Benzaldehyde as a Carcinostatic Principle in Figs

Setsuo TAKEUCHI, Mutsuyuki KOCHI,* Kenji SAKAGUCHI,** Kuniko NAKAGAWA and Takeo MIZUTANI

The Institute of Physical and Chemical Research, Wako-shi, Saitama-ken
**Ichijo-Kai Hospital, 4-26-1, Kita-kokubu, Ichikawa-shi, Chiba-ken*
***Mitsubishi-Kasei Institute of Life Sciences, Minamiooya, Machida-shi, Tokyo*

Received April 12, 1978

The use of fig fruit (*Ficus carica* L.) as a traditional carcinostatic drug is widespread all over the world.[1] One of us (M. Kochi) has long been interested in the clinical utility of fig and has observed suppression of Ehrlich carcinoma in mice by its extracts. The results of clinical treatment of malignant tumor in human with the steam distillate of fig fruit are reported.[2]

In the present study, the effective component in the steam distillate was pursued by chemical means, mainly based on its bioactivity using tumor-implanted mice.

Two kg of frozen fig fruit (harvested Kanagawa-prefecture, Japan) was homogenized with 1000 ml of water and subjected to steam distillation to obtain 600 ml of distillate. In order to determine the carcinostatic activity the distillate was daily injected (1 ml/day) intraperitoneally to each of seven BDF_1 mice which had been implanted subcutaneously with adenocarcinoma 755 (AC 755) 24 hr prior to administration with the fig sample. After 11 days from the tumor implantation, mice were sacrificed and the mean weight of the tumor was compared with those from the control group. The steam distillate of fig fruit reduce the mean weight of tumors by 39%.

The following studies showed that the active agent is benzaldehyde. The steam distillate of fig fruit condensed to 1/100 volume by repeated fractional steam distillation was the starting material for purification of active factor. Its high-speed liquid chromatogram with Shimadzu 830 instrument (Permaphase ETH 8 mm × 500 mm; H_2O, UV 254 nm, 25°C, 50 kg/cm^2) showed four main peaks. Each of the preparatively isolated fractional effluents was injected (1 ml/day) to tumor-implanted mice to determine their carcinostatic activities by the same method as above, and the fourth of the fractional effluents was found to have reduced the mean tumor weight by 36%. By gas chromatograph-mass spectrometry (GC–MS) with Shimadzu LKB 9000 system (separation column: 30% sorbitol), the component of the fourth peak showed molecular ion peak of $M^+ = 106$, and its fragmentation pattern suggested it to be benzaldehyde. Comparison with the authentic specimen with respect to GC–MS pattern, retention time in high-speed liquid chromatography, UV ($\lambda_{max}^{EtOH} = 244$, 280 nm) as well as the thin-layer chromatography and the IR spectra of the 2,4-dinitrophenylhydrazones unambiguously confirmed its identity. The concentration of benzaldehyde in the original fig distillate described as above was found to be approximately 1 ppm, based on the height of the peak in liquid chromatography. The test with purified authentic benzaldehyde for its inhibitory action on AC 755 in mice by the same method as above showed that a dose of 100 mg/kg/day inhibited the growth of tumor with 40% mean reduction in the tumor weight. Also, as shown in the Table I, a dose of 10 mg/kg/day inhibited the growth of Ehrlich carcinoma (solid type) in Swiss mice by 56%.

We investigated also effects of the fig distillate on the permeability of bacterial cell membrane toward amino acids was conducted. It was interesting to note that only the fourth peak of the fractional effluent was found to increase the ^{14}C-tryptophan uptake by a tryptophan-requiring mutant strain of *Escherichia coli* ($K_{12}B_4$) by 20% at ten-fold dilution and, by 29%, at 100-fold dilution.[3,4] It was also clear that 10^{-1} and 10^{-2} ppm of benzaldehyde had pronounced activity, stimulating the uptake by 36% and 27% respectively (see Fig. 1), while

TABLE I. CARCINOSTATIC EFFECT OF BENZALDEHYDE ON EHRLICH CARCINOMA (solid)

Intraperitoneal treatment with benzaldehyde was conducted at a dose of 10 mg/kg (0.2 ml of 1000 ppm emulsion per mouse) to tumor implanted Swiss mice once a day for 9 days, starting after tumor implantation. Tumor were weighed 11 days after tumor implantation.

Group	$\Delta\bar{x}$ body weight (g)	Tumor weight (g)	Mean weight (g)	T/C (%)
Control	+3.5	9.0, 4.2, 3.4, 2.6, 2.5, 2.4, 1.2, 1.1, 1.0, 0.5	2.79	100
Treated	+3.5	3.8, 2.4, 2.2, 0.8, 0.8, 0.6, 0.6, 0.5, 0.4, 0.3	1.24	44.4

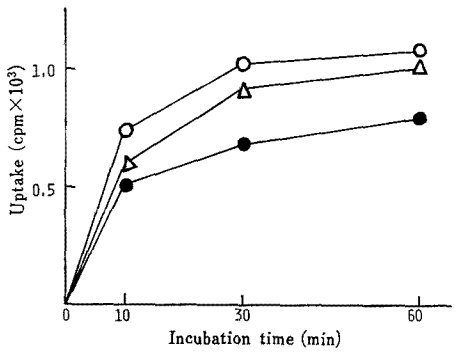

FIG. 1. Influence of Benzaldehyde on ^{14}C-Tryptophan Uptake into *E. coli* ($K_{12}B_4$) Cells.

The cells of the precultured broth of *E. coli* (OD_{660}= 0.3) in 5 ml of Vogel-Bonner's minimal medium containing 20 μg/ml of L-tryptophan at 37°C for 2~3 hr was harvested onto a membrane filter. The collected cells were washed with the same medium but not containing tryptophan was incubated at 37°C with shaking. After addition of 0.3 μg/ml of chloramphenicol, 0.1~0.2 μCi of ^{14}C-tryptophan (4.9×10^{-8} mole), and appropriate amount of benzaldehyde. Radioactivity of the cells collected and washed after incubation was recorded with scintillation counter. ●, control run without benzaldehyde; ○, 10^{-1} ppm benzaldehyde; △, 10^{-2} ppm benzaldehyde.

inhibiting the uptake of glutamic acid and alanine. It seems that this compound acts specifically on individual transport systems for these amino acids. Not only in the presence of benzaldehyde, pretreatment of the cells with benzaldehyde solution followed by washing was equally effective. It has been shown[6] that the separated membrane of the cells incorporated benzaldehyde by pretreatment with 10^{-1} ppm of ^{14}C-benzaldehyde. Results of biochemical studies in this line will be reported elsewhere. The results of determination of the bioactivity of benzaldehyde shown above were all obtained at the respective optimum concentration found by trials. For example, it is characteristic of 1 ppm of benzaldehyde had no stimulating effect on the ^{14}C-tryptophan uptake into *E. coli* cells in the same experiment as above. In order to relate this phenomenon with the carcinostatic activity and to unravel its mode of action, it is essencial to make the experiments with eukaryotic cell membrane, therefore, such studies are now in progress.

Benzaldehyde is an easily oxidizable substance with a pungent odor and strong local irritative property. These problems may be overcome by using benzaldehyde in a form of inclusion compound with β-cyclodextrin (α-1,4-cycloheptaglucan).[6] Pure benzaldehyde was added dropwise to a saturated solution of an equimolar amount of β-cyclodextrin at 45°C, with constant stirring. The mixture cooled to 15°C was filtered to yield white powder having a faint odor of benzaldehyde. Based on ultraviolet absorbance data at 280 nm of its eluate with methanol, this inclusion compound was proved to contain 85 mg of benzaldehyde per gram, which is close to the theoretical value (85.4 mg/g). The above inclusion drug was found to be effective in the test to prevent 4-nitroquinoline-1-oxide (NQO)-induction of papilloma in ICR mice by oral administration at a dose equivalent to 1.6 mg/kg per day of benzaldehyde for 4 months. Incidence of the NQO-induced papilloma was 0% (0/25) in the benzaldehyde-treated mice and 26% (6/25) in the non-treated control mice. This inclusion compound was also clinically tried in a form of enteric-coated tablets. Results of these clinical investigations have been

presented in detail elsewhere.[2] In some cases on cancer patients including those with adenocarcinoma, squamous cell carcinoma *etc.*, oral administration with 30~100 mg/man/day equivalent to benzaldehyde was found to be marked effective in surviving with tumor regression with no adverse reactions. This substance proved more markedly effective on human malignant tumors than on experimental tumors in mice, the reason of which may be attributed to the difference between transplanted tumors and spontaneous ones, or to the physiological difference between mice and man.

The mechanism of action of benzaldehyde on tumors still await clarification. In this connection to be noted is the clinical use of citral and citronellal on the cancer treatment by Osato.[7,8] It was claimed that citral was especially effective on stomach adenocarcinoma. Another instance it is interesting to recall that amygdaline, a carcinostatic drug proposing recently a controversial topic, releases one mole of benzaldehyde by hydrolysis by intestinal microbes, beside hydrogen cyanide to which its antitumor action was also pointed out.[9,10,11] These facts may or may not be related to the present observations with benzaldehyde, but present future problems of general physiological roles of the aldehydes.

Acknowledgement. We are grateful to Drs. A. Kawarada, S. Suzuki, T. Ando, K. Tsuji and T. Mitsuoka at the Institute of Physical and Chemical Research, to Prof. H. Yonehara at the University of Tokyo, and to Dr. S. Esumi and Kaken Chemical Co., Ltd., for their encouragement and advices. We thank also the Shimadzu-Seisakusho Ltd., Mitsubishi-Kasei Co., Ltd., and Sankyo Co., Ltd., for their aid in the present study.

REFERENCES

1) J. L. Hartwell, *Lloydia*, **33**, 184~188 (1970).
2) M. Kochi, S. Takeuchi, T. Mizutani, K. Mochizuki and Y. Matsumoto, presentation of proffered paper to *12th International Cancer Congress*, Buenos Aires, 5–11-October-1978 has been accepted.
3) Y. Anraku, *J. Biol. Chem.*, **242**, 793~800 (1967).
4) K. Sakaguchi and K. Ishikawa, *Annu. Rep. Mitsubishi-Kasei Institute of Life Sciences*, **1**, 14 (1971~1972).
5) T. Miura and S. Mizushima, *Biochim. Biophys. Acta*, **193**, 268 (1969).
6) D. French, *Adv. Carbohydr. Chem.*, **12**, 189~260 (1957).
7) L. C. Strong, a) *Am. J. Cancer*, **32**, 227~239 (1938), b) *Science*, **87**, 144~145 (1938), c) *Am. J. Cancer*, **35**, 401~407 (1939).
8) S. Osato, *Tohoku J. Exp. Med.*, **86**, 102~147, (1965).
9) D. Burk, A. R. L. McNaughton and M. Von Ardenne, *Panminerva Med.*, **13**, 520~522 (1971).
10) B. J. Culliton, *Science*, **182**, 1000~1003 (1973).
11) N. Wade, *ibid.*, **198**, 1231~1234 (1977).