NOTE *Laboratory Animal Science*

Comparison of Toxicity between Saxitoxin and Decarbamoyl Saxitoxin in the Mouse Bioassay for Paralytic Shellfish Poisoning Toxins

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ABSTRACT. The mouse bioassay (MBA) for paralytic shellfish poisoning (PSP) toxins has been used in the AOAC Official Method and the official Japanese method. In the AOAC Official Method, the saxitoxin (STX) standard provided by the U.S. Food and Drug Administration (FDA) is used, but no standard is used in the official Japanese method. The objective of this study was to compare the toxicity of decarbamoyl STX (dcSTX), one of the derivatives of STX and a candidate standard for the MBA for PSP toxins in Japan, to that of FDA STX in the MBA platform. In this study, the toxicity of dcSTX was 918.0 ± 44.9 mouse units/μmol, and the relative toxicity ratio of dcSTX to FDA STX based on moles was 0.478.

KEY WORDS: comparative toxicity, decarbamoyl saxitoxin (dcSTX), mouse bioassay (MBA), saxitoxin (STX)

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The mouse bioassay (MBA) has been used as the official method for detecting paralytic shellfish poisoning (PSP) toxins in Japan since 1980 [7]. Briefly, the MBA for PSP toxins is performed as follows: Five or more male ddY mice, weighing 19–21 g (about 4 weeks old of age), are intraperitoneally (i.p.) injected with 1 m*l* of acid extract of a shellfish sample, and the time of death (the time from the end of the injection to the last gasp of breath) is observed. If the median death time is <5 min, the assay is repeated by diluting the extract so that the animals die 5–7 min after injection. The toxicity of the sample, expressed in mouse units (M.U.), is calculated from the median death time of a total of 5 or more mice by using Sommer's table (table concerning the relationship between death time and M.U. for PSP toxins). If mice, weighing <19 g or >21 g, are included in the assay, weight correction using a correction table for the weight of the mice is needed [4, 6]. The official Japanese method basically conforms to AOAC Official Method 959.08 [1]. In AOAC Official Method 959.08, however, use of a saxitoxin (STX) standard provided by the U.S. Food and Drug Administration (FDA, Rockville, MD, U.S.A.), as a control is required, and toxicity is expressed in μg STX equivalent (STX eq), rather than M.U., calculated by using the results obtained with the STX standard, due to differences in such things as mouse strain, sex and conditions. (No details are provided regarding the requirements for mouse strain and sex in the AOAC Official Method.) In Japan, however, possession of STX is restricted by the Act on the Prohibition of Chemical Weapons and the Regulation of Specific Chemicals, so the STX standard cannot be used in most facilities. Accordingly, in the official Japanese method, the strain and sex of the mice used are designated as an alternative to using the STX standard [4].

Decarbamoyl STX (dcSTX), which is one of the derivatives of STX and is not restricted by the Act on the Prohibition of Chemical Weapons and the Regulation of Specific Chemicals, is considered a candidate standard for the MBA for PSP toxins in Japan. In this study, we aimed to compare the toxicity between FDA STX and dcSTX using the MBA platform for PSP toxins.

Toxins: STX (100 μg/m*l*, lot No. 088, purity unknown, as STX·2HCl) was kindly provided by the U.S. FDA. dcSTX (2.55 nmol/m*l*, purity >95.0%, as dcSTX diacetate salt) was kindly provided by the Hatano Research Institute, Food and Drug Safety Center (Hadano, Japan).

Animals: Specific pathogen-free male ddY mice at 4 weeks old of age were purchased from Japan SLC Inc. (Shizuoka, Japan) and kept in our animal facility for 1 day. The mice were kept at a room temperature of 20–26°C and relative humidity of 30–70%, with a 12-hr light (09:00–21:00)-12-hr dark (21:00–09:00) cycle. The mice were housed in plastic cages with wood chip bedding and were fed commercial pellets (CRF-1; Charles River Laboratories Japan Inc., Yokohama, Japan) and tap water *ad libitum*. All animal experiments were conducted with the approval of the Animal Care and Use Committee of the National Institute of Health Sciences, Japan.

Experimental designs: The MBA method was basically in accordance with AOAC Official Method 959.08 [1] and also the official Japanese method [4]. STX and dcSTX, adequately diluted in each experiment, were injected i.p. into 5 mice each, the time of death was measured, and the toxicity results (M.U.) were calculated by using Sommer's table and the correction table for the weight of the mice. In this study, weight correction was applied for more precise calculation, although all of the mice used weighed between 19 g and

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Table 1. Results of 3 experiments using the MBA for STX and dcSTX

1st Experiment

Toxin (dose)	Mouse No.	B.W.[a)]	B.W.C.F.[b)]	Lethal time (sec)	M.U.[c)]	Corrected M.U.	Median M.U.	Ave.± SD of M.U.	C.F.[d)] (μg/M.U.)	Toxicity[e)] (μg STX eq)	Toxicity ratio
STX·2HCl (0.370 μg/ mouse)	1	19.54	0.9862	326	1.764	1.740	1.852	1.814 ± 0.221	0.1998	-	-
	2	20.34	1.0102	397	1.457	1.472					
	3	19.96	0.9988	290	2.000	1.998					
	4	19.86	0.9958	310	1.860	1.852					
	5	20.27	1.0081	291	1.992	2.008					
dcSTX diacetate salt (0.673 μg/ mouse)	1	19.72	0.9916	372	1.552	1.539	1.678	1.610 ± 0.159	-	0.3353	0.498
	2	20.03	1.0009	430	1.364	1.365					
	3	19.97	0.9991	342	1.680	1.678					
	4	20.04	1.0012	335	1.715	1.717					
	5	20.39	1.0117	332	1.730	1.750					

2nd Experiment

Toxin (dose)	Mouse No.	B.W.[a)]	B.W.C.F.[b)]	Lethal time (sec)	M.U.[c)]	Corrected M.U.	Median M.U.	Ave.± SD of M.U.	C.F.[d)] (μg/M.U.)	Toxicity[e)] (μg STX eq)	Toxicity ratio
STX·2HCl (0.357 μg/ mouse)	1	19.78	0.9934	365	1.580	1.570	1.846	1.811 ± 0.169	0.1934	-	-
	2	20.41	1.0123	299	1.928	1.952					
	3	20.17	1.0051	295	1.960	1.970					
	4	19.86	0.9958	311	1.854	1.846					
	5	19.63	0.9889	331	1.735	1.716					
dcSTX diacetate salt (0.673 μg/ mouse)	1	19.71	0.9913	363	1.588	1.574	1.695	1.714 ± 0.274	-	0.3278	0.487
	2	19.70	0.9910	283	2.056	2.037					
	3	20.21	1.0063	439	1.339	1.348					
	4	20.29	1.0087	342	1.680	1.695					
	5	19.93	0.9979	300	1.920	1.916					

3rd Experiment

Toxin (dose)	Mouse No.	B.W.[a)]	B.W.C.F.[b)]	Lethal time (sec)	M.U.[c)]	Corrected M.U.	Median M.U.	Ave.± SD of M.U.	C.F.[d)] (μg/M.U.)	Toxicity[e)] (μg STX eq)	Toxicity ratio
STX·2HCl (0.333 μg/ mouse)	1	20.14	1.0042	373	1.548	1.555	1.773	1.741 ± 0.241	0.1878	-	-
	2	20.06	1.0018	307	1.878	1.881					
	3	20.25	1.0075	402	1.440	1.451					
	4	19.83	0.9949	283	2.056	2.046					
	5	19.95	0.9985	324	1.776	1.773					
dcSTX diacetate salt (0.640 μg/ mouse)	1	20.15	1.0045	344	1.670	1.678	1.474	1.493 ± 0.121	-	0.2768	0.433
	2	19.96	0.9988	438	1.342	1.340					
	3	20.10	1.0030	393	1.470	1.474					
	4	19.83	0.9949	381	1.516	1.508					
	5	20.15	1.0045	396	1.460	1.467					

a) Body weight (g), b) Body weight correction factor by the correction table for the weight of the mice, c) Mouse unit by Sommer's table, d) Conversion factor, calculated as the amount of toxin injected divided by the median M.U., e) Toxicity was expressed as μg STX equivalent (STX eq), but it more precisely reflects μg STX·2HCl eq.

21 g. Then, the toxicity of dcSTX expressed in μg STX eq, was calculated by using the conversion factor (CF) for STX. The experiments were performed 3 times.

The results of the individual experiments are shown in Table 1, and statistical summaries are shown in Table 2. In each of 3 experiments, the toxicity of dcSTX diacetate salt was calculated using the CF for STX (·2HCl), followed by the AOAC Official Method. It was calculated that 0.640–0.673 μg of dcSTX diacetate salt represented 0.277–0.335 μg STX eq (officially called STX eq in the AOAC method but should be called STX·2HCl eq, as the FDA STX standard is not STX but is STX·2HCl), and the toxicity ratio of dcSTX diacetate salt to STX·2HCl based on weight was 0.473 ± 0.035 (0.433–0.498) (Tables 1 and 2). The molecular weights of STX·2HCl, STX, dcSTX diacetate salt and dcSTX are 372.21, 299.29, 376.37 and 256.26, respectively, and it was calculated that the toxicity ratio of dcSTX to STX based on weight was 0.559 ± 0.042 and that that of dcSTX to STX based on moles was 0.478 ± 0.036 (Table 2).

Previously, Oshima [3] reported that the toxicity values

Table 2. Comparative toxicity of dcSTX to STX calculated by the median values of the 3 experiments

	Toxins	Toxicity (M.U./μmol)	Toxicity ratio of dcSTX diacetate salt to STX·2HCl (weight based)	Toxicity ratio of dcSTX to STX (weight based)	Toxicity ratio of dcSTX to STX (mole based)
1st Exp.	STX[a)]	1863.0	-	-	-
	dcSTX[b)]	939.0	0.498	0.589	0.504
2nd Exp.	STX	1924.6	-	-	-
	dcSTX	948.5	0.487	0.576	0.493
3rd Exp.	STX	1981.7	-	-	-
	dcSTX	866.5	0.433	0.511	0.437
Ave.± SD	STX	1,923.1 ± 59.4	-	-	-
	dcSTX	918.0 ± 44.9	0.473 ± 0.035	0.559 ± 0.042	0.478 ± 0.036

a) The STX standard was provided by the FDA, but more precisely, it was STX·2HCl., b) As dcSTX diacetate salt.

of STX and dcSTX were 2,483 and 1,274 M.U./μmol and that the relative toxicity ratio of dcSTX to STX was 0.513. Recently, Munday *et al*. [2] reported that the toxicity values of STX and dcSTX were 2,090 and 1,330 M.U./μmol and that the relative toxicity ratio of dcSTX to STX was 0.636. In this study, the toxicity values of FDA STX and dcSTX were 1,923.1 ± 59.4 and 918.0 ± 44.9 M.U./μmol, and the relative toxicity ratio of dcSTX to FDA STX was 0.478. In our present results, the relative toxicity ratio of dcSTX to STX was quite similar to that in Oshima's report, although the toxicity values of dcSTX and STX were slightly lower than those in the other 2 studies.

As mentioned above, there is no standard used in the official Japanese method, and dcSTX is considered as a candidate standard for the MBA for PSP toxins in Japan [5]. Use of dcSTX as a standard would help minimize individual differences among assays. The relative toxicity ratios of dcSTX to STX were not so different among the studies (0.478 in this study, 0.513 in Oshima's report [3] and 0.636 in Munday's report [2], based on moles); however, an interlaboratory collaborative study is needed to determine the official conversion factor of the dcSTX standard to the STX (·2HCl) standard provided by the FDA in the future.

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