Experiments on the Chemotherapeutic Treatment of Carcinoma with Citral and Citronellal and those Combined with PCMB

By
Shungo Osato, Hajime Mori and Michio Morita

The Central Laboratory of Fukushima Medical College
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At the last annual meeting (December 18, 1960) of the Japan Cancer Society, we reported on changes of fine structure of tumor cells suffered by the application of several cancer chemotherapeutic remedies. We demonstrated many electron micrographs concerning animal experiments on this problem. There is a remarkable contrast seen between Citral and PCMB (p-chloro-mercuri-benzoic acid.) The former causes changes of nuclear elements (nuclear membrane, chromatin granules and nucleolus) as well as cytoplasm (ergastoplasmic reticulum and ground substance), while the mitochondria are relatively well reserved, although not free from change. On the contrary PCMB causes a degeneration of mitochondria in a high degree, but not much of the other elements. Accordingly we suggested combined use of Citral and Citronellal with PCMB in cancer chemotherapy.

In this investigation we used C3H/dd. F₁ mice. We have experienced these two years that C3H MH134 (adenocarcinoma) is quite sensitive to aldehyde. We are indebted to Dr. H. Sato, formerly professor of Pathology of Fukushima Medical College and now professor of the Research Institute for Tuberculosis and Leprosy of Tohoku University of Sendai, for his kind advice and providing of the strain of mice.

EXPERIMENT I

On December 11, 1959 a lot of mice C3H-F₁ of about 20 g were inoculated into the tail subcutaneously with 0.05 cc. of diluted tumor ascites of MH134 (cells were not counted). The animals were divided in three groups.
Group I. 9 mice were given 1/52 cc, 10% d-Citronellal in olive oil per os every day.
Group II. 9 mice were given dl-Citronellal in the same way as Group I.
Group III. 10 mice served as control.
The administration of the remedies was begun on the same day as inoculation. On Dec. 23, 1959, the 11th day of inoculation, the tails of animals were cut off at the root. The animals continued to live, many of them getting metastatic tumor in the retroperitoneal lymph nodes.

Fig. 1 shows survival duration of these animals. Among 9 animals of Group I, 2 died within 50 days, 3 died after some 60 days. One died about 175 days after inoculation. And 2 survived 190 days, when they were killed.

Fig. 1. Life's span of each animal of the 3 groups in the Experiment I.

Of the Group II, 2 died some 30 days after inoculation. 3 died between 50 and 70 days. And 4 survived 190 days after inoculation.

Among 10 control animals (Group III) 9 died within 60 days after inoculation and one survived 190 days and then killed.

EXPERIMENT II

A lot of mice were inoculated with diluted ascites of MH134, cells were not counted, on March 5, 1960. Anticancer remedies were administered on and after the same day. They were divided in 5 groups.

Group I. 8 animals received 1/52 cc. of 10% d-Citronellal in olive oil per os every day.

Group II. 8 animals received the same dose of dl-Citronellal per os every day.

Group III. 8 animals received Chromomycin 0.2γ intraperitoneally every other day.

Group IV. 8 animals received dl-Citronellal and Chromomycin as above.
Fig. 2. Life's span of each animal of the 5 groups in the Experiment II.

Table I. Macroscopic Aspects (Photos) of the Tail of Each Animal of the 4 Groups Cut Off on the 19th Day after Inoculation of Tumor in the Experiment II.

Citronellal administration to MH 134. (Experiment II)

Comparative figures of the tumor growth in the tails which were cut in 18 days after inoculation of tumor cells.
Table II. Autopsy Findings of Each Animal in the Experiment II, When Died or Sacrificed.

Experiment 2
Citronellal A

Group I

Experiment 2
Citronellal B

Group II

Experiment 2
Chromocychsia

Group III
Group V. 8 animals. Control.

On the 18th day of inoculation the tails were cut off at the root.

Fig. 2 shows durations of life in all of the animals. In Table I, macroscopic aspects of the tails are given. As we see in it, animals of treated groups had in general less tumefaction of the tails.

There were some surviving animals among each treated groups, when they were sacrificed in Sept. 11, 1960, 190 days after inoculation. Group I (d-Citronellal) 5; Group II (dl-Citronellal) 4; Group III (Chromomycin) 3; Group IV (Chromomycin + dl-Citronellal) 4; Group V (Control) 0.

Table II shows autopsy findings of all groups. Animals free from tumor; Group I (d-Citronellal) 5; Group II (dl-Citronellal) 4; Group III (Chromomycin) 3; Group IV (dl-Citronellal) + Chromomycin 4; Group V (Control) 0.

From the Experiment II, consulting the Experiment I, we would be able to conclude a remarkable sensitivity of MH134 to Citronellal. It seems that there is little difference between d-Citronellal and dl-Citronellal in their efficacy on MH134.
tumor. As we described in the previous report, Chromomycin causes somewhat more obvious changes in mitochondria than Citronellal, while the latter is effective on other elements (nuclear membrane, nucleolus, chromatin substance; ergastoplasmic reticulum ground substance of cytoplasm). So we made combined use of Citronellal and Chromomycin with great expectation. Chromomycin itself was really effective but less than Citronellal. Dl-Citronellal+Chromomycin seems to be not more strongly active than dl-Citronellal alone.

EXPERIMENT III

On Dec. 7, 1960 a lot of mice of C3H/dd F1 of about 23 g were inoculated and divided into 6 groups.

Group I, 11 mice, d-Citronellal; Group II, 11 mice, dl-Citronellal; Group III, 10 mice, dl-Citronellal+PCMB; Group IV, 10 mice, Citral+PCMB; Group V, 10 mice PCMB; Group VI, 10 mice, Control. Administration of remedies begun on the 3rd day of inoculation. Citronellal 1/52 cc. of 10% dilution in olive oil. 0.05 cc of 5% emulsion of Citral was injected into the muscle. Citronellal and Citral were given every day. PCMB was injected into the muscle on the 5th day and 7th day. It was used as $10^{-3}$ mol. in n/10 NaOH made pH7.3 by n-HCl. The dose was 0.2 ml at one time.

For the inoculation of tumor MH134, ascites fluid of the 5th day of the inoculation of tumor into peritoneal cavity, diluted 1:4 with sodium citricum, was used, and injected in the tail at 1/3 site from the root. On the 8th day the

Fig. 3. Life's span of each animal of the 6 groups in the Experiment III.
tails were simultaneously cut off at the root.

Fig. 3 shows the life's span allotted for the animals of each group. Table III shows the macroscopic photos of the tails. All the animals had more or less tumefaction of the tails at the injection site. Some difference between the groups could be detected if carefully examined, but we did not go further in it.

At the end of March 1961, 114 days after inoculation, the surviving animals were sacrificed.

Group I (d-Citronellal) 4; Group II (dl-Citronellal) 4; Group III (dl-Citronellal +PCMB) 6; Group IV (Citral + PCMB) 5; Group V (PCMB) 3; Group VI
Table IV. Autopsy Findings of the Animals at Their Death or When Sacrificed.

Group I

Group II

Group III
Chemotherapeutic Treatment of Carcinoma
Table IV shows autopsy findings of all animals of each group: In Group I (d-Citronellal), 4 are free from tumor; in Group II (dl-Citronellal), 4 are free from tumor; in Group III (PCMB+dl-Citronellal), 6 free from tumor; in Group IV (PCMB+Citral): 5 free from tumor; in Group V (PCMB), 4 free from tumor; and in Group VI (Control), 2 free from tumor.

It is to be noticed in this place, that there were some mice, which had white blotch on the tail. In the Group VI (control group) there were 3 with white blotch or spot among 11. Two of them survived the experiment. Group II: 2, Group III: 1, and Group IV: 1, these had such tails respectively. They survived for the most part. It is very probable that such a mouse as with white blotch on the tail may have inherited some character of the white mouse, and that those mice with white blotches on the tails might have somewhat greater resistance against MH134 tumor.

Notwithstanding the circumstance, judging from above data it would be quite evident that PCMB is a good co-operator of Citronellal and may be also of Citral. For the time being we do not know the mechanism of the co-operation of PCMB to Citronellal. The results of our electron-micrographic investigation of tumor cells under anticancer agents might be considered as an explanation of the mechanism.

DISCUSSION

It is tendency in chemotherapeutic researches into bacterial infection to select remedies of wide spectrum. The same trend is in the field of cancer chemotherapy. For this purpose screening tests are done with earnestness. Of course it would be very desirable, if we could get a panacea in the chemotherapy of tumors. Generally speaking an infectious disease is caused by a foreign body, (bacteria, protozoa, or virus) invading from outside. Cancer is quite different from infectious diseases. Some tumors are, it is known, proved to be viral. But carcinoma is believed to have been derived from a soma cell: so called soma mutation. From this point of view, a carcinoma cell stands in very near relation to the soma cell.

A remedy against carcinoma cells might be very likely to be more or less inimical to soma cells. Makino advocate astem-line-cell theory of cancer as a result of his researches into the karyotypes of the tumor cell. He is of opinion that each human carcinoma may be different from others in origin. Here is much difficulty in getting a cancer chemotherapeuticum of wide spectrum without injuring soma cells, especially bone marrow. Osato, one of us, concluded from his chemotherapeutic experiences of 15 years in human carcinoma, that Citronellal and Citral are most effective in adenocarcinoma. His clinical experience taught us that Citronellal and Citral were not good for the
treatment of sarcoma, leukemia or Hodgkin’s disease. Citronellal and Citral are quite efficacious against types of carcinoma other than adenocarcinoma. But Osato has never seen consecutive healing in squirrus and mucinous carcinoma owing to Citronellal and Citral. Fortunately enough a patient stands long administration of these remedies and does not show leukopenia. as seen by other anticancer agents. From all these, it would be quite natural, if we call Citral and Citronellal anticancer remedies of narrow spectrum. Combined use of Citronellal and Citral with other anticancer agents would be preferable, if amore conspicuous effect is desired. Our previous paper as well as the present have been started from this stand point. Animal experiments with combined use of Aldehydes and PCMB seem to be quite promising. As a next step, it must be verified in the clinical use, the result shall follow the present paper.

SUMMARY

1. In the present paper, animal experiments of the chemotherapy with Citronellal in MH134 (adenocarcinoma) of C3H F1 strain are reported.
2. About 30 to 50% of tumor animals were cured when the chemotherapy with Citronellal was started on the same day as inoculation of tumor.
3. When the administration of Citronellal was begun on the third day of tumor inoculation, the result of chemotherapy was lower, yet the effect was quite obvious. In this case the efficacy was very much promoted with the combined use of PCMB.
4. Our expectation in the co-operation of PCMB with Citronellal and Citral, based upon the electron microscopic study of tumor cells mentioned in the previous paper, is verified in this report.

References