Chronic Toxicity and Carcinogenicity of Antibacterial Zeolite "Zeomic"to Mice and Rats by Oral Administration

(Received March 20,1995)

Yukio Takizawa [a)*)], Fujiko Hirasawa [a)],
Shiro Uesugi [b)], Junsuke Yamashita [c)],
Hiroshi Tsunoda [d)], and Masami Fujii [a)]
a) Department of Public Health,
b) Department of Laboratory Examination,
c) Radioisotope Research Center, Akita University School of Medicine
d) Niigata Prefectural Institute of Cancer Research

*Present address: National Institute for Minamata Disease
(Hama, Minamata City, Kumamoto 867)

Abstract

The antimicrobial activity of silver ions has been well known for a long time. Products in which zeolite ions are replaced with silver ions - taking advantage of the ease with which zeolite ions can be exchanged in this way - are used in simple water purifying equipment, for example. In recent years, moreover, they have also been adapted for use as plastic additives for use in the manufacture of a growing range of products including kitchen utensils, hospital pencils and telephone ear and mouthpieces - a class of goods now generally referred to as antimicrobial goods. Other applications as indirect additives for use, for example, in the manufacture of containers designed to prevent food from going off are also currently under consideration.

In light of the above, the authors decided to examine the growing use of zeolites with silver content in the manufacture of household equipment from the point of view of the possible effects of such equipment on public health. To this end, and in strict adherence to the international rules of experimentation, we carried out a series of experiments designed to evaluate the long- term toxicity and carcinogenic characteristics of this type of product.

For the purpose of our experiments, we selected silver, zinc and ammonium composite substitute type A zeolites with high silver and zinc content. The average silver content of the materials used was 2.6%and the average zinc content 14.5%. We tested the selected materials on 600 four week old B6C3F1 mice (300 males and 300 females) and on 700 four week old Fischer 344 rats (350 males and 350 females).We monitored the groups by selecting and dissecting 10 animals from each group at the end of the third, sixth and 12th

month. The rest was used as the breeding group for a period of 24 months. The doses for the experimental groups were determined in accordance with experiments on the subchronic toxicity of silver composite zeolites which were carried out separately. The mice were divided into three dose groups which were administered 0.1%,0.3%and 0.9%dose levels respectively of the test substance mixed in with their feed. The rats were divided into four experimental groups which were similarly administered doses of 0.01%, 0.03%, 0.1% and 0.3% of the test substance, respectively. The results of our experiments are summarized below.

1. In terms of factors such as survival rates and weight change, there was no observable difference between the control and experimental groups of both mice and rats and similar control group (0%) of mice and rats. There was equally no significant change observed in the general physiological condition of the rats and mice in the control and experimental group. However, although increases in body weight were found to be inhibited in the medium and high dose male mice, in all the male rat groups and in the highest dose female rat group throughout the experimental period, a simultaneous tendency towards long-term reduction of the amount of intake was observed only in the male rats during their fastest growth phases. The tendency amongst rodents that have taken in large amounts of silver and zinc over long periods to reduce intake and so to suffer growth retardation has, in fact, been previously reported [9), 10)] (see Figure 1-1, 1-2, 2-1, 3-1, 3-2, 4-1 and 4-2).

2. In clinical biochemical tests on the rats' serum, there was little evidence of changes in the rats' serum protein and albumin levels and pathological tests showed no evidence whatsoever of organic change in the form, for example, of inflammation or decidua of the intestinal membranes. Although a tendency to anemia was identified in the medium and high dose male mice and also in the highest dose female rats, there was no evidence of an increase in thrombocytes or of abnormalities in the shapes of the red blood cells. In the case of animals that were given zinc orally, the ensuing competition between copper and zinc led to a reduction in the amount of copper absorbed from the intestines and cases of abnormal metabolism of copper within the body and of hypochromic anemia were observed [12)]. In the case of rats given silver, alteration of copper metabolism in the body and reduction of the Cp oxidase activity [11)] suggested the excessive formation of bone marrow and the onset of anemia as a result of abnormal red blood cell formation [6)] (see Table 2 and 4).

3. From the standpoint of morbid histology, changing levels of serum lipids and damage to the walls of veins and blood vessels contribute to the development of heart disease [7)] and, while zinc does not bring about a change in the overall level of serum lipids, it does cause a reduction in HDL- C and an increase in LDL-C [8)]. During the course of the present series of experiments, coronary thrombuses were found to develop in animals in both control and

experimental group of both types of animal. There were, moreover, no significant differences observed between the distributions in each case. In the case of the rats, simultaneous deterioration in four serum lipid related categories was found only in the highest dose female group and there was no evidence of an increased risk of developing coronary disease in any of the groups.

During the course of the current series of experiments, discoloration was observed in all the groups administered dosages of 0.1% and upwards in the form of dark rounded granules which were detected not only in the liver, kidney, pancreas, stomach and lymph nodes but in all the internal organs and bodily systems. In the highest dose group, similar changes were clinically observed in the form of a "darkening" of the epidermis. A breakdown of the number of instances of such darkening of the organs revealed a statistically significant difference between the control and experimental groups and significant differences were also detected in the incidence of such darkening between experimental groups. Of the three intestinal absorption related constituents derived from the test substances and absorbed from the intestines, there have been no reports of darkening of the organs and/or bodily systems of animals to which they have been given in the case of silicon and zinc [13), 9)]. There have, however, been cases reported of the accumulation of dark granular silver related substances throughout the organs and bodily systems of rats and dogs given silver alone [11)]. Again, when silver and zinc were simultaneously administered directly into the abdominal cavity of rats over a period of six consecutive days, an accumulation of silver was observed in all the organs and systems of the body [14), 15)]. From this it is possible to confirm the accumulation of silver throughout most of the body. The point to note here is that no organic changes of any sort were observed even in systems which exhibited high levels of silver accumulation. A similar situation has also been reported in the case of a person who took a medicine containing a high concentration of silver over a period of 19 years and who developed substantial accumulations of silver throughout his body [16)]. From this, we may reasonably conclude that the accumulation of silver in the body as a result of taking silver orally or otherwise does not harm the bodily systems in any way, and that, as a means of reducing the toxicity of silver, one of the body's natural defense mechanisms would appear to involve the accumulation of silver in the bodily systems in an undissolved form.

4. Non-tumorous changes associated with the intake of the test substances were observed in the case of Langerhans islet swellings in the male mice, of renal cysts in the female mice and of liver and biliary duct swellings in the male rats. However, although there would appear to be a possibility that the change in the case of the Langerhans islet swellings may have been encouraged by administration of the test substances during the course of these experiments, the frequency of occurrence in the control group was found for

some reason or other to be low with the result that we were obliged to detect a statistically significant difference in the case of the experimental groups. The risk remains that there may be a hidden "skew in the statistical distribution", however, and the most sensible course would appear to be leave the case for a dosage related effect as yet unproven. Little evidence was found during the current series of experiments for the swelling or contraction of pancreatic fiber in either animal and we concluded that no connection with Langerhans islet swelling had been found. In the case of renal cysts in male and female mice, Llobet et al. observed lesions consisted in glomerular Bowman's capsule with flattened epithelial cells and proximal convoluted tubules with desquamation of tubular epithelial cells of SD rats treated with 640 mg/kg b.w./day of zinc acetate over a period of three months [17]. These changes were diagnosed as renal cysts originating in the urinary tubules. Similar problems were diagnosed in both the male and female rats administered medium and large doses of the test substance but, at between 0 and 4 cases, the number of observed instances was extremely small and no statistically significant difference was observed between the various test and control groups.

5. With regard to the accurrance of spontaneous tumors in mice and rats fed for long periods on silver composite zeolites, the frequent occurrence of cancerous renal nodules, renal cellular cancer and malignant lymphatic cysts in male and female B6C3F1 mice has been reported. Although the development of these type of tumor was also found to be more common in the present series of experiments, the frequency of occurrence remained within the natural range and we did not find a significant difference between the test and control groups mice or between mice in the different test groups. In the case of other tumors with a low rate of occurrence, we identified tumors in the same sorts of places, of the same type and with the same frequencies of occurrence as have been previously reported. No special or dramatic deviations from these patterns were observed. Our conclusion, therefore, is that there is no evidence for the development or encouragement of cancerous growths in either male or female B6C3F1 mice due to the administration of the test substances (see Table 3-1, 3-2).

Tumors reported to occur commonly in Fischer 344 rats include leukemia, mammary fibroadenomats and pituitary adenomas in both male and female rats, testicular interstitial cell tumers, adrenal brown cell tumors and foreskin adenomats in male rats, and endometrial polyps in female rats [4]. During the course of the current research, we observed a high frequency of occurrence of leukemia in both male and female rats, and of pituitary adenomas and endometrial polyps in female rats. However, although a significant difference was thus found between the test and control groups, a significant difference was not found between the current research and earlier reports insofar as the location and systematic definition of the tumors was concerned. Moreover, the frequency of

occurrence of these three types of tumor was not much greater than that indicated in earlier reports for each of the different dose levels and there was no significant difference observed between the different dose groups and between the test and control groups. It was therefore impossible to conclude that the administration of silver composite zeolites contributed to the growth of tumors (see Table 5-1, 5-1).

As for the toxic effects of silver composite zeolites, silver, zinc and silicon have been regarded as the most important constituents to be absorbed through the main intestine when such zeolites are taken orally. In tests for cancer development carried out to date using silicon dioxide and type A zeolites, the silicon constituents taken orally have been found to have no carcinogenic effects [13] and the administration of silver colloids i.m. to rats has not been found to result in the development of carcinomas [4]. By contrast, in cases of zinc deficiency, the growth of transplanted Walker 256 carcinomas, liver cancer, leukemia and Lewis lung cancer was found to be inhibited. The implication is that the presence of large quantities of zinc may well induce and subsequently assist the development of a wide range of carcinomas and there have, in fact, been reports of a tendency for doses of zinc chloride to encourage the development of mammary cancers in rats [19]. This phenomenon is thought to be connected with the essential role played by zinc in the synthesis of DNA and the division of cells [19].

As outlined above, we administered mice and rats with oral doses of silver composite zeolites for a continuous period of two years and observed some minor effects on part of the medium and high dose groups. A summary of the results of this series of experiments shows two groups of animals, namely the 0.03% and 0.01% groups of female rats to have suffered no observable effects of any sort. When the monitored data on the 60th week from the start of the 0.03%dosing program was set as the average indicator, the "no obsevable-effects level" (NOEL) was calculated as 0.011 g/kg b.w./day.

Corresponding author:
Masami Fujii, Faculty of Pharmaceutical Sciences,
Kobe- Gakuin University, 518 Arise, Ikawadani-cho, Nishi-ku Kobe 561-21
*Present address:
National Institute for Minamata Disease (Hama, Minamata City, Kumamoto 867)