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Original Article

Oxytetracycline Residues in Farmed Shrimp: The Re-emerging Threatening Issue to Human Health and Economics

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ABSTRACT

Drug residues in food entering international trade caused panic to consumers, health authorities and food industry. In 1992 several frozen farmed shrimp consignments from Thailand were rejected by Japanese quarantine stations due to contamination of antimicrobial agent residues. This study was an attemp to seek for appropriate solutions to cope with the problems of an antimicrobial agent residue-oxytetracycline in cultured shrimp. Scientific data generation and gathering of technical information were performed. Planning was made for short-term and long-term resolutions. Information concerning the use and toxicoligical study of oxytetracycline was also reviewed. Recommendations for setting up an international Code of Hygienic Practice for Aquaculture and a worldwide maximum level of oxytetracycline residues in farmed shrimp were made.

INTRODUCTION

Antimicrobial agents are substances that have the properties of inhibiting growth or killing of microorganisms or both. A wide range of chemical and biological substances are used as bacteriostats and/or bactericides. Antimicrobial agents in relevance to foods are classified within the group of food additives and contaminants by the Joint FAO/WHO Expert Committee on Food Additives[1]. One of the most commonly used antimicrobial agents in food producing system is the tetracycline group. Tetracycline antibiotics were discovered as the result of a systematic screening of soil samples collected from many parts of the world for antibiotic-producing microorganisms[2].

Tetracyclines are usually bacteriostatic in action, but may be bactericidal in high concentrations against highly suseptible organisms. They are broad spectrum antimicrobial agents. Tetracycline, oxytetracycline and demeclocycline are naturally derived compounds from various spe-

cies of Streptomyces[2]. All tetracyclines contain a hydronaphthacene nucleus consisting of four fused rings. Differences among the various analogues are determined by different substitutes on the basic structure. They are usuually subdivided into short-acting (tetracycline, oxytetracycline), intermediate-acting (demeclocycline) and long-acting (doxycycline, minocycline) analogues.

Oxytetracycline is an odourless yellow to tan-coloured crystalline powder produced by the growth of certain strains of Streptomyces. It is very slightly soluble in water but dissolves well in dilute acids and alkalis. Oxytetracycline hydrochloride is more water soluble. Both forms deteriorate in solutions having a pH of less than 2 and are rapidly destroyed by alkali hydroxide solutions. Their selective toxicity for bacteria appears to depend, in part, on energy-dependent uptake of antibiotic by bacteria, but not mammalian cells. This results in a greater accumulation of tetracyclines by bacterial cells.

How do they come into foods? Tetracyclines can be illegally added to foods as preservatives. Their residues are carried over to food chain from animal husbandary and aquaculture practices. They are used in farmed animals primarily for the treatment and control of infectious diseases caused by bacterial pathogens as well as in aquaculture including shrimp farming. They are also introduced to Thai shrimp farmers as feed premix and soluble powder and are very popular among them. Improper use of the substances in shrimp farming causes the problems in international trade. Their impacts to human health are known in two categories. High concentration of tetracyclines can inhibit mammalian protein synthesis in cell-free systems and they can cause acquired resistant to microorganisms. The usual daily dose of oxytetracycline as drug is 250 mg administered once every 24 hours or 300 mg given in divided doses at 8 to 12 hour intervals for adults.[4]

The problem concerning drug residues in cultured shrimp in the Far East was remarkable. One of those most interesting episodes related to the residues of antimicrobial agents used intensively in aquaculture practice in the Far East was oxytetracycline residues in cultured shrimp from Thailand. Japan, one of the most important exported markets for Thai seafood products, has rejected every consignment of shrimp found to contain oxytetracyline residues. This event has caused economic loss to shrimp farmers, frozen cultured shrimp processors and to exported shrimp business of Thailand.

In 1992, the Ministry of Health and Welfare of Japan had banned a few consignments of frozen shrimp from Thailand because of the presence of antimicrobial residues. The rest had to go through strict sampling procedures and laboratory examination where contaminated consignments were rejected. During June 1992 to April 1994, 30 shipmemts of frozen cultured shrimp from Thailand were found to contain antimicrobial agents by Japanese quarantine stations. Nine out of the 30 cases were due to oxytetracycline residues, the rest were because of oxolinic acid residues. Economic loss of 100 million Bahts (US$ 4 million) was estimated. Claims had been made to Thai frozen seafood processors and responsible authority in Thailand mentioning that it has been prescribed in Japanese Food Sanitation Law that food shall contain no antibiotic. Some countries in the Far East also had the experinces of collapse in shrimp farming and exporting business due to failure in the operation of good aquaculture practice.

Since the antimicrobial agent residue issues in farmed shrimp was quite new to Thai Health Authority, scientific approach was selected by the responsible agent, the Division of Food-for-Export Analysis, the Department of Medical Sciences, Ministry of Public Health, to reveal the critical control points in order to reduce or eliminate the problems. This study was planned to cope with short term resolution and long term remedy.

MATERIAL AND METHODS

Laboratory studies consisting of the selection of appropriate methods of determination for oxytetracycline residues, a methodology efficacy comparison between High Pressure Liquid Chromatography (HPLC) and bioassay (microbiological assay) techniques including laboratory monitoring of the residue in shrimp for both raw material and exported consignments were assigned to the Division of Food-for-Export Analysis, the Department of Medical Sciences, Ministry of Public Health, Thailand.

A total of 2,250 samples of cultured shrimp composed of frozen products from various manufacturing locations and raw unprocessed samples from shrimp farms were collected during January 1992 to March 1994 for oxytetracycline residue determinations using HPLC technique.[5,6]

A comparative study on the efficacy of HPLC and bioassay was carried out.[7] Statistical process was performed for the interpretation of laboratory results.

Three interventions were applied during the period of the study as the short term resolution. They were an education program for shrimp farmers emphasizing the proper use of aquaculture drugs run with the collaboration of relevant Thai government agencies and the Thai Frozen Foods Association; a laboratory training course on bioassay technique arranged at the Department of Medical Sciences, for 23 in-plant laboratory analysts and recommedation for more precise production lot identification.

RESULTS

Selection of methodology for laboratory determination of oxytetracycline residues in shrimp. The appropriate sample extraction and residue analysis procedure was found to be the one using Mc Ilvaine buffer for extraction then the filtrate was cleaned up through a Sep-Pak C18 column with methanolic oxalic acid as an eluent and followed by HPLC technique for qualitative and quantitative determination of the substance. HPLC seperation was carried out on a Lichrosorb RP-18 column using a mixture mobile phase of methanol, acetronitrile and 0.01 M oxalic acid at the ratio of 1 :1.5 :2.5 by volume. UV detector wavelength was set at 350 nm. The precision of this procedure gave percentage recovery of 90.8, 88.0 and 85.0 at the concentrations of residues at 0.2 mg/kg, 0.5 mg.kg and 1.0 mg/kg respectively. The limit of determination was 0.10 mg/kg.

Statistical analysis of the data obtained from laboratory studies showed that there was no significant difference in the efficacy between HPLC and bioassay techniques in the determination of oxytetracycline residues in cultured shrimp, seeTable 1).

The incidences of oxytetracycline residues in cultured shrimp products for export during 1992-1994 were presented in Table 2,3. The incidence of positive cases was greater in 1992 than those in 1993, and those for 1993 and 1994 were not significantly different.

Table 4 compared the results of pre-export findingsby the Department of Medical Sciences (DMSc) and the findings of Japanese Quarantine authority for the year 1992 - 1994. The incidences of positive cases detected by the DMSc were greatest in 1992, and the lavel detected by the Japanese authority was greatest in the same year.

DISCUSSION

Statistical analysis of the data obtained from laboratory studies showed that there was no significant difference in the efficacy between HPLC and bioassay techniques in the determination of oxytetracycline residues in cultured shrimp which confirmed the fact that due to limited metabolism of oxytetracycline, the determination of the parent compound in edible tissues by microbiological methods provides sufficient residue information for safety assessment. A microbiological assay would be expected to detect all residues of oxytetracycline in tissues from animals. Although no useful oxytetracycline metabolism papers have been published, the data available for other tetracycline allows the con-

Table 1 A comparision on efficacy of HPLC and BIOASSAY techniques by statistical analysis

statistical summary	HPLC	BIOASSAY
mean (mg/kg)	0.4500	0.4000
standard deviation(std)	0.3835	0.4291
std squared	0.1471	0.1841
Sample size	31	20
Test of hypotheses - use 5 % level of significance		
Test of hypotheses of variances (two tailed)		
S2 square/ S1 square	0.7989	
F Distribution	1.93	
Hypotheses accepted H0		
Pooled est. of std squared	0.1614	
Standard error of diff.	0.1152	
t Statistic	0.0364	
To determine superiority, use	To determine equality, use	
t Distribution(1 tailed) 1.6770	t Distribution(2 tailed) 2.0100	
Hypotheses accepted H0	Hypotheses accepted H0	
HPLC not better than Bioassay	HPLC not different from Bioassay	

Table 2 Incidences of oxytetracycline residues in cultured shrimp products for export, 1992-1994.

Level of residues	Mean (mg/kg)	Number of samples		
		1992	1993	1994
0.1 - 0.19	0.15	46	10	9
0.2 - 0.29	0.25	48	20	8
0.3 - 0.39	0.35	24	2	6
0.4 - 0.49	0.45	4	1	4
0.5 - 0.59	0.55	3	2	0
0.6 - 0.69	0.65	1	1	0
0.7 - 0.79	0.75	5	3	0
0.8 - 0.89	0.85	0	0	0
0.9 - 0.99	0.95	4	0	0
1.0 - 1.09	1.05	1	0	0
1.1 - 1.19	1.15	0	1	0
1.2 - 1.29	1.25	1	0	0
1.3 - 1.39	1.35	1	1	0
1.4 - 1.49	1.45	0	2	0
1.5 - 1.59	1.55	0	0	0
1.6 - 1.69	1.65	0	0	0
1.7 - 1.79	1.75	0	0	0
1.8 - 1.89	1.85	0	0	0
1.9 - 1.99	1.95	0	0	0
2.0 - 2.09	2.05	0	1	0
Sum of positive samples		138	44	27
total sample determined		418	952	880
mean levels (mg/kg)		0.1019	0.0201	0.0082
Standard deviation (mg/kg)		0.5374	0.2177	0.1201

Table 3 Incidence of oxytetracycline residues in shrimp as raw material for frozen shrimp plants during June 1992 - March 1994

Year	No. of samples examined	No. of positive samples	Amount of oxytetracycline detected (mg/kg)
1992	7	4	0.16, 0.67, 0.54, 0.40
1993	12	0	-
1994	37	1	0.13

Table 4 A copmparison on the results of pre-export checking by Department of Medical Sciences, and import quarantine checking by Japanese authority

Year	Total samples	DMSc. findings				Japanese Quarantine findings		
		Positve samples		Levels detected		No. of positve	Levels detected	
		number	%	mean value mg/kg	SD	sample	mean value mg/kg	SD
1992	418	138	33.0	0.1019	0.537	2	1.255	0.945
1993	952	44	4.6	0.0201	0.218	4	1.445	0.343
1994	880	27	3.1	0.0082	0.12	3	0.3067	0.391

clusion to be reached that no metabolism occurs in animals.[8] Bioassay method was recommended as a screening procedure for in-plant laboratories in Thailand. A workshop on microbiological determination of oxytetracycline residues in farmed shrimp was also arranged for 23 in-plant laboratory analysts.

The incidences of positive cases in 1992 was greater than those in 1993 which indicated that the interventions applied did improve the situation. Although incidences in 1993 and 1994 were not significantly different, the mean value of residues and standard deviation found in 1994 was less than those in 1993. Those figures in table 4 concerning DMSc findings and Japanese findings revealed that the situation of oxytetracycline contamination in cultured shrimp from Thailand had been improved.

Antibiotics had been tried experimentally as preservatives used on fish, usually in a dip or in ice. Chlortetracycline and oxytetracycline seemed best. Beyond 1978, the American and Canadian governments permitted the incorporation of the tetracyclines in ice to be used by fishermen to kill or inhibit microorganisms on the surfaces of the fish on trawlers and during transportation.[9] Tetracyclines are active in vitro against a great variety of bacteria, including gram-positive, gram-negative, aerobic, and anaerobic organisms. High concentrations of tetracyclines are active against the protozoans.[3]

Oxytetracycline animal health products are available in four major dosage form product groupings: 1) feed premixes 2) injectables 3)

soluble powders and 4) tablets. These products are used in farm animals primarily for the treatment and control of diseases caused by bacterial pathogens. Most fish drugs are delivered through feeds. Active form concentration of Oxytetracycline in feed premixes as quaternary salt (220 g/kg) for Species of catfish and Salmonids recommended maximum use level of 8.25 mg/kg daily with maximum dosage of 8.25 mg/kg body weight for 10 days and the U.S. withdrawal time before slaughter was 21 days. Disease claims were bacterial hemorragic septicemia, and ulcer diseases.[9]

A study with catfish involved dosage at 10 mg/kg of body weight daily for 10 days.[9] Dosage was approximately 1.2 times the highest use level and gave only one positive muscle tissue assay. Liver residues, although quite low and frequently less than assay sensitivity limits, did persist through day 14. The highest single liver assay was only 0.475 microgram/kg on day 1; therefore, all residues were less than 1.0 microgram/kg during the entire study. It is readily apparent that residues following dosage with oral dosage forms are rapidly cleared from tissues. With the exeption of small residues in kidney tissues, all tissues were cleared of detecteable levels of oxytetracycline within 5 days following dosage. Information concerning oxytetracycline resistance revealed that several species of bacteria have become increasingly resistant to the tetracyclines. Many Enterobacteriaceae and most Pseudomonas aeruginosa are resistant. Emergence of high-level tetracycline-resistant N. gonorrhoeae strains is common in some geographic areas. Bacterial resistance to clinically useful tetracyclines is primarily acquired, as resistance strains have emerged due to the selective pressure exerted on bacteria by widespread use of these antibiotics in humans and animals. Resistance may occur through several mechanisms. A primary mechanism of resistance is decreased accumulation of tetracyclines by the bacterial cell. This is due to alterations in bacterial cytoplasmic membrane-located proteins that result in an energy-dependent increased efflux of antibiotic from the bacterial cell. This type of resistance is common for the Enterobacteriaceae. Resistance to one tetracycline usually implies resistance to all with few exceptions. The genes determining resistance to tetracyclines usually reside in plasmids and/ or transposons. Thus, tetracycline resistance usually can be passed readily from one organism to another.[3]

The tetracyclines are bound to plasma proteins to varying degrees. They penetrate variably into many different body fluids and tissues including bile, liver, lung, kidney prostate, urine, cerebrospinal fluid, brain, sputum, and bone. The highest concentrations are found in bile and are 5 to 20 times those in the serum. Although these drugs undergo enterohepatic circulation and are, in part, recoverable in the feces, their half-lifves are determined primarily by rates of renal excretion. Oxytetracycline and tetracycline are eliminated rapidly by glomerular filtration as unchanged drugs and thus have the shortest half-lives.[3]

In assessing the microbiological effects of oxytetracycline, the Joint FAO/WHO Committee on Food Additives considered the results of studies on the induction of drug-resistant organisms in dogs and humans.[1] The data on the induction of bacterial resistance in dogs, when recalculated on the basis of a 60-kg person, yielded a similar no effect dose of 3 mg per day. An Acceptable Daily Intake (ADI) of 0-0.003 milligram per kilogram body weight was established based on a no-observed-effect level of 2 milligram per person per day from the study with human volunteers and a safety factor of 10. A repeat study using doses between 2 to 20 mg per person per day may result in a higher no-observed-effect level.[1]

Antibiotic residues in shrimp not only had impacts on Japanese consumers and Thai economy but also to the American health authority. In November 1994, the U.S. Seafood Imports News

reported that the Food and Drug Administration continued sampling for oxolinic acid in salmon and oxytetracycline in shrimp aiming at reducing of illegal use of aquaculture drugs.[10]

For long term remedy on the issue, the Royal Thai Government has invested a sum of 250 million Bahts (US$ 10 million) through the Ministry of Agricuture and Cooperatives to conduct a monitoring program to ensure that processing plants would be provided with drug-free farmed shrimp. For international role concerning the issue, a Code of Hygienic practice in aquaculture established by Codex Alimentarius Commission would be much beneficial to global market of farmed shrimp. The Royal Thai Government has also proposed to relevant Codex Committees for the establishment of maximum residue limit (MRL) for oxytetracycline residue in farmed shrimp. Joint FAO/WHO Expert Committee on Food Additives reported in the thirty-sixth Report (Joint FAO/ WHO, 1990) that the Committee recommended MRLs for milk, muscle, fat and eggs at the detection level of the microbiological method : 0.1, 0.1, 0.01 and 0.2 mg/kg respectively and 0.3 mg/kg for liver and 0.6 mg/kg for kidney in all species. The estimated maximum daily intake of oxytetracycline is 150 ug in milk, 30 ug in muscle, 0.5 ug in fat, 20 ug in eggs, 30 ug in l iver, and 30 ug in kidney yielding a total of approximately 260 ug, taking account of daily intake values of 300 g of meat as muscle tissue, 100 of liver, 50 g of kidney, 50 g of tissue fat 100 g of egg and 1.5 l of milk. The Committee concluded that the recommended MRLs are conservative and do not present a risk for the consumer and no further studies on residues of oxytetracycline were required. Thailand needs support from other shrimp producing countries worldwide on the proposal of MRL for oxytetracycline in farmed shrimp. The international acceptable MRL could help a lot in dispute cases with the importing countries where zero telerance is applied.

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