Bioavailability of Tauropine After Oral Ingestion in Mouse

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Keywords

Tauropine · Mass spectrometry · Bioavailability

1 Introduction

Sea animals and seaweed produce various taurine derivatives, such as methyltaurine and tauropine) (Impellizzeri et al. 1975; Sato et al. 1985). There is some evidence that taurine derivatives demonstrate health benefits in mammals. For example, homotaurine inhibits amyloid aggregation and is effective against Alzheimer's disease (Caltagirone et al. 2012). Moreover, N-methyltaurine attenuates steroid-induced muscle atrophy in mice (Nguyen et al. 2020).

Tauropine (D-rhodoic acid) is a kind of opine, which are anaerobic metabolites in abalone and algae (Sato et al. 1985, 1987). Tauropine is produced from reductive condensation of taurine and pyruvate, as catalyzed by tauropine dehydrogenase (TADH, Fig. 1) (Gäde 1986). We are interested in the health effects of tauropine in mammalians. However, it has not been elucidated whether orally ingested tauropine from seafood can be absorbed by the intestines and transported into the blood. In the present study, we tested the bioavailability of tauropine in mice.

2 Methods

2.1 Synthesis of Tauropine

Taurine was synthesized as previously reported (Haque et al. 2000). Taurine (12 g) was reacted with L-bromopropionic acid (25 g) in 350 mL of 1M NaOH solution for 7 days. The reaction mixture was applied to a Dowex 1-X2 column (OH-form, Fuji film, Tokyo, Japan). The column was washed by water and then by 0.1M HCl. Then tauropine was eluted with 0.2M HCl. The eluant was concentrated by rotary evaporator, and the residue was recrystallized by adding ethanol and then lyophilized. The yield was confirmed by electrospray ionization mass spectrometry (ESI-MS) (Esquire 4000, Bruker) (Fig. 2).

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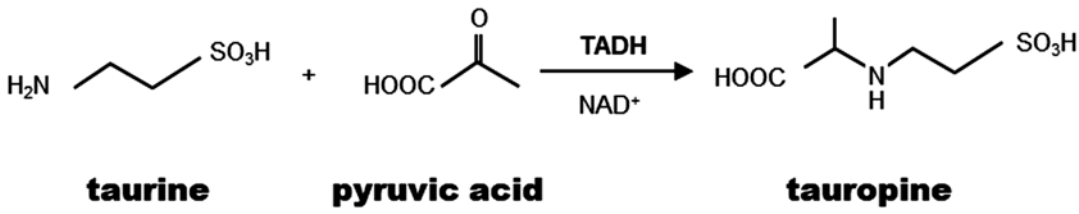

Fig. 1 Production of tauropine from taurine and pyruvic acid in abalone

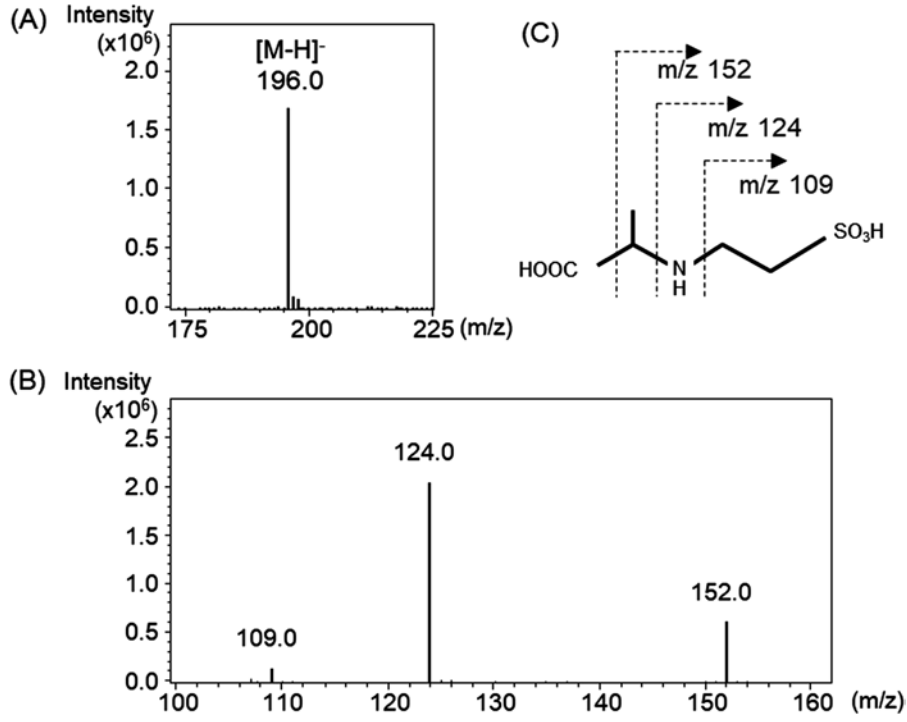

Fig. 2 Detection of tauropine. (**a**) MS spectrum of synthesized tauropine (negative ion mode). (**b**) MS/MS spectrum of daughter ion of tauropine ([M-H] = 196.0). (**c**) Fragmentation of tauropine expected from daughter ions

2.2 HPLC Determination of Tauropine by Phenyl Isothiocyanate Derivatization

Tauropine was derivatized with phenyl isothiocyanate (PITC) as previously reported (Sato et al. 1988). Tauropine solution was mixed with equal amounts of ethanol-water-triethanolamine (2:2:1) and dried by a concentrating centrifuge. The dried sample was dissolved by ethanol-water triethanolamine-PITC (7:1:1:1) and incubated for 20 min at room temperature. The sample was dried again and kept at 4 °C until measurement.

The PITC derivatives were analyzed by high-performance liquid chromatography (HPLC)-ESI-MS (maXis plus, Bruker) equipped with a reversed-phase column (Shim-pack XE-ODS 3 μm, 75 × 2.6 mm; Shimazu, Kyoto, Japan) at 40 °C at a flow rate of 0.3 mL/min and with a linear gradient of acetonitrile in 20 mM acetic acid run over 20 min (5–40% (v/v) acetonitrile for 20 min).

2.3 Animal Experiment

The experimental procedures were approved by the Institutional Animal Care and Use Committee of Fukui Prefectural University. Male ICR mice (8-week-old) was purchased from Japan Crea Inc. (Japan). Mice had access to water ad libitum and maintained on a 12-h light/dark cycle. Tauropine solution in saline was administered intraperitoneally (i.p.) or orally (p.o.) to mice at 50 mg/kg body weight. The mice were anesthetized to collect blood sample and then killed by cervical dislocation. Tissues were isolated and were kept at −80 °C until use.

2.4 Blood Sample Preparation for HPLC Analysis

Samples were deproteinated by using 5% sulfosalycylic acid (SSA, Nacalai). Serum samples were mixed with equal volume of 5% SSA. Tissues were homogenized in 10 volumes of 5% SSA using a polytron homogenizer. Samples were centrifuged, and the supernatant was filtered with a 0.45 microm filter and then neutralized with NaHCO3. Next, the primary amine compounds were removed after being derivatized with o-phthalaldehyde (OPA, 5 mM) in boric acid buffer (pH 10.4) containing 2-mercaptoethanol (29 microM); the OPA-derived amines were removed by a Monospin C18 Column (GL Science, Tokyo, Japan). The flow-through eluant was then derivatized with PITC as described above.

2.5 HPLC Determination of Tauropine-PITC in Blood Samples

The PITC-derivatized blood samples were analyzed using an HPLC-photodiode array detector (Shimazu) equipped with a reversed-phase column (COSMOSIL 5C18-MS-II Packed Column 5 μm, 250 × 4.6 mm; Nacalai tesque, Kyoto, Japan) at 40 °C at a flow rate of 1 mL/min and with a linear gradient of acetonitrile in 10 mM phosphate buffer (pH 7.3) run over 45 min (3–97% (v/v) acetonitrile for 45 min). PITC-derivatized tauropine was detected by absorption at 254 nm.

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3 Results

3.1 Detection of Tauropine by HPLC Method

Since tauropine contains a secondary amine, tauropine can be derivatized by PITC. First, we analyzed whether PITC-tauropine can be separated using HPLC-MS (Fig. 3a, b). We confirmed the peak of PITC-tauropine, and the molecular weight of the compound included in this peak was 314. Therefore, we expected that the reaction of PITC with tauropine may have yielded a dehydration product as shown in Fig. 3c.

Meanwhile, we tested whether FMOC-Cl, another amine-derivatizing agent that is readily detectable by a fluorometric detector, could be used to measure tauropine. However, it did not produce FMOC-tauropine (data not shown). Therefore, we decided to use PITC to measure tauropine.

Next, we tried to separate the peak of tauropine from that of the other amino acids found in the blood. Since the blood contains many primary and secondary amines, as well as amino acids, which all can react with PITC, many HPLC peaks could overlap with the peak of PITC-tauropine. Therefore, we removed the primary amines from the blood samples by using OPA, which reacts with primary amines but not secondary amines. We then reacted the samples with PITC. This protocol allowed successful separation of the PITC-tauropine peak from that of the other compounds in the blood sample (Fig. 4).

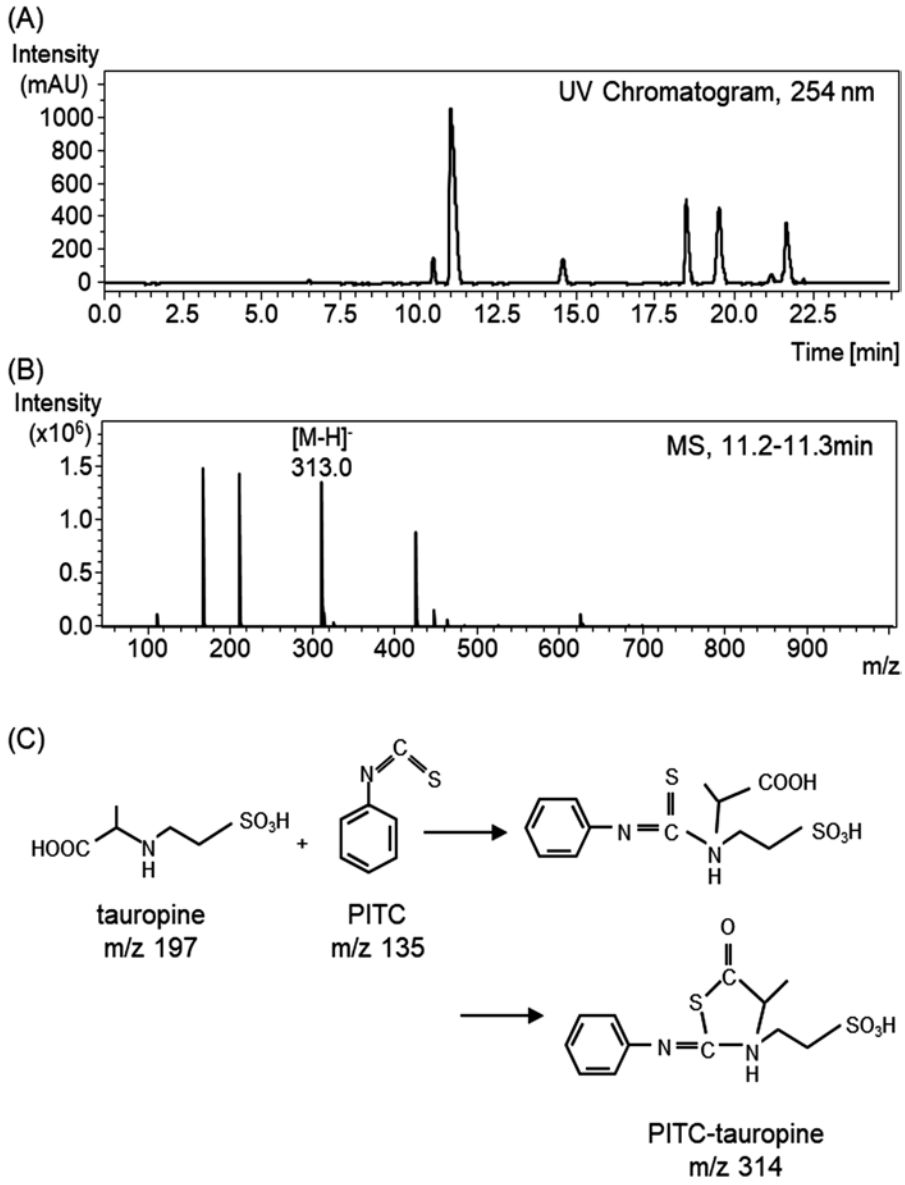

Fig. 3 Detection of PITC-labeled tauropine by HPLC-MS. (**a**) HPLC chart of PITC-labeled tauropine. (**b**) MS spectrum of PITC-labeled tauropine. (**c**) The reaction between tauropine and PITC, which is expected from the m/z of the product

3.2 Pharmacokinetics Study of Tauropine After Oral Injection in Mouse

Tauropine (500 mg/kg body weight) was orally or intraperitoneally injected into mice, and then blood was collected after 1 h. We then confirmed by HPLC whether tauropine was present in the blood (Fig. 4). Although PITC-tauropine was found in the blood of mice intraperitoneally injected with tauropine, it was not detected in the blood of mice administered tauropine orally. Moreover, we maintained mice for 5 days on drinking water containing tauropine (0.5%), and

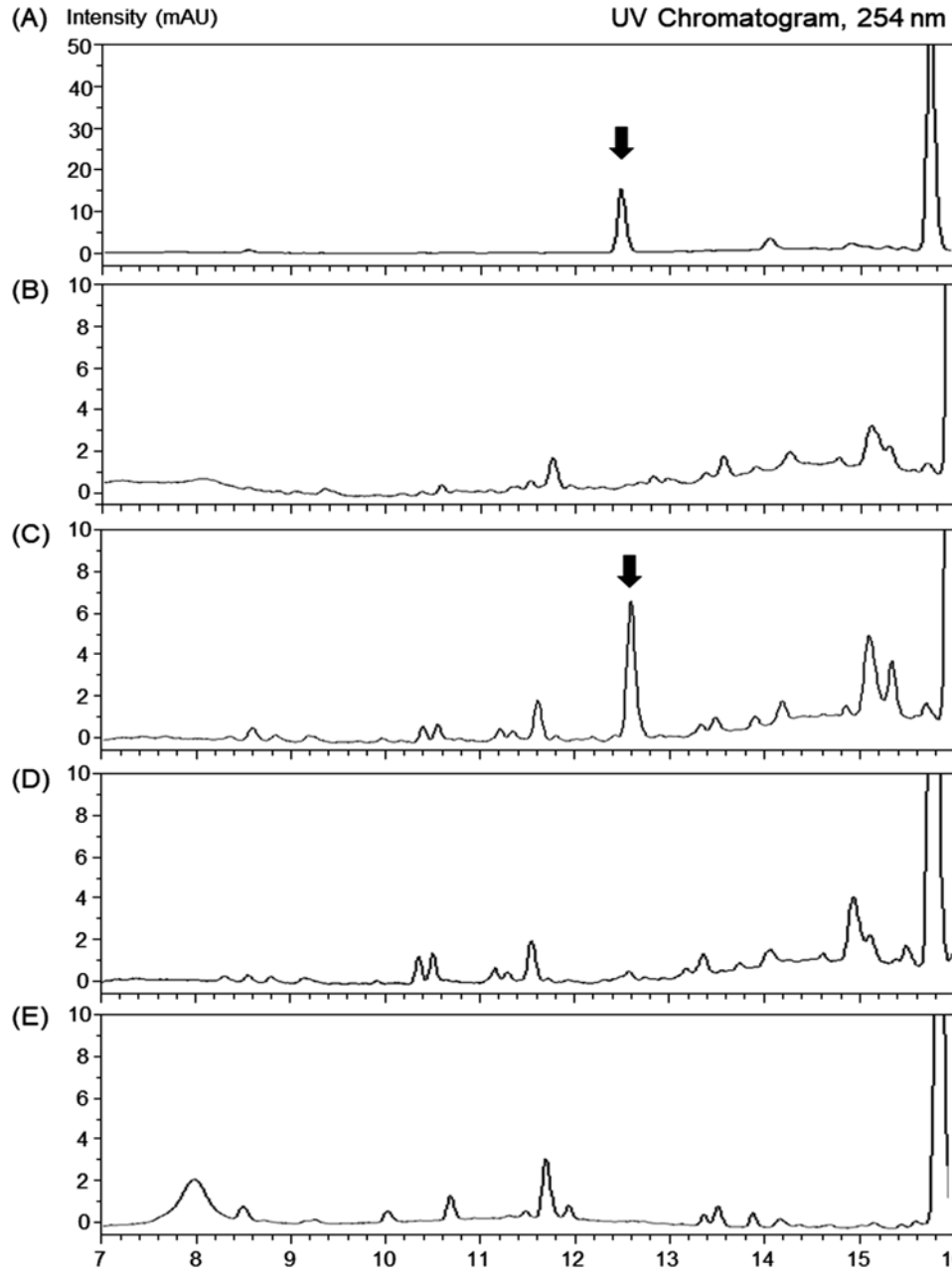

Fig. 4 HPLC charts of PITC-labeled tauropine and blood metabolites. (**a**) Synthesized tauropine. (**b**) Blood from untreated mice. (**c**) Blood from tauropine-treated (i.p.) mice. (**d**) Blood from tauropine-treated (p.o.) mice. (**e**) Blood from mice drinking 0.5% tauropine-containing water for 5 days. Arrows indicate the peaks for PITC-labeled tauropine

then blood tauropine was measured. However, tauropine was not detected in these mice (Fig. 4e). These results indicate that orally administered tauropine cannot be detected in the blood.

There are several possible reasons why orally administered tauropine cannot be detected in the body. (i) It may be degraded in the gastrointestinal tract. The pH of the GI tract is very acidic.

Moreover, there are a lot of enzymes and microbiota that may cause tauropine destruction. Tauropine is stable in acidic condition, since it was eluted by 0.1M HCl during purification. Moreover, since it has no ester-bond, general esterases cannot alter it. Tauropine is formed from pyruvate and taurine in the presence of the enzyme, tauropine dehydrogenase. The catalytic enzymes for tauropine would exist in the gut or gut microbiota. (ii) It may not be absorbed by the intestines and then transferred to the blood. Taurine is hydrophilic; it should be transported by specific transporter to be absorbed by the intestines. We expected that it would be transported by the taurine transporter, but that proved not to be the case.

4 Conclusion

Orally ingested tauropine from certain types of seafood is barely present in the blood. Therefore, the potential health benefit or toxicity of tauropine in the body would not be detected. Interestingly, another opine compound, strombine, contributes to the taste of dried scallops (Starkenmann et al. 2009). There is the possibility that tauropine contributes to the taste of the abalone and other tauropine-containing seafood. If so, information of the pharmacokinetics obtained from this study would be useful in applying tauropine to umami seasoning.

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