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Tetrodotoxin-Associated Food Poisoning due to Ingesting Fish

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ABSTRACT

A food poisoning incident due to ingestig some unknown fish occurred in Hualien County, Taiwan, in December, 1992. Three persons were poisoned having symptoms which were marked by severe numbness of the lips, vomiting and difficult breathing. One of the patients died the next day. The uneaten fish meat was analyzed for toxicity. Later the fish meat showed a high toxicity (150 mouse units per gram). The fish meat toxin was purified by Diaflo YM-2 membrane ultrafiltration and Bio-Gel P-2 chromatography. The purified toxin was shown to contain tetrodotoxin and anhydrotetrodotoxin by analyses of thin layer chromatography, electrophoresis and high performance liquid chromatography. The research, by analysis, disclosed the sole causative toxic agent to be tetetrodotoxin.

Key words : Anhydrotetrodotoxin, Food poisoning, Puffer, Tetrodotoxin.

INTRODUCTION

A serious food poisoning incident due to ingesting fish occurred in Hualien County, Taiwan, in December, 1992. Three victims, including of one death, were recorded. A pregnant woman developed numbness over her extremities and experienced difficulty in breathing soon after eating the fish. She was promptly sent to a local hospital, and had severe respiratory symptoms upon reaching the intensive care unit. Despite intensive treatment including mechanical ventilation and dopamine drip, she expired one day later. Her two children, who ate a lesser amount of fish, quickly became quite cyanotic, and lapsed into shock and a coma. They too, were also promptly sent to the hospital and received the same intensive treatment. After 1-week, they were discharged from the hospital. The causative fish was a gift from their relative, who lives in the same county.

These symptoms reminded us of those caused by tetrodotoxin (TTX) which has been recorded as causing three food poisoning incidents in Taiwan [1,2,3]. Therefore, coercing the present study into this situation, the remaining fish meat was assayed for toxicity, with attempts made to identify the responsible toxin.

MATERIALS AND METHODS

I . *Materials*

The remaining fish meat weighed about 25g. It was kept frozen below -20°C until analysis.

II. *Assay for toxicity*

The fish meat was partially thawed, then examined for toxicity by the official assay method for TTX using 18-21g ICR (Institute of Cancer Research) strain mice[4].

III. *Purification of toxin*

Toxic fish meat (about 25g) was homogenized for 5 min with 3 volumes of 1% acetic acid in methanol and centrifuged. This step was repeated two times. The supernatants were combined, concentrated under reduced pressure and defatted thrice with dichloromethane. The aqueous layer was ultrafiltered with Diaflo YM-2 membrane (Amicon, U.S.A.) to cut off substances of more than 1000 daltons. The filtrate was examined for toxicity and then lyophilized. The lyophilizate was dissolved in 2 ml of 0.03 M acetic acid, and chromatographed on a column (2 × 98 cm) of Bio-Gel P-2 (Bio-Rad, U.S.A.) using 0.03 M acetic acid as eluent. The toxic fractions were combined and freeze-dried . The partially purified toxin thus obtained was subjected to various analyses as described below. Authentic TTX and anhydrotetrodotoxin (anh-TTX) were prepared from the liver of a puffer *Fugu ablongus* by following the methods of Goto *et al.*[5] and the previous paper[6], and used as standards.

IV. *Thin-layer chromatography*

Thin-layer chromatography (TLC) was performed on 5 × 20 cm silica gel 60F_{254} precoated plates (Merk, Germany) with two solvent systems, pyridine - ethyl acetate - acetic acid - water (15:5:3:6) and 1-butanol - acetic acid - water (2:1:1). Toxins were visualized as a yellow fluorescent spot under UV (365nm) light after spraying the plate with 10% potassium hydroxide and heating at 110°C for 10 min, or as a pink spot by

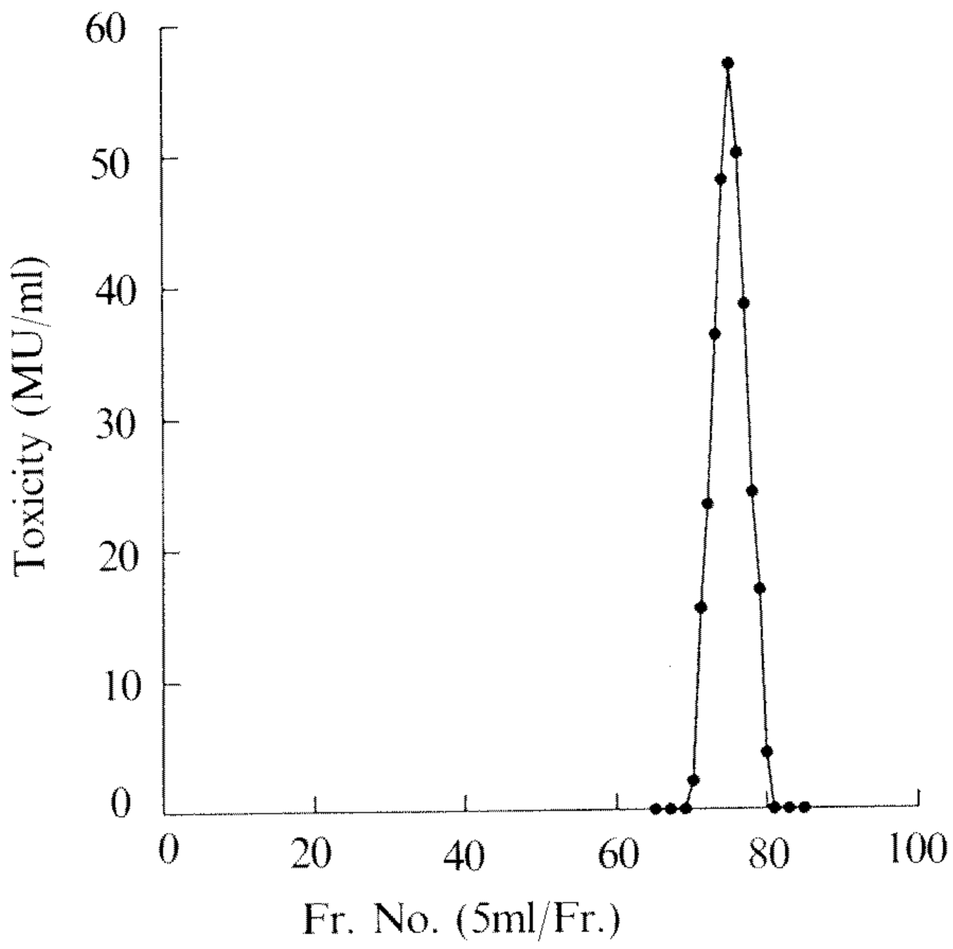

Figure 1. The elution profile of the fish toxin on a Bio-gel P-2 column using an eluent of 0.03 M acetic acid.

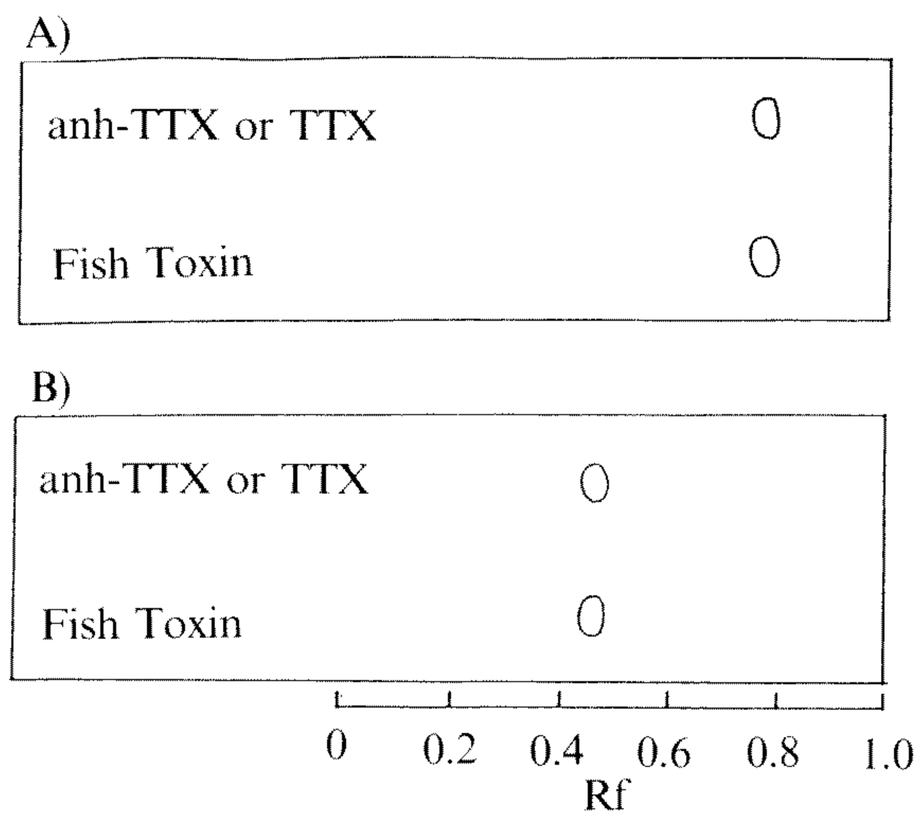

Figure 2. TLC of fish toxin, along with authentic tetrodotoxin (TTX) and anhydrotetrodotoxin (anh-TTX). Solvent System: A) pyridine - ethyl acetate - acetic acid - water (15:5:3:6); B) 1-butanol - acetic acid - water (2:1:1). The plate was sprayed with 10 % KOH or weber reagent.

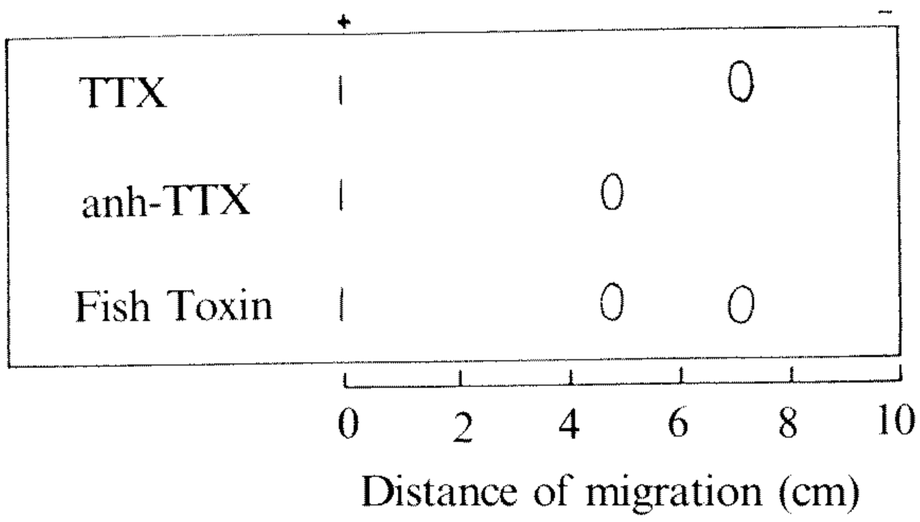

Figure 3. Electrophoresis of fish toxin, along with authentic tetrodotoxin (TTX) and anhydro-tetrodotoxin (anh-TTX).

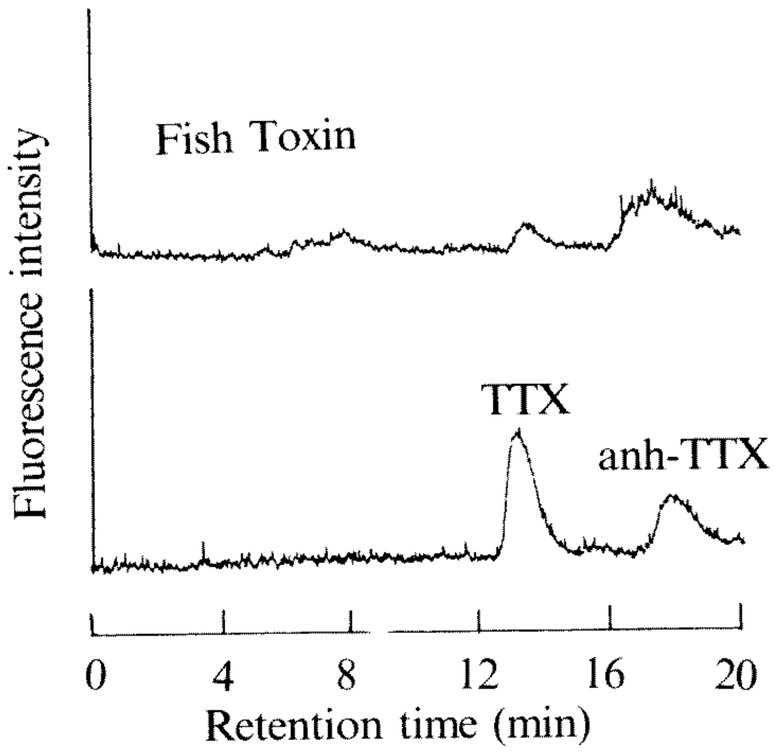

Figure 4. HPLC eluting profile of the fish toxin, along with a authentic tetrodotoxin (TTX) and anhydrotetrodotoxin (anh-TTX).

spraying the plate with Weber reagent[7].

V. *Electrophoresis*

Electrophoresis was performed on 5 × 18 cm cellulose acetate strips (Chemetron, Italy) using 0.08 M Trishydrochloric acid buffer (pH 8.7), under a constant current of 0.8 mA/cm width for 60 min. Toxins were visualized as in the case of TLC.

VI. *High performance liquid chromatography*

The toxin obtained was examined for TTX and its related substances by reversed phase high performance liquid chromatography (HPLC) on an AM-314 column (YMC, Japan) with sodium heptanesulfonate as an ion-pairing reagent[8]. Toxin was detected by mixing the eluate with 3 N sodium hydroxide at a ratio of 1:1, followed by heating at 99°C for 0.4 min, and monitoring the fluorescence at 505 nm with 381 nm excitation. The HPLC system was composed of a Hitachi 655A-11 HPLC, a Hitachi F-1000 Fluorescence Spectrophotometer, a Hitachi L-5000 LC controller, a Hitachi 655A-52 column oven, a Hitachi D-2000 Chromato-Integrator and an Oriental pump.

RESULTS AND DISCUSSION

The toxicity of the remaining fish meat was 150 MU/g. The elution profile of the toxin on a Bio-Gel P-2 column chromatography is shown in Figure 1, and exhibits olny one peak. The total toxicity of the eluate was about 3500 MU. The specific toxicity of partially purified toxin was 87 MU/mg.

As shown in Figure 2, the toxin gave one spot in TLC despite the different solvent system and method of detection. The spot whose Rf value resembled or coincided with that of TTX or anh-TTX was visualized by spraying the plate with 10% KOH or Weber reagent.

As shown in Figure 3, the toxin presented two spots in electrophoresis, which moved with the same mobilities as those of TTX and anh-TTX.

Figure 4 shows the HPLC patterns of toxin and authentic toxin preparations. The toxin gave rise to two peaks whose retention times coincided well with those of authentic TTX and anh-TTX.

Judging from the present results, it is concluded that the food poisoning incident which occurred in Hualien County, Taiwan, in December, 1992, was caused by TTX. It is well known that TTX is generally contained in puffer . Hence, the toxic fish is supposed to have been a toxic puffer fish.

The minimum lethal dose of TTX for a human by oral administration is assumed to be 10, 000 MU[9]. In the food poisoning case, the pregnant woman died by ingesting the toxic fish meat. She may have eaten more than 67 g of fish meat, because the toxicity of fish meat was found to be 150 MU/g.

Although, TTX-associated food poisoning has sporadically been reported in Taiwan[1,2,3,10,11], our people generally are unable to distinguish puffer species. The puffer family Tetraodontidae, which possess 4 teeth in the mouth as a unique character, have more than 27 species in Taiwan[12]. Most puffers, except a few nontoxic species such as *Lagocephalus gloveri* and *L. wheeleri,* contain TTX, especially in viscera as well as the liver, ovary and intestine[13]. Hence, it is very important that the puffer should be eliminated from the diet except those species definitely known to be nontoxic.

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REFERENCES

1. Hwang, D.F., Wang, W.C., Chung, H.M. and Jeng, S.S. 1989. First identification of acute tetrodotoxin-associated food poisoning in Taiwan. J. Formosan Med. Assoc. 88 : 289-291.
2. Hwang, D.F., Chung, H.M., Lin, M.C., Mok, H.K. and Jeng, S.S. 1989. Food poisoning due to ingestion of the puffer *Lagocephalus lunaris*. Fish. Soc. Taiwan. 16 : 1-6.
3. Hwang, D.F., Cheng, C.R., Chung, H.M., Lin, L.C. and Jeng, S.S. 1989. Tetrodotoxin-associated food poisoning due to ingesting fish roe. J. Biomed. Lab. Sci. 4 : 278-283.
4. Hwang, D.F. and Jeng, S.S. 1991. Bioassay of tetrodotoxin using ICR mouse strain. J. Chinese Biochem. Soc. 20 : 80-86.
5. Goto, T., Hish, Y., Takahashi, S. and Hirata , Y. 1965. Tetrodotoxin. Tetrahedron. 21 : 2059-2088.
6. Hwang, D.F., Noguchi, T., Arakawa, O., Abe, T. and Hashimoto, K. 1989. Toxicological studies on several species of puffer in Taiwan. Nippon Suisan Gakkaishi. 54 : 2001 -2008.
7. Weber, M.S. 1928. The determination of the quanidine bases in urine. J. Biol. Chem. 78 : 465-473.
8. Nagashima, Y., Maruyama, J., Noguchi, T. and Hashimoto, K. 1987. Analysis of paralytic shellfish poison and tetrodotoxin by ion-pairing high performance liquid chromatography. Nippon Suisan Gakkaishi. 53 : 819 -823.
9. Tani, I. 1945. A Study of the Toxicity of Japanese Fugu. Teikoku-tosho Co. Tokyo, (In Japanese).
10. Shiau, C.Y. Chueh, M.C. and Chuang, J.L. 1982. Utilization of puffer in Taiwan. China Fisheries Monthly. 351 : 10-13 (In Chinese).
11. Hwang, D.F. 1985. Marine toxin. China Fisheries Monthly. 386 : 4-17 (In Chinese).
12. Yang, H.C. 1975. Studies on the Tetraodontidae fish in Taiwan. Scientific Annual Taiwan Provincial Museum. 14 : 131-165 (In Chinese).
13. Hwang, D.F., Kao, C.Y., Yang, H.C. Jeng, S.S., Noguchi, T. and Hashimoto, K. 1992. Toxicity of puffer in Taiwan. Nippon Suisan Gakkaishi. 58 : 1541-1547.

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食用魚肉引起之河魨毒中毒

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摘　要

1992年12月，臺灣花蓮地區發生一件食物中毒事件，其中毒原因爲食用不明魚種所致，患者三名，其中一名孕婦患者死亡，另兩位小孩患者則治療後，痊癒出院。患者中毒症狀爲嘔吐、噁心、口唇麻痺和呼吸困難。這些症狀與河魨毒相似，因此將患者所食之殘餘魚肉依河魨毒之毒性生物檢定法測其毒性，得知魚肉每公克具有150個老鼠單位之河魨毒毒性。進一步爲鑑定食物之毒成分，乃將魚肉以1%醋酸甲醇溶液抽取後，經二氯甲烷脫脂、Diaflo YM-2濾膜超過濾和Bio-Gel P-2管柱層析法精製，部分精製之毒素則以矽土膠體60 F254薄板之薄層色層分析、醋酸纖維片之電泳分析和AM-314分析管柱之逆相高效能液態層析儀分析，得知此魚肉中之毒素含有河魨毒和其關連物質脫羥河魨毒。綜合上述結果，得知本次食物中毒事件之原因物質乃爲河魨毒所致。