# STUDIES ON ANTI-MUTAGENICITY OF ENZYMATIC HYDROLYZED ANIMAL PROTEINS (EHAPs) FROM LIVESTOCK AS REVEALED BY THE MICRONUCLEUS TEST WITH MS/Ae MICE.

KOJI NAKADE, NAOHIKO HIGASHIKUNI, MASAHIRO NUMATA, HITOMI KOMABA, and TOYOO NAKAMURA
ITOHAM Central Research Institute, 1-2 Kubogaoka, Moriya, Kitasouma, Ibaraki 302-01, Japan.

Keywords: anti-mutagenicity, enzymatic hydrolyzed animal proteins (EHAPs), micronucleus test, micronucleated reticulocytes, MS/Ae mouse

## BACKGROUND

Various substances in foods suppress the formation or action of carcinogens and mutagens. Many naturally occurring compounds of plant origin are known to inhibit chemical carcinogenic and mutagenic activities (Ramel et al., 1986).
Especially, "inactivation factors" in vegetable foods such as greens and fruits, have been shown to prevent cancer. However, there are only a few reports on the anti-carcinogenic and anti-mutagenic activity of substances from animal foods. Using chlorogenic acid (CGA), a typical plant anti-mutagen, as a reference test substance, the anti-mutagenicity of four EHAPs was tested in the mouse micronucleus test.

## OBJECTIVE

For efficient use of livestock, we investigated the anti-mutagenic activity of enzymatic hydrolyzed animal proteins (EHAPs).

## MATERIALS AND METHODS

*Animals*

The high sensitivity of the MS/Ae mouse, a mutagen-sensitive strain derived from the CD-1 strain (Aeschbacher et al., 1979) in the micronucleus test has been reported (Aechbacher, 1986; CSGMT, 1988; Hayashi et al., 1982, 1989; Sutou and Sato, 1990). Male and female MS/Ae mice at 8-12 weeks of age were used. Animals were housed 5 per cage and given free access to water and food pellets.

*Samples*

Four types EHAPs prepared from livestock and chlorogenic acid were used as follows.

1. EHAP was prepared from swine liver (ESL)
2. EHAP was prepared from swine blood (ESB)
3. EHAP was prepared from bovine meat (EBM)
4. EHAP was prepared from bovine cartilage (EBC)
5. Chlorogenic acid (CGA, polyphenol of plant origin) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan)

*Mutagens*

Two types of mutagens were used. One was 2-amino-3,8-dimethylimidazo- [ 4,5-f ] quinoxaline (MeIQx, Wako), a heterocyclic amine which is formed by baking meat. The other was cyclophosphamide (CP, Sigma Chemical Co., St. Louis, MO, U.S.A.), an alkylating agent.

*Dose levels*

All the EHAPs were dissolved in water, and mice were given doses of 0.341, 1.706, and 8.528 mg/kg body weight in terms of nitrogen content. CGA was dissolved in water, and mice were given doses of 0.8, 4.0, 20.0, and 100.0 mg/kg body weight. MeIQx was suspended in physiological saline and given at a dose of 8.0 mg/kg body weight. CP was dissolved in physiological saline and given at a dose of 20.0 mg/kg body weight.

*Test procedure*

EHAP and CGA were administered orally twice at an interval of 24 h and a mutagen (MeIQx or CP) was injected intraperitoneally 24 h after the second dose of EHAP or CGA. Peripheral blood cells were collected just before injection of the mutagen (0 h) and at 48 h after treatment with the mutagen, because most peak responses were seen 48 h after administration (CSGMT, 1992). Peripheral blood was obtained by pricking the tail ventrally with an injection needle from a tail blood vessel of a mouse without killing it. Care was taken to use a needle with a sharp tip. A blood sample (5 $\mu$ l) was placed on a glass slide previously coated with acridine orange and was covered with a coverslip. The blood samples were allowed to stand for several hours and then examined by fluorescence microscopy.
Ocular ( x 15) and objective ( x 40) lenses were used. One thousand erythrocytes with red fluorescing reticulum structures (reticulocytes) were examined per animal for the presence of micronucleated reticulocytes (MNRETs). Mice in the treatment groups were treated with EHAPs or CGA and a mutagen. The mutagen-control mice were treated with water instead of test substance and a mutagen.
The significance of the difference between groups was evaluated by the Kastenbaum and Bowman's method (1970) with significance levels of $P<0.05$ and $P<0.01$. Treatment groups were compared with the mutagen-control. Five mice were assigned to each treatment and the mutagen-control group.

## RESULTS AND DISCUSSION

Table 1 shows the effect of EHAPs against two types of mutagens (MeIQx and CP). All four EHAPs showed a significant difference from the mutagen-control. ESB and EBC were more anti-mutagenic against MeIQx than the other EHAPs, and ESL against CP than the others. There were no significant differences among dose levels. The mechanism of anti-mutagenic activity is not clear, but the present findings suggest that factors prepared from animal proteins, have anti-mutagenic activities.
Table 2 shows the effect of CGA on the clastogenic activity of CP. The micronucleus frequency was significantly lower than the mutagen-control at all four doses.
The micronucleus test is a useful tool to investigate the clastogenic potential of chemicals in vivo. The most characteristic feature of the peripheral blood micronucleus test is that there is no need to kill the test animal. This provides us with two major advantages. One is

that blood samples can be taken before administration. The other is that time-course samples, e.g., at 24, 48 and 72 h, can be taken from the same animal, which can lessen the number of animals needed for a test under a multi-sample program.

## CONCLUSION

All four EHAPs showed a significant anti-mutagenic effects against two types of mutagens (MeIQx and CP), which suggests that the EHAPs have preventive effects against cancer. Thus we speculate, that a multitude of anti-carcinogenic and anti-mutagenic factors is formed from animal foods.

## REFERENCES


Ramel, C., U.K. Alekperov, B.N. Ames, T. Kada and L.W. Wattenberg (1986) Mutat. Res., 168, 47-65.

Aeschbacher, H.U., U.D. Gottwick, H. Meier, and A.W. Poot (1979) Mutat. Res., 59, 301-304.

Aeschbacher, H.U. (1986) Mutat. Res., 164, 109-115.

CSGMT (Collaborative Study Group for the Micronucleus Test) (1988) Mutat. Res., 204, 307-316.

Hayashi, M., T. Sofuni, and M. Ishidate Jr. (1982) Mutat. Res., 105, 253-256.

Hayashi, M., S. Sutou, H. Shimada. S. SATO, Y. F. Sasaki, and A. Wakata (1989) Mutat. Res., 223, 329-344.

Sutou, S., and S. Sato (1990) Environ. Mol. Mutagen., 15, 125-130.

CSGMT (Collaborative Study Group for the Micronucleus Test) (1992) Mutat. Res., 278, 83-89.

Kastenbaum, M.A. and K.O. Bowman (1970) Mutat. Res., 9, 527-549.


Table 1
Effect of EHAPs against two types of mutagens (MeIQx and CP)

| Sample name | Nitrogen content (mg/kg) | MNRETs( ‰ )(Group mean ± SD) | | | |
|---|---|---|---|---|---|
| | | MeIQx | | CP | |
| | | Sampling time * (h) | | Sampling time * (h) | |
| | | 0 | 48 | 0 | 48 |
| ESL | 0.000 | 3.40 ± 1.14 | 7.20 ± 0.84 | 3.40 ± 1.82 | 22.00 ± 3.16 |
| | 0.341 | 2.20 ± 0.45 | 2.60 ± 2.80 ** | 2.80 ± 0.84 | 14.00 ± 1.58 ** |
| | 1.706 | 2.20 ± 1.48 | 1.60 ± 1.14 ** | 3.20 ± 1.10 | 14.20 ± 1.48 ** |
| | 8.528 | 2.60 ± 1.82 | 4.20 ± 2.95 * | 2.40 ± 1.14 | 11.60 ± 2.07 ** |
| ESB | 0.000 | 3.00 ± 1.41 | 8.40 ± 1.10 | 2.80 ± 1.48 | 18.60 ± 1.82 |
| | 0.341 | 3.20 ± 2.17 | 2.00 ± 0.71 ** | 3.00 ± 1.58 | 12.60 ± 2.70 ** |
| | 1.706 | 2.60 ± 1.82 | 4.20 ± 1.64 ** | 2.80 ± 1.92 | 13.20 ± 1.92 * |
| | 8.528 | 1.60 ± 1.52 | 3.00 ± 1.00 ** | 3.40 ± 1.52 | 16.20 ± 3.11 |
| EBM | 0.000 | 2.75 ± 1.71 | 5.75 ± 1.50 | 2.40 ± 1.34 | 19.40 ± 2.51 |
| | 0.341 | 3.40 ± 1.95 | 2.60 ± 2.07 * | 3.40 ± 1.52 | 12.40 ± 1.95 ** |
| | 1.706 | 3.60 ± 1.95 | 2.50 ± 0.58 ** | 2.60 ± 0.80 | 14.20 ± 2.95 * |
| | 8.528 | 2.20 ± 1.79 | 2.60 ± 1.14 * | 3.80 ± 2.59 | 12.80 ± 3.70 ** |
| EBC | 0.000 | 3.00 ± 1.41 | 8.40 ± 1.10 | 3.40 ± 1.82 | 22.00 ± 3.16 |
| | 0.341 | 2.60 ± 1.14 | 1.75 ± 0.96 ** | 3.00 ± 1.22 | 15.40 ± 1.67 ** |
| | 1.706 | 2.40 ± 1.82 | 2.40 ± 1.82 ** | 2.60 ± 1.95 | 16.40 ± 4.45 * |
| | 8.528 | 2.60 ± 1.52 | 1.80 ± 1.10 ** | 3.20 ± 1.10 | 14.80 ± 1.30 ** |

* Sampling time was just before injection of the mutagen (0) and 48 h after injection of the mutagen (48).
* P<0.05, ** P<0.01 compared with the mutagen-control (Kastenbaum and Bowman, 1970).

Table 2
Effect of CGA on the clastogenicity of CP

| Sample name | Dose at a time (mg/kg) | MNRETs( ‰ )(Group mean ± SD) | |
|---|---|---|---|
| | | CP | |
| | | Sampling time * (h) | |
| | | 0 | 48 |
| CGA | 0.0 | 3.60 ± 2.30 | 20.60 ± 3.20 |
| | 0.8 | 1.40 ± 1.50 | 8.00 ± 3.30 ** |
| | 4.0 | 3.40 ± 2.50 | 13.60 ± 3.80 ** |
| | 20.0 | 1.80 ± 1.60 | 7.80 ± 2.30 ** |
| | 100.0 | 3.00 ± 1.90 | 11.40 ± 7.70 ** |

* Sampling time was just before injection of mutagen (0) and 48 h after injection of mutagen (48).
** P<0.01 compared with the mutagen-control (Kastenbaum and Bowman, 1970).