SHORT REPORTS

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THE PRESENCE OF DIMETHYLNITROSAMINE IN FERMENTED FISHES AND ITS MUTAGENICITY

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ABSTRACT

Partially purified dimethylnitrosamine (DMN) from sixty batches of uncooked fermented fish extracts from the northeast of Thailand was quantitatively investigated. Only 8.33 percent tested negative for this carcinogen, and levels of positive samples were in the range of 42 to 1,228 ppb. Its mutagenic response was also assayed by S. typhimurium *with a microsomal system. A fairly good correlation was found between the presence of DMN and its mutagenesis action in these extracts.*

INTRODUCTION

It is now more than three decades since the toxic effect of dimethylnitrosamine (DMN) in animal species was first demonstrated by Barnes and Magee,[1] followed by the reports of hepatocarcinogenic activity of this particular compound in rat two years later.[2,3] A large number of reports have since appeared on the occurrence of N-nitrosamines in foods,[4-7] mostly cured meat products, smoked fish and Chinese salted fish.

Since fermented fish is used as a principal constituent of daily meals by over 85 percent of the Thai households in northeastern Thailand,[8] we felt that it was the most likely source of dimethylnitrosamine ingestion for the local people. We thought, therefore, that a study of commonly consumed Thai food products would be worthwhile. This communication reports the determination of DMN in uncooked fermented fishes from four different areas, and the assay of its mutagenic action by a method that has been employed for a wide range of N-nitrosamines and shows high reliability in the prediction of carcinogenicity.[9-11]

EXPERIMENTAL

Sixty samples of uncooked fermented fishes were purchased from local markets in Khon-Kaen and three other nearby provinces, namely Maha-Sarakam, Kalasin and Udorn-Thanee (fifteen samples from each area). One hundred grams of each batch was used for methylene chloride extraction at an alkaline pH, and the extract was then passed through an ion-exchange polyamide column.[12,13] Definite determination was performed by the technique of thin-layer chromatography and spectrophotometry.[14] One gram of each sample was separately extracted with two millilitres of dimethylsulfoxide (DMSO) for an assay of mutagenic activity.[9,15] TA 98 and TA 100 were employed as bacterial tester strains throughout the experiments.

RESULTS AND DISCUSSION

The presence of DMN in uncooked fermented fishes obtained from the local markets of four northeastern provinces, viz : Khon-Kaen, Maha-Sarakam, Kalasin and Udorn-Thanee, is shown in Table 1. Positive detection ranges from 42 to 1,228 ppb. Only five samples out of sixty (8.33%) tested negative for this particular carcinogenic substance by semiquantitative analysis using thin-layer chromatography. The levels of DMN in our extracts were higher than those found by Fong and Chan$_7$ (10-300 ppb), but were remarkably lower than the range of up to 40 ppm observed in nitrite-pretreated smoked fish by Ender and Ceh.[16] However, the DMN level in the present study was apparently close to our recently reported previous findings.[17] A possible reason for this is that estimation of this particular compound in different types and sources of food, employing different sample and analytical procedures, may lead to dissimilar results. The method of TLC-spectrophotometry employed for detection of DMN contents in this report showed low validity, thus, confirmation of results by GC-MS or HPLC should have been performed. Chemically, the participating factors which have to be considered in the formation of nitrosamine (nitrosation) in foods are the nitrosating agents and nitrosatable amines.[18,19] It was reported that the most important nitrosating agent involved in nitrosamine production in food systems is nitrous anhydride which can be easily formed from nitrites in aqueous acidic solutions, Nitrites are probably produced from the reduction of nitrates by bacterial reductase which occur in the system. Another group of precursors for nitrosamine formation[18-20] are the secondary and tertiary amines which are commonly found in preserved food products.

The preparation and processing of fermented fishes are traditionally carried out under primative village conditions without any particular concern for modern standards of hygiene. It is interesting to note that nitrites and nitrates were detected in various locally-preserved protein foods, including fermented fishes, as well as in crude salt, rock salt and in the water[21] used in the preparation of fermented fishes. During the processing of fermented fishes and their storage of several months, secondary and tertiary amines are produced. Although the pH range of 4.9 to 6.1 in fermented fish preparations is not strongly acidic enough to enable nitrosamine formation by straightforward chemical reaction, it has

TABLE 1 Levels of DMN and mutagenic activity (modified Ames' test using TA 98 and TA 100 S.*typhimurium* tester strains) in uncooked fermented fish extracts from Khon-Kaen, Maha-Sarakam, Kalasin and Udorn-Thanee

Provinces : Khon-Kaen			**Maha-Sarakam**			**Kalasin**			**Udorn-Thanee**		
DMN	No. of His$^+$ revertants/plate		DMN	No. of His$^+$ revertants/plate		DMN	No. of His$^+$ revertants/plate		DMN	No. of His$^+$ revertants/plate	
(ppb)	TA 98	TA 100	(ppb)	TA 98	TA 100	(ppb)	TA 98	TA 100	(ppb)	TA 98	TA 100
ND	–	–	ND	–	–	ND	–	–	ND	–	–
66	NS	NS	51	NS	NS	56	NS	NS	ND	–	–
98	NS	NS	82	NS	282	86	NS	NS	42	NS	NS
109	NS	NS	298	140	576	235	148	520	57	NS	NS
120	95	NS	320	155	526	284	122	557	65	NS	NS
326	139	526	355	139	544	301	150	482	73	NS	NS
366	135	536	396	159	628	317	144	569	86	NS	296
388	129	607	421	166	632	386	170	678	306	140	539
411	186	628	448	188	634	398	166	670	326	150	528
429	160	678	486	190	689	402	318	830	359	176	549
465	218	705	502	197	706	495	179	832	413	186	698
520	258	826	536	202	804	590	340	988	432	198	702
763	380	1,225	624	198	966	602	247	915	511	190	712
862	405	1,265	688	228	980	903	405	1,284	658	243	962
982	448	1,342	1,048	398	1,432	1,228	532	1,627	734	395	1,126
*421.79 ±73.90			*446.79 ±64.04			*448.79 ±80.40			*312.46 ±63.14		

DMN = Dimethylnitrosamine
ND = Not detectable
NS = Not significant
ppb = Parts per billion
* Mean ± SEM

been indicated that certain bacteria possibly activate nitrosation under these pH conditions. Bacterial contamination supports the feasibility of formation of dimethylnitrosamine via reduction of nitrate as mentioned above.

The DMN present in the positive samples was *in vitro* tested for mutagenesis action by using two strains of Salmonella *typhimurium* (TA 98 and TA 100) as shown in Table 1. It was significantly mutagenic to TA 100 which showed base-pair change mutation after preincubation with a rat liver microsomal enzyme system. The number of revertant colonies of TA 98 which showed frameshift mutation was far smaller than that of TA 100. Our results support the previous reports of Yahagi *et al.*,[9] and of other workers. [15,17,22] Moreover, the findings in Table 1 reveal that there seems to be a fairly good correlation in general, between the DMN levels and the mutagenic response of the extracts, eventhough the DMN levels do not represent the amounts of DMN in induced revertants in plate, since it derives from different procedures. In addition, it is possible that other mutagens are also present in these extracts, for instance, diethylnitrosamine and N-nitroso pyrrollidine.

In conclusion, the consumption of preserved protein foods, including fermented fishes which are rich in secondary or tertiary amines and nitrites or nitrates through the action of certain bacteria under the slightly acidic pH, should be considered as a health hazard due to nitrosamine production in these foods. Thus, contamination of nitrosamines in foods is perhaps a contributory factor linked to the high incidence of primary liver carcinoma in Thailand[2,3] and some other parts of the world.[24-26] Further investigations of DMN in these fermented protein foods in relation to other factors, i.e. cooking effect, duration of fermentation and storage, types of fish, are still needed in order to obtain more concrete information on the association of human liver carcinogenesis with this particular mutagenic compound.

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REFERENCES

1. Barnes, J.M. and Magee, P.N. (1954). Some Toxic Properties of Dimethylnitrosamine. *Br.J. Ind. Med.* **11**, 167-174.
2. Magee, P.N. (1956). Toxic Liver Injury: The Metabolism of Dimethynitrosamine. *Biochem. J.* **64**, 676-682.
3. Magee, P.N. and Barnes. J.M. (1956). The Production of Malignant Primary Hepatic Tumours in the Rat by Feeding Dimethylnitrosamine. *Br. J. Cancer* **10**, 114-122.
4. Panalaks, T., Iyengar, J.R. and Sen, N.P. (1973). Nitrate, Nitrite, and Dimethylnitrosamine in Cured Meat Products. *J. Assoc. Off. Analyt. Chem.* **56**, 621-625.
5. Sen, N.P. (1972). The Evidence of the Presence of Dimethylnitrosamine in Meat Products. *Food Cosmet. Toxicol.* **10**, 219-223.
6. Fazio, T., Damico, J.N., Howard, J.W., White, R.H. and Watts, J.O. (1971). Gas Chromatographic Determination and Mass Spectrometric Confirmation of N-Nitrosodimethylamine in Smoke-Processed Marine Fish. *J.Sci. Food Agric.* **14**, 134-140.
7. Fong, L.Y.Y. and Chan, W.C. (1973a). Dimethylnitrosamine in Chinese Marine Salt Fish. *Food Cosmet. Toxicol.* **11**, 841-845.
8. Koonanuwatchaidet, P. and Srisuwan, C. (1985). Patterns of Fermented Fish Consumption of Villagers in Khon-Kaen: Preliminary Survey. *J. Nutr. Assoc. Thailand* **19**, 207-218.
9. Yahagi, T., Nagao, M., Seino, Y., Matsushima, T., Sugimura, T. and Okada, M. (1977). Mutagenicities of N-Nitrosamines on Salmonella. *Mutation Res.* **48**, 121-130.
10. Andrews, A.W. and Lijinsky, W. (1980). The Mutagenicity of 45 Nitrosamines in Salmonella *typhimurium*. Teratogenesis, Carcinogenesis. *Mutagenesis* **1**, 295-303.
11. Andrews, A.W. Thibault, L.H. and Lijinsky, W. (1978). The Relationship between Mutagenicity and Carcinogenicity of Some Nitrosamines. *Mutation Res.* **51**, 319-326.
12. Stepheny, R.W., Freudenthal, J. and Schuller, P.L. (1976). Quantitative and Qualitative Determination of Some Volatile Nitrosamines in Various Meat Products. *IARC Sci. Publ.* No. **14**, Lyon, pp. 343-354.
13. Fong, L.Y.Y. and Chan, W.C. (1976). Methods for Limiting the Content of Dimethylnitrosamine in Chinese Marine Salt Fish. *Food Cosmet. Toxicol.* **14**, 95-98.
14. Cross, C.K., Bharucha, K.P. and Telling, G.M. (1978). Determination of Volatile N-Nitrosamines in Bacon Cook-Out Fat by Nitrite Releases and Thin-layer Chromatography of Fluorescent Amine Derivatives. *J. Agric. Food Chem.* **26**, 657-660.
15. Fong, L.Y.Y., Ho, J.H.C. and Huang, D.P. (1979). Preserved Foods as Possible Cancer Hazards: WA Rats Feb Salted Fish have Mutagenic Urine. *Int. J. Cancer* **23**, 543-546.
16. Ender, F. and Ceh, L. (1968). Occurrence of Nitrosamines in Foodstuffs of Human and Animal Consumption. *Food Cosmet. Toxicol.* **6**, 569-573.
17. Koonanuwatchaidet, P., Srisuwan, C. and Waropastrakul, N. (1987). Qualitative, Quantitative Analysis and Biological Assay of Dimethylnitrosamine in Fermented Fishes. *J. Nutr. Assoc. Thailand* **21**, 287-308.
18. Scanlan, R.A. (1983). Formation and Occurrence of Nitrosamine in Food. *Cancer Res.* (Suppl.) **43**, 2435S-2440S.
19. Hotchkiss, J.H., Vecchio, A.J. and Ross, H.D. (1985). N-Nitrosamine Formation in Fried-Out Bacon Fat: Evidence for Nitrosation by Liquid-Bound Nitrite. *J. Agric. Food Chem.* **33**, 5-8.
20. Lijinsky, W. and Singer, H.M. (1974). Formation of Nitrosamines from Tertiary Amines and Nitrous Acid. In: *Nitroso Compounds in the Environment* (Bagovski, P. and Walker, E.A., eds.), IARC Sci. Publ.,No. **9**, Lyon.
21. Migasena, P., Reaunsuwan, W. and Changbumrung, S.(1980). Nitrates, Nitrites in Local Thai Preserved Foods. *J. Med. Assoc. Thailand* **63**, 500-505.
22. Sugimura, T. and Sato, S.(1983). Mutagens-Carcinogens in Foods. *Cancer Res.* (Suppl.) **43**, 2415S-2421S.
23. Migasena, P. and Changbumrung, S. (1974). The Role of Nitrosamines in the Causation of Primary Carcinoma. *J. Med. Assoc. Thailand* **57**, 175-178.
24. Craddock, V.M. (1983). Nitrosamines and Human Cancer. *Nature* (London) **305**, 638.

25. Magee, P.N. (1982). *Nitrosamines and Human Cancer.* Banbury Report No. **12**, Cold-Spring Harbor Laboratory, New York.
26. Bartsch, H. and Montesano, R. (1984). Relevance of Nitrosamines to Human Cancer: Commentary. *Carcinogenesis* **5**, 1381-1393.

บทคัดย่อ

การแยกสกัดปลาร้าดิบจำนวน 60 ตัวอย่าง ซึ่งซื้อมาจากตลาดสดของ 4 จังหวัดในภาคตะวันออกเฉียงเหนือ และทำให้บริสุทธิ์อย่างไม่สมบูรณ์เพื่อตรวจวิเคราะห์หาปริมาณของสารไดเมทธิลไนโตรซามีน และตรวจวัดความสามารถในการกลายพันธุ์ของสารก่อมะเร็งชนิดนี้ พบว่ามีตัวอย่างที่ไม่สามารถตรวจพบไดเมทธิลไนโตรซามีนได้คิดเป็นร้อยละ 8.33 ของจำนวนตัวอย่างทั้งหมด ในบรรดาตัวอย่างที่ตรวจพบสารชนิดนี้ ปรากฏว่ามีปริมาณอยู่ในช่วงระหว่าง 42 ถึง 1,228 ส่วนในพันล้านส่วน สำหรับความสามารถในการกลายพันธุ์โดยสารชนิดนี้ พบว่าค่าความสามารถจะสูงในกรณีของสายพันธุ์ TA 100 มากกว่าสายพันธุ์ TA 98 และความสัมพันธ์ระหว่างระดับของสารไดเมทธิลไนโตรซามีนที่เจือปนอยู่ในปลาร้าดิบกับค่าความสามารถในการกลายพันธุ์ดูเหมือนจะดีพอสมควร