The Combined Carcinogenic Effect of Cigarette Smoke Condensate and Urethan

JOSEPH A. DI PAOLO AND PAUL R. SHEEHE

(Roswell Park Memorial Institute, Buffalo, New York)

SUMMARY

The role of tobacco tar in the development of lung adenomas was investigated in A mice of both sexes given a single intraperitoneal threshold or subthreshold dose of ethyl carbamate. Incidence of lung tumors indicated a highly significant increase in response to urethan from subthreshold to D50. When additional groups of mice were given urethan and the oral area was painted 5 times weekly for 6 months with a brush full of cigarette-tar solution, the adenoma incidence in groups of mice receiving urethan plus tobacco condensate was greater for each urethan dose than for the corresponding group receiving urethan alone. The difference in tumor incidence between the tar-added and urethan-alone groups is highly significant (P < 0.0001). All tumors were alveolar. Furthermore, the addition of tar produced significantly greater than additive effects in terms of incidence. The addition of tar produced effects as though the urethan dose had been increased by a factor of 8.

The effect of two carcinogenic hydrocarbons applied to the same skin area during successive periods indicates that the carcinogenic process initiated by one hydrocarbon can be completed by a second one (10, 11). The resulting number of skin tumors is considered an additive effect of the hydrocarbons and equivalent to their known carcinogenic potencies on mouse skin. Gellhorn (9) has demonstrated that, when a threshold dose of benzpyrene is followed by tobacco applications to mouse skin, a larger number of papillomas and carcinomas occurs than could be explained by the additional effect of any benzpyrene which might be present in the tobacco tar used in the experiments.

The use of two dissimilar carcinogens has resulted in both synergism and no increase in carcinogenicity. Rous and associates (14) found that intravenous injection of Shope papilloma virus to rabbits with benign ear warts induced by tar application resulted in large and invasive tumors. Neither agent by itself induced malignant tumors so quickly. Ahlstrom and Andrews (1) also found that the application of two different carcinogens to different areas of a rabbit resulted in an intensified response to fibroma virus if coal tar were injected intramuscularly. Lesions due to the combination did not regress as rapidly, and some of the fibromas grew to resemble malignant neoplasms. Until recently combinations of physical and chemical carcinogens have been unsuccessful in potentiating carcinogenesis (8, 13, 15); however, several reports on synergism and antagonism of radiations and chemicals have become available (2, 3, 12, 16).

Experimental lung tumors are usually pulmonary adenomas which result from the administration of a carcinogen at some remote site by painting on the skin, by injecting it intravascularly, intraperitoneally, or intratracheally, or sometimes by including it in the diet. The purpose of the present experiment is to determine the effects of cigarette-smoke condensate ("tar") and relatively small amounts of ethyl urethan on the induction of pulmonary adenomas.

MATERIALS AND METHODS

The albino A mice used in these experiments are derived from a colony of Strong A mice first obtained from Dr. L. C. Strong in 1958. They develop a high number of spontaneous adenomas, and their responsiveness to urethan has been established (5). Five groups of A mice were given a single intraperitoneal dose of urethan at doses of 0, 0.0005, 0.002, 0.008, or 0.05 mg/gm body weight. Four other groups received oral applications of tobacco tar 5 times weekly for 6 months in addition to the treatment with urethan.
to a single urethan dose of 0, 0.0005, 0.002, or 0.008 mg/gm body weight. The urethan was dissolved in distilled water, and solutions were made so that each animal received 0.01 ml solution/gm body weight. Lips and oral area of mice were painted with ¼ camel-hair brush. The daily dose received by each animal was in the range of 0.02–0.06 gm. tar. This was determined by finding the difference between weight of brushes before and after painting.

Animals serving as controls were given injections intraperitoneally with water, maintained for 6 months, then examined for incidence of lung tumors. Mice of both sexes were included in every group, which consisted of males only. Animals were 4–6 weeks old at the beginning of the experiment. They were kept in small metal cages housing four to six mice each and fed Rockland chow and water ad libitum.

The tar was obtained from a manifold-type smoking machine with the negative pressure source from a rotary vacuum pump controlled by an automatic valve. Cigarette smoke from a variety of popular brands was collected at low temperature (—20° C.), washed from collection flasks weekly with the smallest convenient volume of acetone, condensed under reduced pressure, and redissolved in 15 ml. acetone per carton of cigarettes. Tobacco tar, when not in use, was refrigerated and replaced every 10–14 days.

Group sizes were based on statistical and practical principles. Relatively large numbers of mice were assigned to the zero dose and tar-only groups because these groups were used as common controls for the entire experiment. The urethan-only groups were assigned relatively more mice than the corresponding tar-plus-urethan groups because of savings in treatment time. Thus larger numbers of mice were treated than would have been the case if the mice had been equally distributed.

All mice were autopsied 6 months after initiation of treatment. Two mice in the group which received tar plus 0.0005 mg/gm urethan died before 6 months and are excluded from the results. The lungs of all mice were given injections intra-tracheally of approximately 1 ml. of Fekete’s modification of Tellyesnicky’s fixative before the chest cavity was opened. Lungs were then excised, and the tumors in each lobe counted in saline under 12X magnification. Questionable masses, as well as tumor nodules selected at random, were sectioned, stained with hematoxylin and eosin, and the diagnosis was confirmed histologically.

RESULTS

The incidence of lung adenomas in Strain A mice 6 months after the intraperitoneal administration of urethan and the oral painting of tobacco tar is presented in Table 1. Analysis of lung-tumor effects revealed no significant variation in incidence among similarly treated cages. Comparison of response of similarly treated males and females showed no sex differences.

The observed incidence of spontaneous lung adenomas was 8.6 per cent in the no-urethan–no-tobacco group. The tar-only group had an incidence of 20 per cent lung adenomas which was not significantly greater than that in the distilled-water control group. Studies on a considerably greater number of mice would be needed to establish the significance of the tar-only group.

However, the average difference in incidence of adenomas between all the tar-added and urethan-only groups was significant (P < 0.0001). In the urethan-only groups, as the urethan dose was increased from 0.0005 mg/gm to 0.05 mg/gm, the lung-tumor incidence increased significantly (P < 0.001).

The primary purpose of this experiment was to test whether the addition of tobacco tar after a low dose of urethan produces greater lung-tumor effects than does tar alone. The effect of tar is defined here as the difference between the incidence for the tar-plus-urethan group and the corresponding urethan group, at a given dose level of urethan. In the case of tar-only treatment, the effect of tar is defined similarly as the difference between the tar-only and no-urethan groups. The synergistic effect of tar treatment when applied after initial threshold or subthreshold dose of urethan is demonstrated in Table 1. The observed effects of tar when applied after urethan are considerably greater than the effect of tar when no urethan has been given. The differences in per cent...
obtained by subtracting urethan-only incidences from the urethan-plus-tar ones are 11.4 with no urethan; 28.9, 32.5, and 32.5 with the 0.0005, 0.002, and 0.008 mg urethan/gm body weight, respectively. The average effect of tar at the three threshold doses is significantly (P < 0.05) greater than the effect of tar at the no-dose level of urethan. The effect of tar also appears to increase when applied after increased doses of urethan, at the levels tested, although this increase is not statistically significant.

The dose-response relationship is also reflected in the average number of tumors per mouse. For the untreated and tar-only groups this average was 0.086 and 0.240, respectively. The average number increases slightly with 0.0005 and 0.002 mg urethan/gm body weight (0.100 and 0.125, respectively) then rises more rapidly with the 0.008 mg/gm dose (0.325). When groups of mice were treated with tar in addition to the urethan doses above, the average number of tumors per mouse was 0.445, 0.500, and 0.750. The differences between urethan-and-tar groups and urethan-only groups demonstrate the greater-than-additive effect of tobacco. The additional treatment with tar increased the number of tumors per mouse. The 0.05 mg/gm dose of urethan produced lung tumors in 45 per cent of the animals, with an average number per mouse of 0.50. This showed excellent agreement with the predicted response.

**DISCUSSION**

The effect of combined treatment with urethan and tobacco-tar condensate is greater than would be expected on the basis of the summation of sequential carcinogenic effects. The explanation of this phenomenon is complicated, because both compounds are capable of producing tumors when used alone. The carcinogenicity of this tobacco tar has been proved by Bock and Moore (4) in experiments in which skin tumors developed in 27 per cent of the animals after repeated application of tar. DiPaolo and Moore (7) demonstrated that daily administration of the tar to the oral region of mice produced skin papillomas as well as a significant increase in the number of pulmonary adenomas compared with acetone control animals. The specificity of urethan for lung tissue is a well established fact. Numerous investigators have recently demonstrated that urethan is no longer a unique carcinogen but must be considered a multipotential carcinogen (6, 17).

In terms of either incidence or average tumors per mouse, application of tar after low doses of urethan produces the same effects as do much higher doses of urethan only. For example, tar following the 0.0005-mg/gm and 0.002-mg/gm doses of urethan produces about the same effects as does the dose of 0.05 mg/gm urethan alone. The 0.05 dose is 100 times the lowest dose and 25 times the next highest dose of urethan. Thus the addition of tar produces tumors as if the dose of urethan were increased by a factor of 100:1 and 25:1, respectively. The average potentiating factor is estimated to be 50-fold and is statistically significant (P < 0.05). This potentiating factor produces the greater than additive effects seen at low doses of urethan.

**ACKNOWLEDGMENT**

The authors are indebted to Dr. Fred G. Bock for supplying the cigarette-smoke condensate.

**REFERENCES**

The Combined Carcinogenic Effect of Cigarette Smoke Condensate and Urethan

Joseph A. DiPaolo and Paul R. Sheehe


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/22/9/1058

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.