring (SIM) mode, monitoring ions were m/e 58, 121, 215 for pyrilamine, m/e 58, 167, 218 for carbinoxamine, m/e 106, 167, 218 for nor-carbinoxamine and m/e 86, 174, 202 for bis-nor-carbinoxamine.

The concentration of carbinoxamine was calculated based on the calibration curve. Owing to the lack of authentic standards for nor-carbinoxamine and bis-nor-carbinoxamine, the excretion profiles of these compounds were observed via height ratio (peak height of nor-carbinoxamine or bis-nor-carbinoxamine/peak height of internal standard).

In order to hydrolyze glucuronide conjugates, another 5 ml urine was applied to a XAD-2 column, it was washed with an equal volumn of water and adsorbed materials were eluted with methanol(3×1 ml). The eluent was evaporated to dryness with rotary evaporator.

Phosphate buffer and 5 units of β-glucuronidase were added to the residue and the solution was heated at 50 ℃ for 1 hr, cooled to room temperature, and extracted and derivatized as described above.

RESULTS

Identification of metabolites

The results of the GC/MS analysis of urinary metabolites of carbinoxamine in man is shown in Fig. 1-5. Two metabolites and the parent drug were detected in the free fraction. Each metabolite was characterized by mass analysis.

When urine samples were examined by CI mode GC/MS, the parent drug and two metabolites, nor-carbinoxamine and bis-nor-carbinoxamine, were all detected in the urine of only one subject by monitoring the m/e 202 ion characteristic of these three compounds (Fig. 1).

However, EI mode GC/MS detected the three compounds in the urine of all subjects, by monitoring m/e 167 (Fig. 3).

Metabolites detection was performed by using m/e 167 extracted ion that was expected as one of major fragment ion of the parent drug and metabolites in electron impact (EI) mode. CI mass spectrums of carbinoxamine, nor-carbinoxamine and bis-nor-car-

binoxamine are in Figure 2 and EI mass spectra of them are shown in Fig. 4.

Several minor components were detected by EI and CI GC/MS which were absent from pre-dose samples. These components were not identified.

Carbinoxamine, nor-carbinoxamine and bis-nor-carbinoxamine were also found by EI GC/MS in the glucuronide conjugated fraction of the urinary extract with derivatization. Their chrorhatographic profiles are shown in Fig. 5.

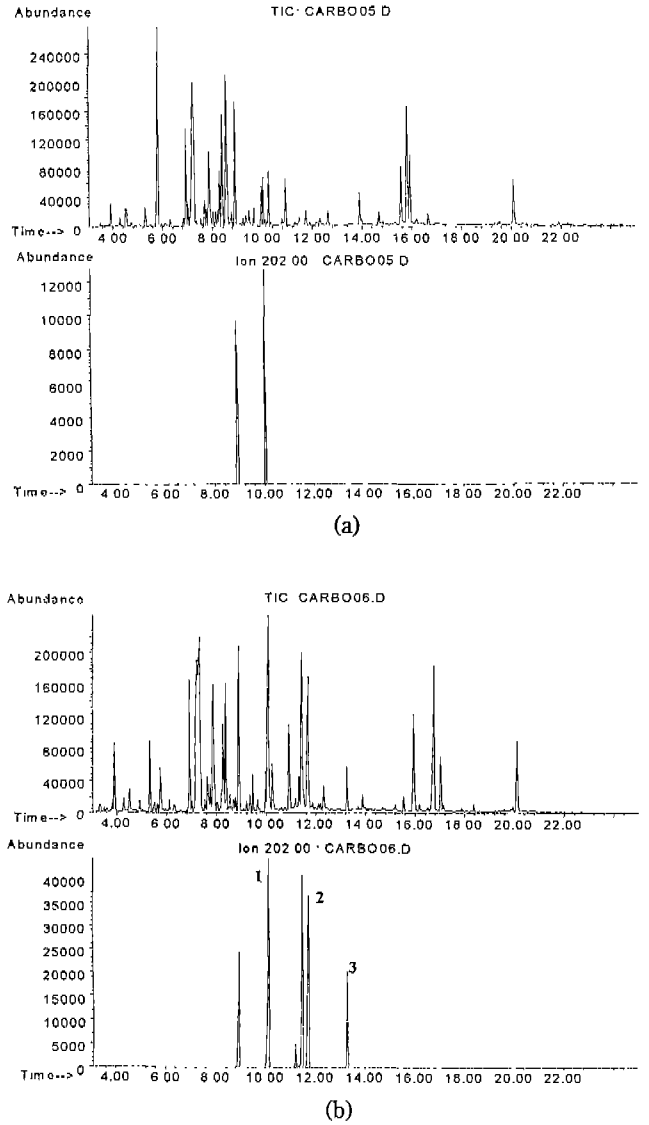


Fig. 1. Total ion chromatogram and extracted ion chromatograms of m/e 202 of (a) an extract of human urine collected before carbinoxamine, and (b) an extract of human urine collected for 24h after 15 mg carbinoxamine administration. 1: carbinoxamine, 2: nor-carbinoxamine, 3: bis-nor-carbinoxamine. in the chemical ionization mode.

Urinary excretion study

Carbinoxamine concentrations in each urine sample were quantitated by using a calibration curve (r=0.999).

Excretion profiles of nor-carbinoxamine and bis-nor-carbinoxamine were observed by peak height ratio described above. The excretion rate of carbinoxamine after a single oral administration is shown in Fig. 6. The urinary recoveries of the total carbinoxamine was 8.1-12.0% dose with free and conjugated drug representing 4.9-8.1% and 3.2-3.9% respectively. The excretion rates of total carbinoxamine and its metabolites are shown in Fig. 6 and Table I, respectively.

DISCUSSION

The proposed metabolic routes of carbinoxamine in human are described in Fig. 7. Major metabolites of carbinoxamine were nor-carbinoxamine and bis-nor-car-

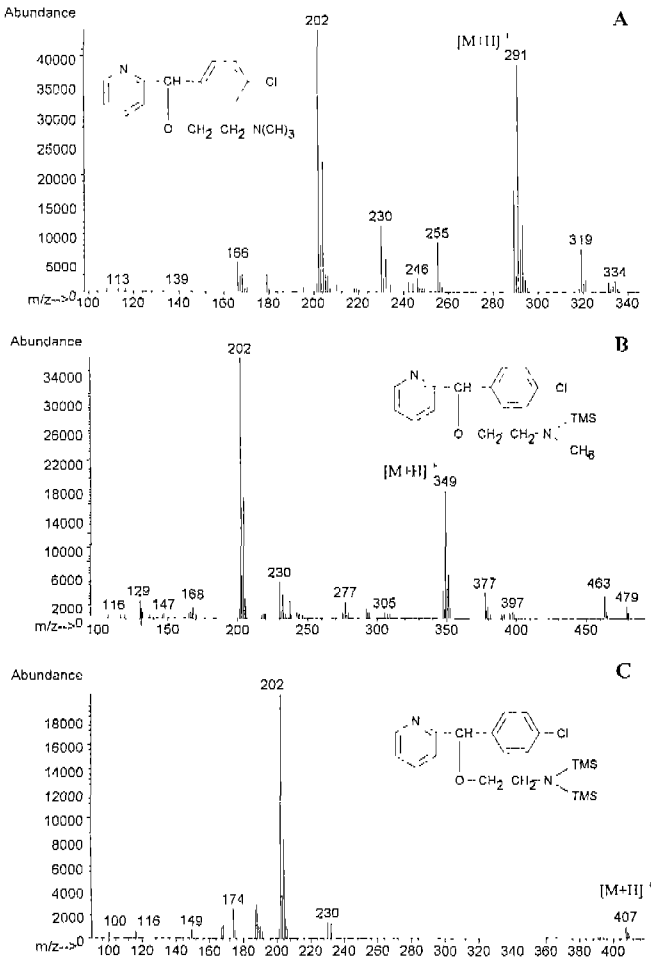
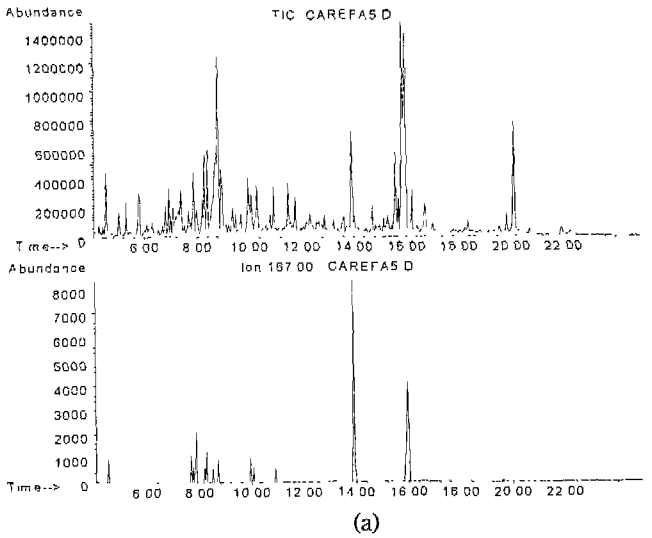
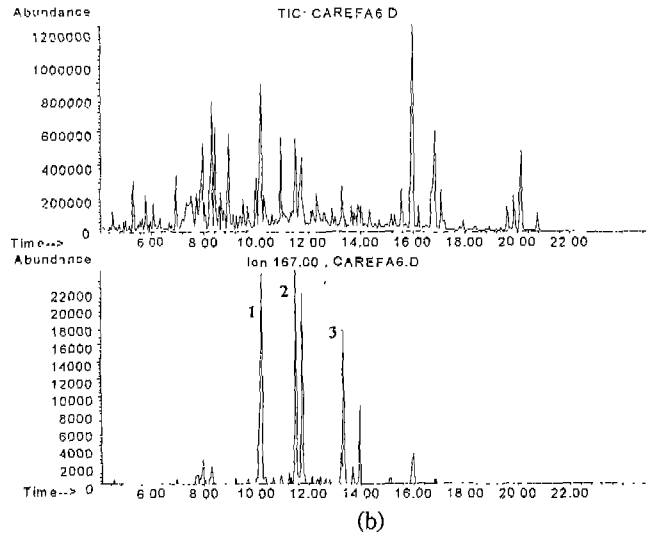


Fig. 2. CI mass spectra of carbinoxamine (A), nor-carbinoxamine (B) and bis-nor-carbinoxamine (C).

binoxamine, and parent carbinoxamine were detected, too. It is suggested that carbinoxamine metabolism in human is subsequent demethylation followed by conjugation with glucuronide. Previous reports showed that carbinoxamine metabolism involved O-dealkylation, deamination and demethylation (Maurer and Pfleger 1988) but in our study only demethylation process was observed. This may result from the different hydrolysis methods. In contrast with human study, carbinoxamine metabolism in rat urine involves demethylation, O-dealkylation and acetyl conjugation (B.H.Jung et al. 1993). This result in-



(a)



(b)

Fig. 3. Total ion chromatogram and extracted ion chromatograms of m/e 167 of (a) an extract of human urine collected before carbinoxamine, and (b) an extract of human urine collected for 24h after 15 mg carbinoxamine administration. 1: carbinoxamine, 2: nor-carbinoxamine, 3: bis-norcarbinoxamine. in the electron impact mode.

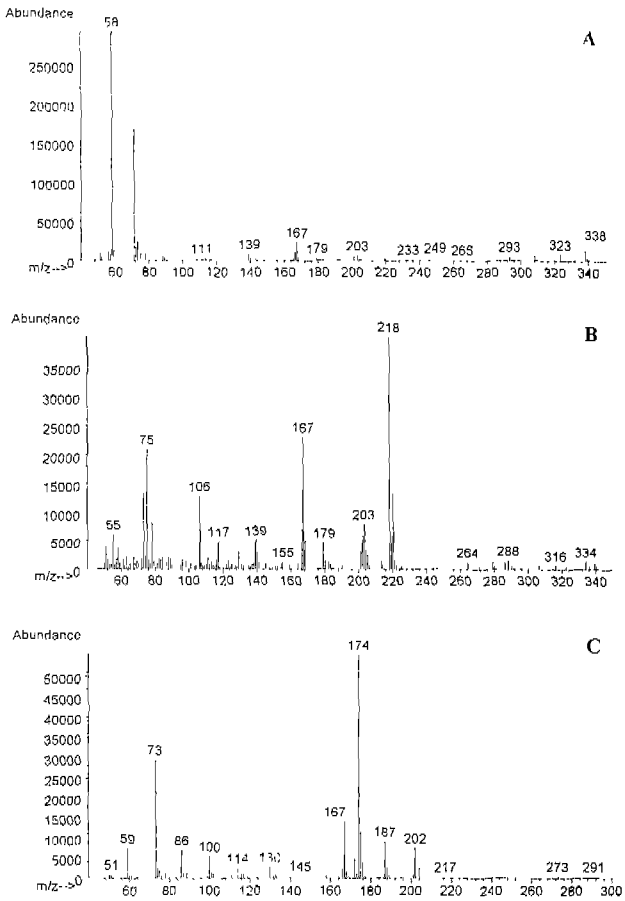


Fig. 4. EI mass spectra of carbinoxamine (A), nor-carbinoxamine (B) and bis- nor-carbinoxamine (C).

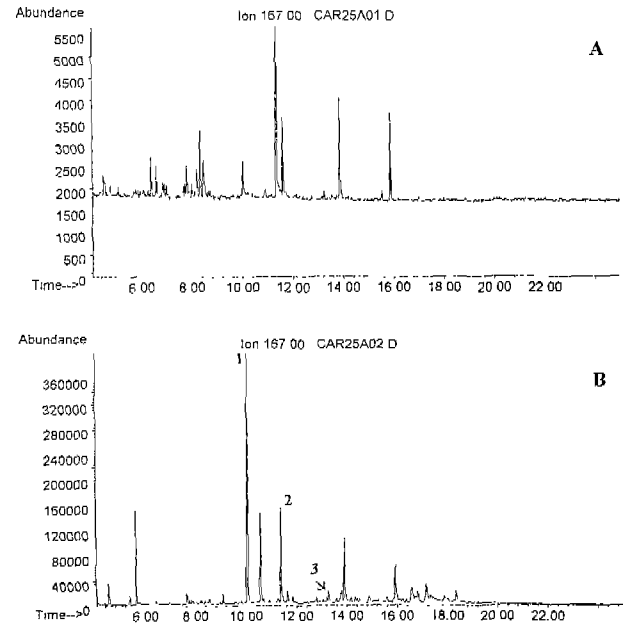


Fig. 5. Extracted ion chromatograms of m/e 167 of 10th extracted fraction of free fraction (A) and hydrolyzed (by β-glucuronidase) fraction (B). 1: carbinoxamine, 2: nor-carbinoxamine, 3: bis-norcarbinoxamine.

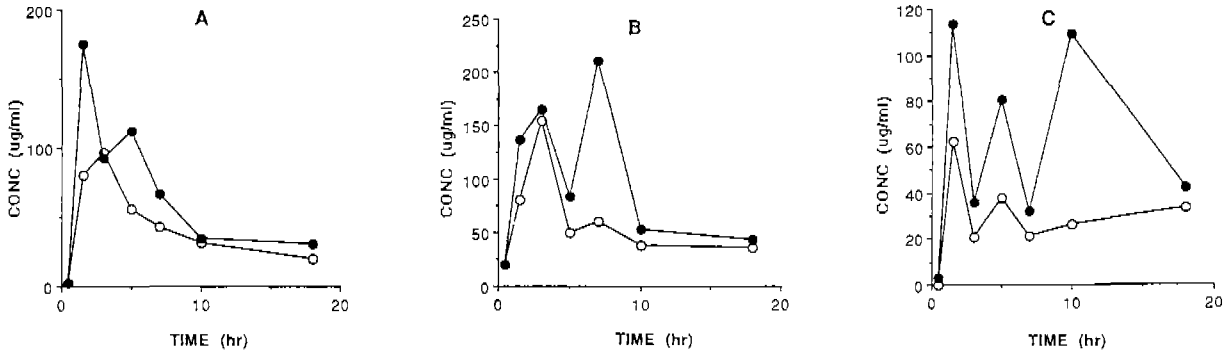


Fig. 6. Excretion rates of carbinoxamine of subject 1 (A), subject 2 (B) and subject 3 (C). open circle; free fraction, closed circle; total fraction.

Table I. Excretion rate of free and conjugated (total) metabolites of carbinoxamine.

TIME (hr)	NOR-CARBINOXAMINE			BIS-NOR-CARBINOXAMINE		
	SUB1	SUB2	SUB3	SUB1	SUB2	SUB3
0-1	ND	ND	0.65	ND	ND	0.73
1-2	ND	2.03	6.44	ND	ND	4.44
2-4	ND	6.62	9.07	ND	ND	3.32
4-6	2.86	5.39	19.46	0.40	0.58	16.03
6-8	4.27	8.08	3.71	0.84	0.99	3.85
8-12	3.70	5.56	23.65	2.14	2.22	25.09
12-24	0.74	6.00	4.68	1.10	5.20	17.28

SUB: subject
ND: Not Detected
Excretion rate unit: height ratio fx urine volumn (ml)/time interval.

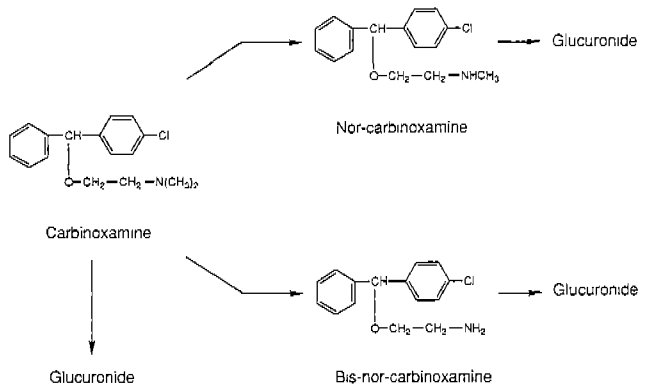


Fig. 7. Postulated metabolic pathways of carbinoxamine in human.

dicates that species differences exist.

Urinary recovery of total carbinoxamine following single oral administration (15 mg) was 8.1-12.0%. Low recovery of the N-demethylated metabolites and their glucuronides indicates the importance of extra-renal elimination routes for carbinoxamine in man.

Mutagenic and carcinogenic potential of carbinoxamine is still in question because of the structural similarity with carcinogenic methapyrilene and induction of unscheduled DNA synthesis in rat hepatocyte culture (Probst and Neal 1980, Budroe et al. 1984). Biological significance of a newly confirmed human meta-bolism of carbinoxamine should be further evaluated in order to understand the toxicological profile of this compound.

Acknowledgement

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