Co-carcinogenic Effect of DDT and PCB Feedings on Methylcholanthrene-induced Chemical Carcinogenesis

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Among the many parameters for the toxicological evaluation of food or environmental contaminants, carcinogenic effects have attracted the attention of many researchers. Many kinds of chemical carcinogens have been recognized and isolated so far from our environment.

Besides the carcinogenic activity of such chemical carcinogen, the protective power of the host against a tumor may be one of the important factors to influence the actual generation and the prognosis of cancer. If a certain contaminant which has a tendency to accumulate in human body suppresses the protective power and consequently promotes the growth of tumor, special attention must be paid to such a contaminant even if it does not have a carcinogenesity itself. However, the conventional procedure to determine the tumor-promotive effect of environmental contaminants is not established satisfactorily yet.

In the present paper we deal with a reproducible, reliable and relatively easy experimental procedure for the confirmation of co-carcinogenic activity, i.e. experimental cervical epithelium changes towards cancer were produced by means of 20-methylcholanthrene(MC)-impregnated thread inserted into the uterus of female mice which were divided into groups and exposed to various levels of contaminants. The magnitude of the progress of epithelium changes was compared among each group. Utilizing this procedure the tumor-promotive effect of DDT and polychlorobiphenyls(PCB) was assayed.

METHODS

Adult virgin female mice of dd-strain weighing 22-25 g were used. The control group was fed with standard laboratory chow, and the experimental groups were fed with the diet containing DDT or PCB(KC-400, equal to Arochlor 1248) in the concentration indicated in the text.

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The application of chemical carcinogen was carried out according to the method of NODA and TOKUNAGA (1972) which was originally devised by MURPHY (1953) and modified by IIJIMA et al.(1964).

A cotton thread of about 15 cm was washed with water, dried completely and impregnated with the mixture of MC and beeswax(1:3). A knot was made in one end of each thread prior to the impregnation. The uterus was exposed under ether anesthesia and the impregnated thread was inserted into the right bifurcated cervical canal from the vagina by the aid of a special needle. The terminal of the thread was pulled out through the wall of a lower uterine horn until the knotted end of the thread reached the cervix. Another knot was made where it passed through the lower uterine horn to fix the thread. The schematic drawing of thread application was shown in Fig. 1.

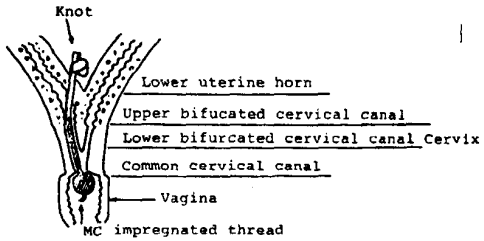


Fig. 1. Application of Methylcholanthrene-impregnated Thread to Mouse Uterine Cervix

RESULTS AND DISCUSSION

Observation of the Changes in the Squamous Epithelium

After operation the animals were all kept breeding under normal conditions and were fed with each experimental diet as before the operation.

At the end of 4 weeks after the operation the thread was pulled out from the uterus and each animal was kept for an additional 3 weeks under the same conditions with

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the exception of the kind of diet. Then, the histological changes in the squamous epithelium were examined in the sections of uteri obtained from each group. The degree of changes was classified in five grades according to the procedure of NODA and TOKUNAGA (1972): Normal, basal cell hyperplasia, atypical dysplasia, carcinoma in situ and invasive carcinoma.

Animals fed with normal diet were sacrificed at the end of each week after the application of MC-thread. The result shown in Fig. 2 indicates the standard progress of cancerous change of cervical squamous area. The duration of MC-thread insertion and subsequent standing period employed in this experiment are identical with the experiment of NODA and TOKUNAGA (1972), in which they tested the relation of the paracervical nerve plexus to the progress of cervical carcinoma. They selected the application period of 4 weeks because no carcinoma was found at the end of 4 weeks, but during the standing period of 3 weeks significant changes were produced depending on the condition of the host. Therefore, this might be the suitable period for judging the progress of precancerous changes caused by other factors. Morsover, it has been confirmed that in mice the spontaneous carcinogenesis occurs in the part of uterine horn, but scarcely occured in cervical tissue. And cervical carcinogenesis in mice induced by chemical carcinogen such as methylcholanthrene has been suggested to be almost identical with human uterine cancer concerning the process of tumor generation and morphological observations.

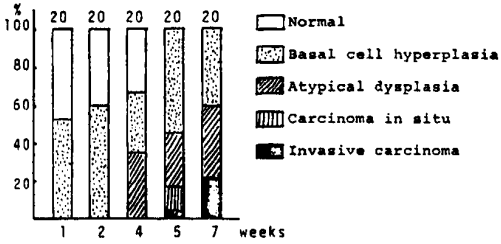


Fig. 2. The Duration of MC-thread Application and the Changes in Cervical Epithelium

The numbers on each bar indicate the number of animals employed.

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The Influence of DDT- and PCB-feeding on MC-induced Carcinogenesis

The carcinogenic changes appearing in cervical epithelium at the end of 7 weeks were summarized in Figs. 3 and 4. Although the number of animals employed in each group was not necessarily enough to reach a definite conclusion, the feeding of 10 or 100 ppm of PCB did not show any significant change in comparison with the control. However, the feeding of 100 ppm of DDT for 8 weeks, it means the feeding from 1 week prior to operation, showed a remarkable tendency towards the induction of carcinoma. By feeding 10 ppm of DDT for 15 weeks adverse condition were induced, but the result was not significant.

The amount of DDT and PCB accumulated in every tissue of treated mice were not determined. But the level of these chlorinated hydrocarbon in the tissue is easily estimated from the experience that we have obtained so far by the feeding of them. As one of the evidences, the elevation of drug metabolizing activity of liver microsomes was expected by the effective accumulation of DDT or PCB. So, liver microsomes were prepared from mice in each group and drug metabolizing activities were

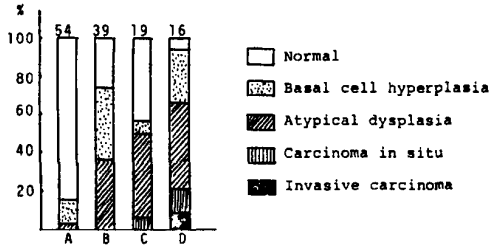


Fig.3. Effect of DDT-feeding on the Cervical Epithelial Change Induced by Methylcholanthrene

A: Sham-operated. B: Fed control diet.
C: DDT(10 ppm) was fed for 15 weeks (from 8 weeks prior ro operation).
D: DDT(100 ppm) was fed for 8 weeks (from 1 week prior to operation).

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analyzed by employing aminopyrine and aniline as substrates. As shown in Table I, the activity was stimulated by the feeding of both DDT and PCB.

TABLE I

Activity of Drug Metabolizing Enzyme of Liver Microsomes Prepared from Mice Employed in the Assay

Group of mice	Aminopyrine N-demethylation	Aniline hydroxylation
Control	100	100
DDT 100 ppm	167.3	241.7
PCB 100 ppm	160.3	178.4

Activity was expressed as % of control group which was operated as same as experimental group and fed with normal diet.

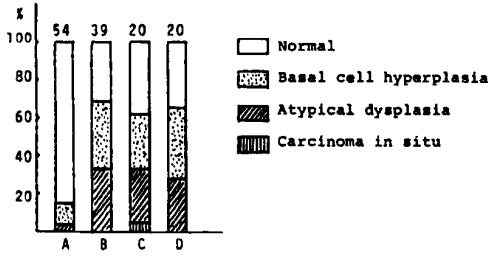


Fig. 4. Effect of PCB (KC-400) on the Cervical Epithelial Change Induced by Methylcholanthrene

A: Sham-operated B: Fed control diet
C: PCB(10 ppm) was fed for 15 weeks.
D: PCB(100 ppm) was fed for 8 weeks.

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From the above results, we would like to conclude that PCB is non-effective up to 100 ppm; however DDT shows co-carcinogenic activity by the feeding of relatively low level. The level of DDT-feeding in this experiment was maximum 100 ppm, which looks like very high compared with the residue level of DDT in food. However, as a result of the feeding of 100 ppm, mice have around 600-700 ppm accumulation of DDT in their adipose tissue and such accumulation is only 30-40 times of the actual residue of DDT in our body fat.

For the present we must rely upon relatively crude studies in experimental animals from which we extrapolate conclusions regarding people (FRIEDMAN 1971). In general, it has been suggested that no essential difference in carcinogenesis between mice and human was detected, although there are some cellular and structural divergences between human and mouse uterus (TAKI 1967).

Thus, the assay method presented in this report might provide reliable information on the co-carcinogenesity of various contaminants.

The mechanism of action of DDT is obscure, but the summation effect of DDT and MC does not seem to be applicable for this case because the experimental period is quite short. The immunosuppressive effect of DDT (WASSERMAN et al. 1969) and PCB (VOS and DEROIJ 1972) may be the most probable cause, but it could not explain the discrepancy between DDT and PCB. The precise investigation is now under way.

SUMMARY

Conventional method for assaying the promoting activity of food contaminants on chemical carcinogenesis was presented. Experimental cervical epithelium changes towards cancer were produced by means of 20-methylcholanthrene-impregnated thread inserted into the uterus of female mice which were fed with the diet containing various levels of contaminants. The magnitude of the progress of epithelium changes was compared with animals fed control diet.

Utilizing this procedure it was made obvious that feeding of 100 ppm of DDT for 8 weeks produced a remarkable tendency towards the induction of carcinoma, while 10 ppm of DDT and 10 and 100 ppm of PCB were not effectiv

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