CASE REPORT

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Puffer Poisoning in Japan—A Case Report

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ABSTRACT: A fatal case of puffer poisoning in an 80-year-old man is reported. By using a bioassay with mice and extracts of crude poisons, the toxicities of the autopsy materials, fresh puffer ovaries, and the remaining cooked ovaries were determined and expressed in mouse units per gram (M.U.). Some medicolegal aspects of puffer poisoning are also discussed. Puffer poisoning is a primary cause of fatal food poisoning in Japan even though its frequency is not high.

KEY WORDS: toxicology, poisons, food poisoning

Hazardous marine animals can be divided into three categories [*1*]: those that present such physical dangers to man as abrasions from coral and attacks by sharks; those that are venomous, passively or actively transferring a toxin to man through a simple to complete venom apparatus such as the stinging capsules of jellyfish or the spines of scorpion fish; and those that are poisonous to eat because of toxins produced by the body or acquired through environment, such as puffer fish and those fish that produce the disease ciguatera. Halstead [*2*] divided poisonous fish into three major subdivisions based on the location of the toxin: (1) ichthyosarcotoxic fish, which contain a poison within the flesh, that is, musculature, viscera, skin, or mucus; (2) ichthyotoxic fish, which produce a poison that is generally restricted to their gonads; and (3) ichthyohemotoxic fish, which have poisonous blood. Among these poisonous fish the ichthyosarcotoxic ones are responsible for most cases of fish poisoning. Of the nine types ichthyosarcotoxism, ciguatera, scombroid, and puffer poisoning are most common. According to Hughes et al [*3*], there has been an increase in the number of outbreaks of fish and shellfish poisoning reported to the Center for Disease Control in Atlanta, Georgia. Ciguatera and scombroid fish poisoning accounted for 99% of vertebrate fish poisoning outbreaks reported to the Center. Although fatality rates of ciguatera poisoning as high as 12% have been reported, no deaths occurred in 184 cases of ciguatera poisoning reported to the Center. In 404 reported

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cases of scombroid fish poisoning, only 8 patients (2%) were hospitalized. None of these died.

Puffer poisoning is the most widespread ichthyotoxism in Japan. The Japanese consider the fish, either cooked or raw, as an epicurean delicacy. In the decade between 1954 and 1963, 1962 instances of puffer poisoning were reported [*4*]. No data are available on the number of individuals involved in each reported incident. Among these instances there were 1153 fatalities. Owing to improved legal control over the marketing and preparation of puffer fish, the incidence of puffer poisoning has recently decreased.

The following study describes the case of an 80-year-old man who died of puffer poisoning. The statistics of puffer poisoning in Japan in the decade between 1967 and 1976 are also presented.

Case Report

A 53-year-old housewife bought 150 g of puffer ovaries (*Fugu vermicularis vermicularis*) at a fish shop that was not licensed to prepare puffer. She did not realize their poisonous character, and she cooked and served them to her family at 1:00 p.m. She ate two pieces of the cooked ovaries, her 23-year-old daughter three pieces, and her 80-year-old father-in-law five or six pieces. After the meal the two women attended a meeting outside their home. At approximately 2:30 p.m. the housewife noticed paresthesia of the lips and tongue accompanied by dizziness, nausea, and vomiting. Her daughter felt only slight nausea. The two victims were admitted to the hospital at 3:00 p.m. and recovered by 9:00 p.m. after receiving medical treatment. Upon hearing of the poisoning, a doctor was dispatched to the father-in-law in his house and arrived at 4:30 p.m. The victim was found unconscious, but his blood pressure was 130/70 mm Hg and his pulse was regular at 76/min. Vomitus was observed around his mouth. Despite intensive medical care his condition worsened and death was certified at 5:00 p.m. A medicolegal autopsy was performed on the victim since there was suspicion of a violation of the Criminal Law (Act 211) and the Food Sanitation Law (Act 4) by the owner of the fish shop.

Autopsy Findings

The autopsy was carried out 23 h after death. The deceased was a poorly built man whose rectal temperature was 27.5°C (81.5°F). A small amount of pale brown froth exuding from his mouth and petechial hemorrhages both in the conjunctivae and skin of the upper thorax were noticed. Cyanosis was not observed and no evidence of trauma was found. On internal examination his lungs and gastrointestinal tract were markedly congested and edematous. Diffuse myocardial fibrosis of the left ventricle and severe atherosclerosis in the aorta, coronary arteries, and cerebral arteries were seen. The blood in the heart was dark red and fluid. Fifty millilitres of brown viscous gastric contents containing a few fish eggs and yellow-brown liquid contents in the jejunum were observed and collected for toxicological investigation. The remaining cooked ovaries and the dried vomitus, which had been collected at the victim's house, as well as some fresh puffer ovaries of the same kind were also submitted for toxicological investigation.

Toxicological Investigation

A bioassay of tetrodotoxin, following the method authorized by the Japanese Ministry of Health and Welfare [*5*], was performed on the autopsy materials, the remaining cooked ovaries, the dried vomitus, and the fresh ovaries.

Determination of Toxicity—After the preparation of crude puffer poisons from the materials to be tested by the authorized method, each original preparation of crude poi-

sons was diluted with distilled water to give the minimum lethal dose. A half millilitre of the diluted preparation was injected intraperitoneally into a mouse; the mice weighed 17 to 26 g, and death within 30 min after injection was observed. The toxicity of each specimen was expressed in mouse units (M.U.) per gram. One M.U. per gram is defined as that 1 g of specimen that is lethal for 1 g of the body weight of the mouse used [*6*]. Consequently, the toxicity of each specimen is expressed as:

$$\text{toxicity (M.U.)} = \frac{P \times M}{S \times I \times D}$$

where

P = volume of the original preparation, ml,
M = weight of the mouse, g,
S = weight of the specimen, g,
I = volume of the intraperitoneal injection, ml, and
D = maximum dilution of the original preparation.

Interpretation of Results—The toxicity of each specimen is shown in Table 1. One gram of fresh ovaries had 8500 M.U. of toxicity. It has been said that 200 000 M.U. of toxicity is the minimum lethal dose for humans [*5,6*]; therefore, the ingestion of 23.5 g of the fresh ovaries would be lethal. This amount could be collected from one puffer involved in the present case. The remaining cooked ovaries in the present case had 8178 M.U. of toxicity, aproximately the same toxicity as the fresh ones. In contrast, the vomitus and the gastric and intestinal contents had 853, 8, and 18 M.U. of toxicity, respectively. These extremely low toxicity values would be explained as follows. The vomitus had been collected in an old newspaper by a neighbor, and hence the water-soluble poisons in the vomitus were absorbed by the newspaper. It was unfortunate that only the dried vomitus was brought to our laboratory. The low value of the toxicity in both the gastric and intestinal contents might be accounted for by the severe vomiting, which is one of the acute toxic symptoms in puffer poisoning.

Cause of Death

Through police investigation, it was confirmed that the victim had consumed five or six pieces of the cooked ovaries, which had a toxicity of 8178 M.U. No gross anatomic findings were observed that might have caused the sudden death of the victim. The two recovered victims showed characteristic symptoms of puffer intoxication. Hence it was concluded that the cause of death was puffer poisoning, even though extremely low toxicities were determined in the vomitus and the alimentary contents of the victim.

Discussion

Detailed information on hazardous marine animals including puffers is available in Banner's text [*1*]. The poisonous components of puffer ovaries are known as tetrodotoxin. A bioassay of tetrodotoxin has been traditionally used in medicolegal cases of puffer poisoning [*7-10*] for the following reasons:

1. The isolation and purification of tetrodotoxin require large amounts of the material and an extremely complicated procedure [*11*].
2. A bioassay of tetrodotoxin using a crude extract of poisons and mice can be easily performed in every laboratory [*5,6*].

TABLE 1—*Toxicological analysis in a fatal puffer poisoning.*

Dilution	Body Weight of Mouse, g	Intraperitoneal Dose, ml	Time of Death, min and s	Toxicity,[a] M.U.	Average of Toxicity, M.U.
		Fresh Ovaries, 5 g[b]			
×100	17.0	0.4	14′ 05″	8 500	
×100	17.0	0.4	19′ 45″	8 500	8500
×100	17.0	0.4	13′ 00″	8 500	
×120	18.0	0.4	not fatal	N.D.[c]	
×120	18.0	0.4	not fatal	N.D.	N.D.
×120	17.0	0.4	30′ 40″	10 200	
		Cooked Ovaries, 2 g[b]			
×20	19.0	0.5	7′ 30″	3 800	
×20	26.5	0.6	7′ 50″	4 417	4100
×20	24.5	0.6	5′ 20″	4 083	
×40	22.5	0.6	33′ 00″	7 500	
×40	26.5	0.6	24′ 30″	8 833	8178
×40	20.5	0.5	21′ 00″	8 200	
×60	19.0	0.5	not fatal	N.D.	
×60	19.5	0.5	not fatal	N.D.	N.D.
×60	20.0	0.5	not fatal	N.D.	
		Vomitus, 5 g[b]			
×5	26.0	0.6	4′ 53″	433	
×5	25.0	0.6	4′ 57″	417	419
×5	24.5	0.6	4′ 58″	408	
×10	21.5	0.5	16′ 00″	860	
×10	20.5	0.5	14′ 30″	820	853
×10	22.0	0.5	16′ 30″	880	
×15	19.5	0.5	not fatal	N.D.	
×15	20.5	0.5	not fatal	N.D.	N.D.
×15	20.0	0.5	30′ 00″	1 200	
		Gastric Contents, 50 g[b]			
×1	17.5	0.5	12′ 30″	7	
×1	20.0	0.5	16′ 30″	8	8
×1	23.0	0.5	16′ 00″	9	
×2	23.5	0.6	not fatal	N.D.	
×2	25.5	0.6	not fatal	N.D.	N.D.
×2	23.5	0.6	not fatal	N.D.	
		Intestinal Contents, 20 g[b]			
×1	19.0	0.5	14′ 30″	19	
×1	21.5	0.6	16′ 10″	18	18
×1	21.5	0.6	20′ 00″	18	
×2	20.0	0.5	not fatal	N.D.	
×2	21.0	0.5	not fatal	N.D.	N.D.
×2	19.5	0.5	35′ 30″	39	

[a] See text for the calculation of toxicity; the volume of each original preparation is 10 ml.
[b] The values indicate the weight of each specimen submitted for the extraction of the poisons.
[c] Not determined.

3. The toxicity of a specimen can be expressed semiquantitatively in M.U. per gram with the bioassay [*5,6*].

4. Extensive data have been compiled in the comprehensive work by Tani [*12*] on the toxicity of puffers using the bioassay.

A method for the conversion of tetrodotoxin into 2-amino-6-hydroxymethyl-8-chinazolinol (C_9-base), which gives a characteristic ultraviolet spectrum, has been reported [*13*]. For instrumental analysis of tetrodotoxin in a fatal poisoning case, a gas chromatographic

procedure using an electron current detector apparatus and purified halogenoacylated C_9-base was reported [*14*]. A gas chromatographic/mass spectrometric determination of trimethylsilylated C_9-base, one of the tetrodotoxin derivatives, was also applied on an extract of the gastric contents of a fatal poisoning victim.[2] These instrumental methods give more sensitivity, reproducibility, and objectivity in results than the biological method described above.

Surveys on food poisonings in the decade between 1967 and 1976 in Japan [*15*] and on administrative controls over puffer preparation and cooking[3] were carried out. Because the Japanese consume large amounts of seafood, both raw and cooked, fish and shellfish and their products have been the greatest single source of food poisoning in Japan. In this decade there were, in total, 12 691 cases of food poisoning, which involved 351 688 patients and 607 fatalities. Seafood and its products have been responsible for 4555 cases of food poisoning (35.9% of all food poisoning cases); 87 890 patients were involved in these cases (25.0% of all victims of food poisoning), and 450 deaths resulted (74.1% of all deaths from food poisoning).

Puffer poisoning is one type of seafood poisoning. The number of outbreaks and patients has not been so high: 610 out of 12 691 cases (4.8%) and 1015 out of 351 688 patients (0.3%). However, as regards the number of fatalities, puffer has been the major single cause of food poisoning deaths: 372 deaths, or 61.3%, of all food poisoning deaths and 82.7% of all seafood poisoning deaths.

The fatality rate of puffer poisoning has also been very high: 372 deaths out of 1015 patients (36.7%), whereas the average fatality rate of all food poisonings was less than 0.2%, 607 deaths out of 351 688 patients. The present statistical figures suggest that puffer poisoning is an unusually life-threatening food intoxication in Japan. In some districts, people who have been poisoned by the ingestion of puffers are referred to as having been "shot." Puffers themselves are sometimes called *Teppo* (guns). The statistics clearly prove that the colloquialisms used to refer to puffers or its poisoning agree with relevant statistical data.

Based on cases of poisoning, the liver, ovaries, and skin, in decreasing order, are considered to be the most toxic parts of the puffer [*4*]. The flesh of the puffer, when prepared properly by a specially trained cook, is entirely safe. Therefore the marketing of puffers and serving of *Fugu Ryori* (puffer dishes) are not against the Food Sanitation Law. It is very difficult for laymen to prepare the fish safely. Consequently, regulations on puffer preparations have been issued throughout the country. An example of such regulation is that passed in 1958 by the Department of Sanitation of Kumamoto Prefecture. According to this regulation, cooks who have attended a special training course [*16*] and passed an examination will be given a license to prepare the fish. This kind of regulation, however, has not been established in all 47 administrative districts of Japan. Thirteen districts, Kanagawa, Chiba, Tokyo, Shizuoka, Aichi, Shiga, Kyoto, Totori, Hiroshima, Ehime, Kochi, Miyazaki, and Kagoshima Prefecture, have issued a similar regulation. In four districts, Osaka, Hyogo, Okayama, and Kagawa Prefecture, a cook cannot prepare the fish without attending a special training course, but he is not required to take an examination. In Kumamoto Prefecture, 456 cooks out of 544 candidates have passed the examination between 1971 and 1977, and the number of licensed cooks, as of April 1978, is 704.

There have been several reports on medicolegal autopsies performed on victims of puffer poisoning [*7–10*]. Most of the cases were accidental poisonings resulting from untrained people, unlicensed cooks, and laymen preparing puffer for their own tables. The

[2]Y. Otsuka, Criminal Investigation Institute of Fukuoka Prefectural Police Headquarters, Fukuoka, Japan, personal communication.

[3]Department of Health, Division of Public Health, Kunamoto Prefectural Office, personal communication.

victims ate the toxic parts of puffer, that is, livers, ovaries, and other parts of the visceral organs. But an unusual homicide by puffer poisoning with the intention of perpetrating an insurance fraud has been reported by S. Makizumi [7].

Barring unexpected and unreliable changes in the Japanese taste for seafood, puffer poisoning will continue to be in the first rank of fatal food poisonings since untrained and uneducated people will prepare puffers for their own tables.

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