

BIOLOGICAL ACTIVITIES OF STERIGMATOCYSTIN

O. De Carli-Izmaylova, MSc Food Science MAS NH ETH, Quality Assurance
Genossenschaft Migros Zurich

N. Izmailova, Associate Professor of the Department of Biochemistry and Biotechnology
Sumy National Agrarian University

The article provides an overview of the data of the world's scientific schools on biological activities of sterigmatocystin (STC). The European Commission requested the European Food Safety Authority (EFSA) to obtain a scientific report on the health risks of animals and people associated with the presence of STC in food and feed. The article presents data on the mechanism of toxicity, carcinogenicity, lethality, mutagenicity of sterigmatocystin.

Key words: *mould, mycotoxins, sterigmatocystin, aflatoxins, toxicity, carcinogenicity, lethality, mutagenicity human and animal health.*

Introduction. Moulds are ubiquitous in nature. Under certain environmental conditions moulds produce secondary metabolites. Those that are toxic to animals and humans are called mycotoxins. Paradoxically, antibiotics isolated from moulds (for example Penicillin from *Penicillium* fungi) are mycotoxins too, but could be used beneficially for humans and animals. Furthermore, some fungal strains are applied for production of foodstuff. Well-known examples are mould-ripened cheeses (camembert, roquefort) and sausages (salami). Thus moulds are friends and foes at the same time. However, mycotoxins are feared food contaminants, which have a negative impact on public health, food security and safety and the economy in many countries, particularly in developing ones. Some mycotoxins exhibit genotoxic, mutagenic, immunosuppressive, carcinogenic and teratogenic effects. To prevent health hazard in humans and animals regular monitoring and control of feed and food occur in almost all countries. There are two different ways in which moulds could affect the humans or animals health. Inhaling of fungal spores could cause diverse allergic reactions or systemic mycosis. The second route of exposure occurs via the digestive tract, due to consumption of food spoiled by mycotoxins. Depending on the amount and duration of exposure mycotoxin can cause acute or chronic toxic effects.

At the present time a great number of mycotoxins are known, but only a limited number of them have an adverse effect on the health and occur in food and feed. Most of them are metabolites produced by fungi of the genus *Aspergillus*, *Penicillium*, *Alternaria* and *Fusarium*. The major mycotoxin groups, which are monitored, are aflatoxins, ochratoxins, trichothecenes, patulin, fumonisins and zearalenon. Due to their high toxicity and carcinogenetic effect, aflatoxins is the most important one among these groups. Their discovery and further investigation appears rather late, in 1960. After this finding, a huge amount of time and effort was invested into toxicological studies, the development of methods for routine control in food and feed. Despite all this preventive events, aflatoxin poisoning is still a problem in certain developing countries. For example in 2004 in Kenya: 125

people died after the consumption of highly aflatoxin-contaminated maize.

The mycotoxin STC is a precursor in the biosynthesis of aflatoxin B₁ (AFB₁). The mechanism of molecular action of STC is very similar to AFB₁. However STC seems to be less toxic than AFB₁. According to the California Environmental Protection Agency and the Office of Environmental Health Hazard Assessment the No Significant Risk Intake (NSRI) value for STC is 0.02 µg/day. This is the intake associated with life time cancer risk of 1: 100 000 or lower for an adult who weighs 70 kg.

Up to now there is no harmonised regulation for STC within the EU. Only the Czech Republic and Slovakia have set maximum allowed levels for STC: 5 µg/kg for rice, vegetables, potatoes, flour, poultry, meat, milk, and 20 µg/kg for other foods. The European Commission has made a request to European Food Safety Organisation (EFSA) for a scientific opinion on the risk for animal and public health related to the presence of STC in food and feed. Depending on the opinion of the EFSA, appropriate changes might be done in the EU Legislation.

Routine analysis of aflatoxins is preferably done by HPLC-FLD in all sectors dealing with food and feed. Therefore the development of a method for the simultaneous and sensitive determination of STC and AFBG would be very beneficial. The primary aim of this investigation was to develop a combined method for STC and AFBG detection.

Materials and methods. As a material for this work the articles of the leading scientists of different countries of the world were used. On the basis of the Federal Technical University of Zurich the articles were collected, compared and analysed. The databases of the research results of advanced universities were used (mainly PubMed). This work presents the scattered results of studies of the biological effect of sterigmatocystin over the past thirty years.

Results and discussion. The unsaturated 2,3 bond in the bis-dihydrofuran ring is closely related to the biological activation of STC. After STC undergoes a metabolic activation, it can covalently bind to macromolecules such as DNA and RNA [1]. Binding to DNA base have been shown to result in

further formation of a major adduct 1, 2-dihydro-2-(N⁷-guany l) - 1 - hydroxylsterigmatocystin (ST-N⁷-Gua) [2].

Lipid peroxidation was described as a possible secondary mechanism of STC toxicity [3].

The mechanism of covalent binding of STC to DNA guanine base pair has been investigated and described by Essigmann et al. For the experiment, STC was metabolically activated in the presence of calf thymus DNA by using phenobarbital-induced rat liver microsomes. Metabolic activation of STC begins with the formation of the electrophilic exo-ST-1,2-oxide, its adduction to DNA and its further hydrolysis to 1,2-dihydro-2-(N⁷-guany l) - 1 - hydroxysterig-matocystin. The bond between the carcinogen and the DNA base links STC to the N⁷ atom of guanine. In this experiment of Essigmann et al. radioactive labelling of STC was not available. That is why the precise quantification of the total level of covalent binding to DNA was impossible [2].

The induced ST-N⁷-Gua-DNA complexes result in the inactivity of the DNA as a template. Secondary effects ensue after the DNA is altered in the form of condensation of the chromatin. The loss of activity of the altered DNA-protein complex is associated with morphological changes in the nucleolus such as macro segregation and fragmentation or fibrous transformation [1]. The p53 tumor suppressor protein is most frequently expressed at the time of transformation to a malignant tumor, and when DNA repair is required. A significant increase in p53 protein expression in the STC treated human gastric cells in vitro was observed by Xie et al. [4]. Another in vitro study showed that the level of unscheduled DNA synthesis in the STC treated human epithelial gastric cells was significantly higher than the one in the control group. By unscheduled DNA synthesis the process of replication of DNA during the nucleotide excision repair of DNA damage is understood. The level of DNA repair of STC groups was significantly increased in a dose-dependent manner. These results suggest that bioactivated STC is a genotoxic compound, and that it may damage the DNA of human gastric epithelial cells [5].

Long-term administration of STC *in vivo* (Mongolian gerbils) and further investigation of p53 gene expression has been conducted by Kusunoki et al. A significant increase of p53 gene expression was detected in a STC treated group compared to a non-treated control group [6].

Carcinogenicity According to IARC (International Agency for Research on Cancer) Monographs STC belongs to the 2B Group. This group is defined as possibly carcinogenic to humans. This category is used for agents for which there is limited evidence of carcinogenicity in humans and less than sufficient evidence of carcinogenicity in experimental animals.

The experimental data in vivo shows that the

exposure to STC can cause cancer. The most effected organ is the liver. Little data on lung and kidney cancer induced by STC is available. Experiments show a dose-response relationship in all tested animals. In general mammals, newborns and male animals are more susceptible to STC than adults and females. In contrast, an experiment with medaka fish showed that the female medaka fish were more sensitive to STC than male one [1]. The susceptibility for STC for different species varies a lot. The biggest number of experiments has been done on mice and rats.

The most susceptible animals for STC are Cynomulgus Monkeys with the TD₅₀ of 0.127 mg per kilogram body weight a day. The most frequent liver diseases reported in all animal studies were liver hemangioendothelioma, angiosarcoma, hepatocellular adenoma, hepatocellular carcinoma (solid), cholangiocarcinoma, and liver hyperplastic nodule formation.

All changes in liver metabolism and diseases were induced by STC. The explanation for this site of action can be as follows: In all this studies the STC solution was fed or force-fed (gavage) to the animals, therefor STC was absorbed though GI tract. The portal vein transports blood from the intestine to the liver for further metabolism. After absorption STC reaches the liver via portal vein blood flow. The metabolic activation is required for STC to enable its binding activity. The metabolic activation derives via CYP enzymes. CYP enzymes are present almost in all tissues of the body, and the liver as the main metabolic organ has the highest concentration of CYP enzymes, taking part in xenobiotic biotransformation. Therefore, STC goes directly to the liver, where it would be activated and would be able to damage the cell directly.

A way for better understanding of TD₅₀ values is a comparison of STC and aflatoxin B₁ potency to cause cancer: In rats the TD₅₀ for AFB₁ is 0.0032 mg/kg body weight per day and for STC 0.152 mg/kg body weight per day. AFB₁ is almost 50 times more potent to cause cancer in rats. Cynomulgus monkeys (*Macaca fascicularis*) were also tested for susceptibility for both toxins. The TD₅₀ for AFB₁ is 0.0201 mg/kg body weight per day and for STC 0.127 mg/kg body weight per day. The different susceptibility after predisposition to the same chemical in different species is common [7].

Lethality The lethality indicator LD₅₀ is the dose of a toxic substance causing the death of 50 percent of the animals taking part in experiment. This indicator is often used to describe the lethal potency of a substance, drug or toxin. LD values differ between animal species and also due to the way of administration. Some species are more susceptible to a given toxin than the others. At the same time different drug administration passway even within the same species can result in a different outcome: for example inhaling or

intravenous administration can show a different effects.

Until present time some publication on LD₅₀ value of STC are available: Purchase et al. have investigated acute toxicity of STC in rats. A big role in the determination of LD₅₀ plays the solubility and further absorption of STC. For this experiment STC was diluted into two different solutions: dimethylformamide and wheat germ oil. According to their results LD₅₀ with dimethylformamide for male rats with oral administration was 166-mg/kg body weight and intraperitoneal 60 mg/kg body weight. Oral administration for female rats with STC diluted in wheat-germ oil resulted into 120 mg/kg body weight. Results of intraperitoneal administration with wheat-germ oil (LD₅₀=65 mg/kg body weight) in male rats were only slightly different from the one with dimethylformamide (LD₅₀=60 mg/kg body weight) [8]. LD₅₀ of STC has been also investigated in young chicken by Sreemannarayana et al. Ten-days-old chicken got intraperitoneal STC in different dosage diluted in olive oil. Two groups, consisting of 10 chickens, were treated identically. LD₅₀ in the first group resulted in 14.0 mg/kg body weight, and in the second in 10.0 mg/kg body weight [9]. These results differs from the ones reported by Salam et al., where LD₅₀ for newborn chicken in oral administration was between 3.4 and 4.0 mg/kg body weight [10]. The LD₅₀ for chicken embryos was investigated by Schoeder et al. and estimated between 5 and 7 µg/egg. STC was diluted in methanol. No difference was detected if STC was injected into the yolk or into the air cell. The no-effect level was 1 µg/egg [11]. Adult mice are much more resistant to STC than other animal models. Their LD₅₀ has been reported to be 800 mg/kg body weight. LD₅₀ determined in Vervet monkeys was 32 mg/kg body weight [1].

The reasons for such difference in LD₅₀ values could be the following: Animals of different species vary in their susceptibility. Adult mice are very resistant to the toxic effects of STC. In general younger animals are more susceptible to chemical carcinogens than old ones. The way of STC administration plays a crucial role in STC bioactivation and the further effects on the target. For example, if STC was administrated intraperitoneal, necrosis around the portal tract has been detected. If oral, severe necrosis around the central veins was observed [8]. Papillomas or squamous cell carcinomas were observed on a rat skin after STC application [12]. If the way of administration is not identical, there is no possibility to compare outcomes. The poor solubility of STC can also be a problem for accurate LD₅₀ assessment. Usage of various solvents for STC administration can result in different solubility and permeability of the cell membrane of STC, solubility in the body fluid, further absorption and toxic action.

Mutagenicity Chemicals with a mutagenic

property can induce or increase the frequency of mutation in an organism. Somatic mutations play an important role in the cancer provocation. McCann et al. tested 300 chemicals for their mutagenic properties using the *Salmonella*/microsome test. The *Salmonella*/microsome test is a simple method designed as a highly sensitive indicator of a chemical's mutagenicity. For indication of reserve mutation several special strains of bacteria are used. The incorporation of mammalian liver enzymes directly into vitro procedure allowed estimating the mutagenicity and carcinogenetic potential of a given chemical substance in mammals. In this work STC was also tested and assigned to the group of the most potent mutagens [13]. With the use of *Salmonella typhimurium* TA 98 test procedure the mutagenic properties of STC were also tested. The maximum STC activity was observed at the amount of 10-µg/plate. AFB₁'s maximum activity was observed at the amount of 0.1-µg/plate [14].

Another study has been undertaken by Sekijima et al. in 1992 to test the mutagenic potency of STC and related compounds. The Ames test in *Salmonella typhimurium* TA 98 and TA 100 and the aberration test in Chinese hamster cell line (CHL) were used for the mutagenicity assessment. The relative mutagenic activity of STC compared to AFB₁ in the Ames test was 38%. The aberration test in CHL was also positive [15]. The extensive work of Ames and his colleagues confirmed that almost all carcinogens tested have a mutagenic property [13]. STC is not an exception. Its mutagenic property was proven in all studies listed.

Conclusion. The article shows a number of experiments, in which the mutagenicity and carcinogenic potential of a given chemical substance was evaluated. According to their results, STC was assigned to the group of the most powerful mutagens.

All this indicates the need to monitor this mycotoxin in food and feed. Due to solubility and other physical and chemical properties of STC the application of existing methods for mycotoxins determination is not possible [17, 18]. Therefore, it is important to develop a sensitive method for determination of the STC within one method combined with other mycotoxins.

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Де Карлі-Ізмайлова Е.В., Ізмайлова Н.А., БІОЛОГІЧЕСКАЯ АКТИВНОСТЬ СТЕРИГМАТОЦИСТИНА

В статье представлен обзор результатов исследований биологической активности стеригматоцистина (STC). Европейская организация по безопасности пищевых продуктов (EFSA) в настоящее время работает над научным заключением о риске для здоровья животных и населения, связанным с наличием STC в пищевых продуктах и кормах. В статье представлены данные о механизме токсичности, канцерогенности, летальности и мутагенности данного микотоксина

Ключевые слова: плесень, продукты питания, корма, микотоксины, стеригматоцистин, афлотоксины, токсичность, здоровье человека и животных.

Де Карлі-Ізмайлова О.В., Ізмайлова Н.О. БІОЛОГІЧНА АКТИВНІСТЬ СТЕРІГМАТОЦИСТИНУ

У статті представлений огляд досліджень, присвячених біологічному впливу стерігматоцистину (STC). Європейська організація з безпеки харчових продуктів (EFSA) працює над науковим висновком про ризик для здоров'я тварин і населення, пов'язаний з наявністю STC в харчових продуктах і кормах. У статті представлені дані про його токсичність, канцерогенність, летальність та мутагенність.

Ключові слова: цвіль, мікотоксины, стерігматоцістін, афлотоксину, токсичність, канцерогенність, летальність та мутагенність здоров'я людини і тварин.