Effect of Commercial Saccharin Preparations on Urethan-induced Lung Tumorigenesis in Strain A Mice

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ABSTRACT

The effect of commercial saccharin preparations on urethan-induced mouse lung tumorigenesis was assessed by gavaging groups of male strain A mice with 1-g/kg doses of each saccharin preparation on a daily basis 5 days/week. Gavage was initiated 1 week before i.p. injection of either a low (0.1 mg/g) or a high (1 mg/g) dose of urethan and continued until the mice were sacrificed 16 weeks after urethan administration. The average number of surface lung tumors per mouse for each group of mice was determined and was compared statistically with the appropriate control group. The commercial saccharin preparations did not produce an elevated lung tumor response when administered alone. One of the four saccharin preparations enhanced the lung tumor response to urethan when given in conjunction with the low dose of urethan, but this enhancement was not statistically significant. At the high urethan dose, all saccharin preparations produced a statistically significant enhancement of the lung tumor response to urethan.

INTRODUCTION

The carcinogenic hazard of saccharin to humans has been the subject of much controversy for a good many years. In 1978, the National Academy of Sciences issued a report which concluded that saccharin itself, and not one or more impurities found in saccharin preparations, is a carcinogen of low potency in rats (11). While saccharin appears to be a carcinogen in rats, it does not possess properties and induce effects which are usually associated with carcinogenically active chemicals. Saccharin is an anionic nucleophilic molecule which is not significantly metabolized by rats even when the carcinogen-metabolizing system has been enhanced (13). It would thus seem that saccharin cannot induce carcinogenic effects in the same way as do the classic electrophilic carcinogens. This is supported by the finding that saccharin is not mutagenic to bacteria (12), does not bind with liver or bladder DNA in the rat (9), and does not produce a synergistic effect on sister chromatid exchange in human lymphocytes when given in conjunction with caffeine (2).

Evidence has recently accumulated which supports the idea that saccharin acts as a promotor of the carcinogenic process rather than as an inducer of this process. In rats, saccharin has been found to enhance the development of bladder tumors in response to methyl-nitrosourea (7, 8) and to N-(4-(5-nitro-2-furyl)-2-thiazolyl)formamide (5). In vitro, saccharin promoted the transformation of C3H/10T1/2 mouse embryo cells in response to 3-methylcholanthrene (10). In mice, the implantation of cholesterol pellets containing saccharin into the bladder produced a greater number of bladder tumors than when pellets containing only cholesterol were implanted (3, 4). This evidence led to the suggestion that the occurrence of bladder tumors in saccharin-treated rats may be due to the promoting effect of saccharin on endogenous initiators of the carcinogenic process and that the lack of carcinogenic activity of saccharin in other tissues of the rat and in other species of animals may be due to the lack of these endogenous initiators (1). The recent demonstration that saccharin induces hyperplasia in the rat urinary bladder lends further support to the idea that saccharin acts as a tumor promotor (8).

These findings raise the possibility that saccharin may act as a general promotor of carcinogenesis in tissues other than the bladder and in species other than the rat when exposure to saccharin is coupled with exposure to an exogenous initiator or low dose of complete carcinogen. To investigate this possibility, the effect of commercial saccharin preparations on urethan-induced lung tumorigenesis in strain A mice has been examined.

MATERIALS AND METHODS

The 4 commercial saccharin preparations used in this investigation were a pharmaceutical preparation marketed by Merck & Co., Inc., (Rahway, N. J.), Sweeta tablets (E. R. Squibb & Sons, New York, N. Y.), Sweet10 liquid (Pillsbury, Minneapolis, Minn.), and Sweet’n Low powder (Cumberland Packing Corp., Brooklyn, N. Y.). The pharmaceutical powder was obtained from a local pharmacy, and the other saccharin preparations were purchased at a local grocery store. No attempt was made to purify these commercial samples because the intent of this research was to determine if the actual saccharin preparations to which humans are exposed may pose a hazard as general promoters of carcinogenesis.

These saccharin preparations were made up fresh weekly in distilled water and administered at a dose of 1 g/kg daily for a period of 5 days each week by gavage. This route of administration was chosen because it is quantitative. It also more closely resembles the exposure of humans to saccharin than does the low constant exposure resulting from putting saccharin in the water or diet, since mice consume water and food almost continually rather than intermittently as humans do.

Three groups of 25 eight-week-old male strain A/St mice (L. Jolla, California 92093) were used. Two groups of mice were initiated 1 week before i.p. injection of either a low (0.1 mg/g) or a high (1 mg/g) dose of urethan and continued until the mice were sacrificed 16 weeks after urethan administration. The average number of surface lung tumors per mouse for each group of mice was determined and was compared statistically with the appropriate control group. The commercial saccharin preparations did not produce an elevated lung tumor response when administered alone. One of the four saccharin preparations enhanced the lung tumor response to urethan when given in conjunction with the low dose of urethan, but this enhancement was not statistically significant. At the high urethan dose, all saccharin preparations produced a statistically significant enhancement of the lung tumor response to urethan.

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were examined microscopically to confirm that adenomas were weighed at the start and finish of the saccharin exposure. One week after initiation of saccharin administration, one of these controls. One group received an i.p. injection of 0.1 mg urethan the same fashion as the saccharin-treated mice to serve as controls. One group received an i.p. injection of 1 mg urethan per g (Matheson, Coleman & Bell, Norwood, Ohio), and another group received i.p. injections of 1 mg urethan per g dissolved in 0.9% NaCl solution. The third group was not exposed to urethan. Three groups of 25 mice were gavaged with distilled water in the same fashion as the saccharin-treated mice to serve as controls. One group received an i.p. injection of 0.1 mg urethan per g, one group received an i.p. injection of 1 mg urethan per g, and one group was not exposed to urethan. All mice were weighed at the start and finish of the saccharin exposure period.

Sixteen weeks after exposure to urethan, all mice were sacrificed, and the adenomas appearing on the lung surfaces were counted under a dissecting microscope. The average number of lung tumors per mouse found in each saccharin-exposed group was then compared to the appropriate control group by Student's t test. A few lungs from each group of mice were examined microscopically to confirm that adenomas were in fact being counted.

RESULTS

The effect of commercial saccharin preparations on urethan-induced lung tumorigenesis in strain A mice is depicted in Table 1. The animals generally survived the treatment well. The majority of deaths which occurred were due to trauma induced by gavaging the mice. Animals exposed to the various saccharin preparations gained somewhat less weight than the control animals during the course of this experiment, but the weight gain was substantial in all saccharin-exposed mice. A mice revealed that the A/St mice used in this saccharin study are somewhat resistant to the carcinogenic effects of urethan.3

C. Strong Research Foundation) were exposed to each saccharin preparation for a period of 17 weeks in this fashion. One week after initiation of saccharin administration, one of these groups received i.p. injections of 0.1 mg urethan per g (Matheson, Coleman & Bell, Norwood, Ohio), and another group received i.p. injections of 1 mg urethan per g dissolved in 0.9% NaCl solution. The third group was not exposed to urethan. Three groups of 25 mice were gavaged with distilled water in the same fashion as the saccharin-treated mice to serve as controls. One group received an i.p. injection of 0.1 mg urethan per g, one group received an i.p. injection of 1 mg urethan per g, and one group was not exposed to urethan. All mice were weighed at the start and finish of the saccharin exposure period.

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The commercial saccharin preparations did not elicit a significant lung tumor response when given alone. The lung tumor response to the various artificial sweeteners followed the same order of potency at both doses of urethan, Sweeta tablets producing the smallest response and Sweeta Low powder producing the largest response. At the low urethan dose, the only sweetener to produce a lung tumor response greater than that in the animals treated with urethan alone was Sweeta Low, which produced a 2-fold increase. This increase was not statistically significant, however. At the high urethan dose, all the saccharin preparations significantly increased the number of lung tumors per mouse over the number found in animals treated with urethan alone. This enhancement ranged from a 2-fold increase for Sweeta tablets to a 4.6-fold increase for Sweeta Low powder. A mice revealed that the A/St mice used in this saccharin study are somewhat resistant to the carcinogenic effects of urethan.3

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DISCUSSION

While the results of this investigation suggest that commercial saccharin preparations may possess significant cocarcinogenic activity, much more work is necessary to confirm that this cocarcinogenic effect is real and to characterize this effect in greater detail. One problem with the results is that a significant enhancement of the mouse lung tumor response occurred only at the high dose of urethan used. Also, only one dose of each commercial saccharin preparation was used; therefore, no information is available concerning the dose-response relationship of the apparent cocarcinogenic effect. Because the commercial saccharin preparations utilized in this study were not pure, it is not known if the cocarcinogenic effect obtained is due to the saccharin itself or to some impurity in the saccharin preparations. The results of this investigation also provide no information concerning the nature of this cocarcinogenic effect. These saccharin preparations could be acting by a variety of mechanisms including alterations in urethan metabolism, sensitization of lung tissue to urethan effects, or promotion of the growth of urethan-induced altered cells into lung tumors. Experiments are being planned to determine the dependence of cocarcinogenic activity both on urethan dose and on saccharin dose. The components of these saccharin preparations which produce the cocarcinogenic effect will also be investigated and possible mechanisms by which this cocarcinogenic effect is produced will be examined. Investigations of the health hazard of saccharin in both experimental studies in animals and epidemiological studies in humans have focused on the risk of contracting bladder cancer

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Table 1

<table>
<thead>
<tr>
<th>Commercial saccharin preparation</th>
<th>Urethan dose</th>
<th>% of tumor incidence</th>
<th>No. of lung tumors/mouse</th>
<th>Survivors/initial</th>
<th>% of tumor incidence</th>
<th>No. of lung tumors/mouse</th>
<th>Survivors/initial</th>
<th>% of tumor incidence</th>
<th>No. of lung tumors/mouse</th>
</tr>
</thead>
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<tr>
<td>None</td>
<td>0</td>
<td>0.04 ± 0.04</td>
<td>25/25</td>
<td>52</td>
<td>0.60 ± 0.13</td>
<td>24/25</td>
<td>71</td>
<td>2.13 ± 0.44</td>
<td></td>
</tr>
<tr>
<td>Sweeta tablets</td>
<td>0.1 mg/g</td>
<td>0.21 ± 0.10</td>
<td>19/25</td>
<td>27</td>
<td>0.32 ± 0.12</td>
<td>25/25</td>
<td>100</td>
<td>4.36 ± 0.70</td>
<td></td>
</tr>
<tr>
<td>Pharmaceutical powder</td>
<td>1.0 mg/g</td>
<td>0.04 ± 0.04</td>
<td>6.0</td>
<td>36</td>
<td>0.44 ± 0.13</td>
<td>20/25</td>
<td>95</td>
<td>5.95 ± 1.29</td>
<td></td>
</tr>
<tr>
<td>Sweeta Low powder</td>
<td></td>
<td>0.12 ± 0.07</td>
<td>4.8</td>
<td>50</td>
<td>0.67 ± 0.20</td>
<td>24/25</td>
<td>100</td>
<td>6.08 ± 1.29</td>
<td></td>
</tr>
<tr>
<td>Sweet'n Low powder</td>
<td></td>
<td>0.14 ± 0.10</td>
<td>4.8</td>
<td>47</td>
<td>1.11 ± 0.57</td>
<td>21/25</td>
<td>100</td>
<td>9.88 ± 3.65</td>
<td></td>
</tr>
</tbody>
</table>

* Average of all animals exposed to each saccharin preparation.

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as a result of ingestion of this artificial sweetener. While further investigations are indicated, the results of the initial study described in this communication do suggest that commercial saccharin preparations to which humans are exposed may have the capacity to promote the development of other types of cancer when saccharin ingestion is coupled with exposure to a chemical carcinogen. If this is supported by additional studies, it would have significant impact on humans, in view of the variety of chemical carcinogens that humans come into contact with in the environment. It would thus seem prudent to investigate more thoroughly the cocarcinogenic activity of commercial saccharin preparations.

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REFERENCES

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