ABSTRACT

Three nitrosoalkylureas, two nitrosotrialkylureas, and three nitrosoalkylcarbamates were given to Syrian golden hamsters by gavage at approximately equimolar doses. Measured by the time to death with tumors as an index, nitrosoethyleurea was the most potent carcinogen, followed by nitroso-2-hydroxyethyleurea, which was less effective in males than in females. The least effective compounds, by this measure, were nitrosooxazolidone and nitroso-5-methylazoxazolidone. The remaining compounds, nitroso-N-ethylurethan, nitroso-2-hydroxypropylurea, nitroso-methylthiethylurea, and nitrosotriethylurea appeared to be of similar potency. All of the compounds induced papillomas or carcinomas of the nonglandular stomach in high incidence, except in the groups given nitrosohydroxyethylurea or nitrosooxazolidone, which were in mid-incidence; exceptionally, only 35% of the latter group had tumors, compared with 70% or more in the other groups. All of the nitrosoalkylureas induced a high incidence of hemangiosarcomas of the spleen, but the nitrosoalkylcarbamates did not. The quite uniform response of the hamster to these compounds contrasts with the great variety of organs and cell types in which they induce tumors in the rat.

INTRODUCTION

Nitrosoalkylamides have attracted great interest as model compounds for studying mechanisms of carcinogenesis because they are directly acting DNA-damaging agents in many systems (2, 5) and appear to be directly acting carcinogens (14). In an examination of their effects in different species, they provide an advantage in the absence of need for metabolic activation, which is quite often a significant factor in determining the carcinogenic response (4). Since the carcinogenic effects of a number of nitrosoalkylamides, particularly nitrosoalkylureas, have been examined, complementing them in several cases with studies of nucleic acid alkylation (3, 6, 13), it seemed likely that additional information would be provided by comparing their carcinogenic effects in Syrian hamsters with those in rats. To this end, a number of nitrosoalkylureas and nitrosoalkylcarbamates were given to Syrian hamsters by gavage at dose rates similar to those administered to rats. The nitrosoalkylureas were nitroso-ethyleurea, nitroso-2-hydroxyethyleurea, nitroso-2-hydroxypropylurea, and the trialkynitrosoureas (which require metabolic acti-

RESULTS

The mortality rates and the tumor incidences for each group are given in Table 1.

The nitrosoalkylureas and nitrosooxazolidones were administered at approximately equimolar concentrations for similar lengths of time, the treatment with nitrosoethyleurea being the shortest, the remainder from 22 to 30 weeks. The total doses

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NITROSOALKYLUREAS AND NITROSOALKYLCARBAMATES IN HAMSTERS

Table 1  
Carcinogenesis by nitrosoalkylamides in Syrian hamsters

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (mg/ml)</th>
<th>Duration of treatment (wks)</th>
<th>Total dose (mg/mmol)</th>
<th>No. of animals with tumors</th>
<th>Survivors at Wk:</th>
<th>Liver</th>
<th>Fore-stomach squamous cell carcinoma/ Other</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Nitrosoethylurea</td>
<td>12</td>
<td>20</td>
<td>48 (0.4)</td>
<td>20 F</td>
<td>20 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Hydroxyethylurea</td>
<td>12</td>
<td>20</td>
<td>48 (0.4)</td>
<td>19 M</td>
<td>18 16 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Hydroxypropylurea</td>
<td>16</td>
<td>24</td>
<td>77 (0.52)</td>
<td>20 M</td>
<td>20 20 16 9 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Ethylethan</td>
<td>14</td>
<td>28</td>
<td>78 (0.6)</td>
<td>15 F</td>
<td>15 15 11 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxazolidone</td>
<td>12</td>
<td>30</td>
<td>72 (0.82)</td>
<td>20 F</td>
<td>20 20 20 19 10 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-Methylazolidone</td>
<td>14</td>
<td>28</td>
<td>79 (0.91)</td>
<td>20 F</td>
<td>20 20 20 16 0 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-Methyl-3,3-diethylurea</td>
<td>40</td>
<td>25</td>
<td>200 (1.25)</td>
<td>20 M</td>
<td>17 17 4 0 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,3,3-Triethylurea</td>
<td>45</td>
<td>25</td>
<td>225 (1.3)</td>
<td>20 M</td>
<td>20 18 10 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20 20 20 20 19 16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* These hemangiosarcomas were metastases from the spleen.
* A, 1 thorax mesenchymoma; 1 hepatocellular adenoma; 8 cervix carcinoma; 3 oiliary gland carcinoma; 2 uterine endometrial stromal polyp; 1 esophageal squamous cell papilloma; skin angiosarcoma; 1 spinal cord neurofibrosarcoma; B, 1 spinal cord osteoma; C, 1 tracheal adenoma; 1 thyroid follicular cell carcinoma; 1 intestine adenocarcinoma; 1 uterine adenocarcinoma; 1 ovarian undifferentiated sarcoma; 1 uterine endometrial stromal polyp; D, 1 colon adenocarcinoma; 1 adrenal pheochromocytoma; 1 adrenal cortical adenoma; 1 adenocortical adenoma; 1 salivary gland undifferentiated sarcoma; E, 1 kidney hemangiosarcoma; 2 lymph node lymphosarcoma; 1 vagina/uterus squamous cell carcinoma; F, 1 uterine adenocarcinoma; 1 ovarian granular/theca cell tumor; 1 multiple-organ malignant lymphoma; G, 1 lymph node lymphosarcoma; 1 subcutaneous undifferentiated sarcoma; H, 1 hepatocellular adenoma; 1 forearm soft tissue sarcoma; 2 tongue squamous cell carcinoma; 2 cerebral astrocytoma; I, 1 splenic undifferentiated sarcoma; 1 tracheal adenoma; 1 oral mucosal squamous cell papilloma; 1 adrenal pheochromocytoma; 1 thyroid folicular cell carcinoma; J, 1 intestine squamous cell carcinoma; K, 1 malignant lymphoma; 2 multiple-organ malignant lymphoma; 1 stomach adenocarcinoma; 1 ovarian undifferentiated sarcoma; 1 uterine endometrial stromal polyp; 0, 1 colon adenocarcinoma; 1 adrenal pheochromocytoma; 1 skin angiosarcoma; 1 spinal cord neurofibrosarcoma; 1 hepatic osteoma; 1 tracheal adenoma; 1 thyroid folicular cell carcinoma; 1 intestine squamous cell carcinoma.

Given ranged from 0.4 to 0.6 mmol. Yet, the time to death with tumors varied quite widely, from a median of 22 weeks for sarcoma, 1 harderian gland adenoma.

Organ lymphosarcoma; K, 1 malignant lymphoma, 1 sarcoma (heart), 1 adrenal pheochromocytoma; 3 adrenal cortex carcinomas -I- 6 adenomas; 1 omentum giant cell sarcoma; 1 esophageal squamous cell papilloma; 1 epiglottis squamous cell carcinoma; 1 spinal meninges neurofibrosarcoma; J, 1 uterine stromal sarcoma; 2 multiple-organ malignant lymphoma; G, 1 adrenal pheochromocytoma; 1 adrenocortical adenoma; 1 adrenocortical carcinomain; 1 salivary gland undifferentiated sarcoma; E, 1 kidney hemangiosarcoma; 2 lymph node lymphosarcoma; 1 vagina/uterus squamous cell carcinoma; F, 1 uterine adenocarcinoma; 1 ovarian granular/theca cell tumor; 1 multiple-organ malignant lymphoma; G, 1 lymph node lymphosarcoma; 1 subcutaneous undifferentiated sarcoma; H, 1 hepatocellular adenoma; 1 forearm soft tissue sarcoma; 2 tongue squamous cell carcinoma; 2 cerebral astrocytoma; I, 1 splenic undifferentiated sarcoma; 1 tracheal adenoma; 1 oral mucosal squamous cell papilloma; 1 cervical lymph node undifferentiated sarcoma; 1 esophageal squamous cell papilloma; 1 epiglottis squamous cell carcinoma; 1 spinal meninges neurofibrosarcoma; J, 1 uterine stromal sarcoma; 2 multiple-organ malignant lymphoma; K, 1 malignant lymphoma, 1 adrenal pheochromocytoma; 3 adrenal cortex carcinomas + 6 adenomas; 1 omentum giant cell sarcoma, 1 harderian gland adenoma.

The dose of compound received by the hamsters was similar, although it was approximately double for the 2 nitrosotrialkylureas. Nevertheless, there were large differences in the rate at which the animals died with tumors, which is one criterion by which potency may be measured but which is not considered significant unless large. Hamsters without tumors in our facility usually live for 60 weeks or more. Clearly, by this measure, nitrosoethylyurethyleneurethane is considered to be more potent than is the former. Nitrosomethylidihydrourylethane and nitrosotrialkylureas are of approximately equal potency. Nitroso-2-hydroxypropylurea is considerably less potent than is the former. Nitrosomethylidihydrourylethane and nitrosotrialkylureas are of comparable potency but are considerably less potent than is nitrosoethylyurethane. The 2 nitrosoalkoxazolines are considerably weaker carcinogens than are the analogous nitrosoalkylurethylurethanes and are less effective than is nitrosoethylyurethane. Nitrosoalkoxazolines not only induces fewer tumors than do the other compounds but also has the least effect in reducing survival of the animals; the mortality rate of this group was essentially the same as that of the untreated female hamsters.

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DISCUSSION

Apart from the neoplasms of the forestomach, which most of these compounds induce in both hamsters and rats, there are profound differences in their effects between rats and hamsters. In the case of the nondirectly acting nitrosoalkylureas, this could be due to differences in enzymatic activation between the 2 species. However, this is presumably not the case with the directly acting nitrosoalkylureas and nitrosoalkylcarbamates, cyclic or acyclic.

The action of the nitrosooxazolidones and of nitrosoethylene than in inducing neoplasms of the forestomach is similar in both rats and hamsters (8, 9); presumably, the induction of these neoplasms is through direct alkylation of the DNA in the progenitor cells by the nitrosoalkylcarbamates. Although they are similar in stability to the corresponding nitrosoalkylureas in simple aqueous medium, the nitrosocarbamates might be entirely consumed in the stomach so that little is distributed systemically.

The homologous nitrosohydroxyethylurea and nitrosohydroxypropylurea induced essentially the same spectrum of neoplasms in the hamster, but survival in the latter group was better than in the group given nitrosohydroxyethylurea, which seems to be the more potent carcinogen in this species. In contrast, in the rat, nitrosohydroxypropylurea caused very rapid death from thymic leukemia (9) while, with nitrosohydroxyethylurea treatment, survival was significantly longer. However, the latter did induce a great variety of tumors in rats, including those in colon, bone, duodenum, kidney, bladder, thyroid, and others (9). This contrasting response of rats and hamsters to the same pair of \( N \)-nitroso compounds has been observed previously, even extending to the cis- and trans-isomers of nitroso-2,6-dimethylmorpholine (7, 16). In both cases in which male and female hamsters were given the same nitroso compound, nitrosohydroxyethylurea and nitrosohydroxypropylurea, the incidence of neoplasms was similar. However, survival in males was significantly better with nitrosohydroxyethylurea, which indicated a lesser carcinogenic potency of this compound in male hamsters than in female hamsters. The difference was not likely due to the difference in dose per unit of body weight between males and females, since there is only a small weight difference between the sexes in hamsters. In F344 rats given this compound chronically, there was a very similar response in the 2 sexes, and nitrosohydroxyethylurea given to male and female hamsters produced a similar response in both sexes.

Similarly, the contrast between hamsters and rats in their response to nitrosomethylidithiurea and nitrosotriithiurea is significantly different. Nitrosomethylidithiurea induced a high incidence of neoplasms of the brain and spinal cord in F344 rats, whereas nitrosotriithiurea induced mammary tumors but very few tumors of the central nervous system (10). In hamsters, on the other hand, both nitrosotrialkylureas induced the same tumors of the stomach and spleen at a very similar rate; there were other tumors induced by both compounds in the hamsters, but these appeared in only a small number of animals.

It is not yet possible to understand how the nitrosotrialkylureas induce tumors in rats or hamsters. They are not directly acting alkylating agents, and both are very weak mutagens to Salmonella typhimurium with rat liver microsomal activation, although they are quite potent mutagens with hamster liver microsomal activation (1). On the other hand, neither of the nitrosotrialkylureas is more potent on a dose per unit of body weight basis in hamsters than in rats. It must be assumed that they are metabolized in the forestomach of both rats and hamsters to active carcinogenic forms, although it is hard to imagine that these are the same as those derived from the directly acting nitrosoalkylureas, which also induce forestomach tumors in both species. However, since the nitrosotrialkylureas both induce hemangiosarcomas of the spleen in hamsters but not in rats, it must also be assumed that the necessary activating enzymes are present in cells of the hamster spleen but not in cells of the rat spleen. Furthermore, it is curious that the splenic vascular system is susceptible to the carcinogenic action of these and the directly acting nitrosoalkylureas but not in general the vascular system of other organs (liver, heart, etc.) of the hamster. This bespeaks a very subtle specificity toward these carcinogens in cells at certain anatomical locations.

As has been discussed elsewhere (9), nitroso-2-hydroxypropylurea, nitroso-5-methylxoxazolidone, and nitrosobis-(2-hydroxypropyl)-amine would be expected to give rise to the same alkylating species, the last through alpha oxidation. Yet, the 3 have vastly different carcinogenic effects in hamsters (and in rats (9)). The nitrosamine induces in hamsters mainly neoplasms of the pancreas, nasal mucosa, and lung, with a few of the liver, none of which were hemangiosarcomas (11). Nitrosohydroxyethylurea induced neoplasms mainly of the forestomach and spleen, while nitroso-5-methylxozaxolidone induced a high incidence of neoplasms of the forestomach and few others. These comparisons suggest that the 3 carcinogenic \( N \)-nitroso compounds do not necessarily act through formation of the same proximate carcinogenic intermediate; instead, it is possible that the distribution and activation of these compounds is determined by their particular chemical structures, which, in turn, determine their affinity for critical cellular receptors. It should be profitable, using radiolabeled compounds, to determine the nature of these differences in localization and intracellular binding, which should lead to a better understanding of the possibly subtle reactions which are involved in carcinogenesis by these compounds.

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\* W. Lijinsky and R. M. Kovatch, unpublished data.

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Carcinogenic Effect of Nitrosoalkylureas and Nitrosoalkylcarbamates in Syrian Hamsters

W. Lijinsky, G. L. Knutsen and R. M. Kovatch


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