EFFECT OF N-(3,5-DICHLOROPHENYL)SUCCINIMIDE ON THE HISTOLOGICAL PATTERN AND INCIDENCE OF KIDNEY TUMORS IN RATS INDUCED BY DIMETHYLNITROSOAMINE

Nobuyuki Ito, Seiichi Sugihara, Sachio Makiura, Masayuki Arai, Kazuya Hirao, Ayumi Denda, and Osamu Nishio

Department of Oncological Pathology, Cancer Center Institute, Nara Medical University*

The effect of the nephrotoxic substance, N-(3,5-dichlorophenyl)succinimide, on the histological pattern and incidences of kidney tumors in rats induced by dimethylnitrosoamine was studied histologically.

Post-treatment with N-(3,5-dichlorophenyl)succinimide markedly increased the induction of tumors, especially renal cell tumors, by dimethylnitrosoamine, but pretreatment with this substance clearly inhibited the induction of tumors, especially embryonal cell tumors in the kidney. Oral administration of N-(3,5-dichlorophenyl)succinimide alone caused severe interstitial nephritis but not development of kidney tumors. Thus, treatment with N-(3,5-dichlorophenyl)succinimide changes the histological type and incidence of kidney tumors in rats induced by dimethylnitrosoamine.

There have been many investigations on experimental conditions for induction of kidney tumors in rats and mice, and on the histology, histogenesis, ultrastructure, biochemistry, and biology of these tumors.3-11,13-16,19,20,22,24,28) Histopathological analysis has been made of kidney tumors in rats induced by various carcinogens.15,27) The kidney tumors in rats induced by dimethylnitrosoamine have been classified into three types; epithelial, mesenchymal, and vascular. Previous reports have shown that dimethylnitrosoamine mainly induces mesenchymal tumors in rat kidney.15,19,24,27) Recent investigations have also shown that oral administration of a high concentration of N-(3,5-dichlorophenyl)succinimide to rats induces severe interstitial nephritis.17,18)

The present paper describes the effect of the nephrotoxic chemical, N-(3,5-dichlorophenyl)succinimide, on the induction of kidney tumors in rats by dimethylnitrosoamine. The effect of this nephrotoxic chemical substance on the histological pattern and incidence of kidney tumors induced by dimethylnitrosoamine was also analyzed histologically.

MATERIALS AND METHODS

Male Wistar strain rats (Fuji Animal Farm, Tokyo), 8 to 10 weeks old, with an average body weight of 185 g, were used. Seventy-four rats were divided into 6 groups.

Group 1: Sixteen rats were fed on the semi-synthetic basal diet described previously,14) supplemented with 500 ppm of dimethylnitrosoamine (Tokyo Kasei Co., Tokyo) for 2 weeks and then on commercial stock diet (Oriental MF) for 22 weeks.

Group 2: Sixteen rats were fed on basal diet containing 500 ppm of dimethylnitrosoamine for 2 weeks, on stock diet for 2 weeks, on basal diet containing 5000 ppm of N-(3,5-dichlorophenyl)succinimide (Sumitomo Chemical Co., Osaka) for 8 weeks, and then on stock diet for 12 weeks.

Group 3: Twelve rats were fed on basal diet for 2 weeks, on stock diet for 2 weeks, on basal diet containing 5000 ppm of N-(3,5-dichlorophenyl)succinimide for 8 weeks, and then on stock diet for 12 weeks.

Group 4: Twelve rats were fed on basal diet containing 5000 ppm of N-(3,5-dichlorophenyl)succinimide for 8 weeks, on stock diet for 2 weeks, on basal diet containing 500 ppm of dimethylnitrosoamine for 2 weeks, and then on stock diet for 22 weeks.

Group 5: Twelve rats were fed on basal diet containing 5000 ppm of N-(3,5-dichlorophenyl)succinimide for 8 weeks, on stock diet for 2 weeks, on basal diet for 2 weeks, and then on stock diet for 22 weeks.

Group 6: Six rats were fed on stock diet for 10 weeks, on basal diet for 2 weeks, and then on stock diet for 22 weeks.

* 840 Shijō-cho, Kashihara, Nara-ken 634 (伊東信行, 杉原誠一, 牧浦幸男, 荒井昌之, 平尾和也, 傳田阿由美, 西尾 治).
The feeding schedules of these groups are summarized in Fig. 1. Rats were housed in wire cages in an air-conditioned room at 24°C and weighed weekly. Animals dying before the time of sacrifice were not included in the final effective number of rats. Surviving animals were killed by ether 24 weeks after the start of dimethylnitrosoamine diet or basal diet and their liver and kidneys were weighed. Any other organ appearing grossly abnormal was also examined histologically. Tissues were fixed in 10% buffered formaldehyde solution, and sections were routinely stained with Hematoxylin and Eosin, van Gieson's stain, and Mallory's stain.

**Results**

The average changes in the body, liver, and kidney weights of the rats in the 6 groups are summarized in Table I. All the rats gained weight. In groups 1 to 5, liver weights increased slightly. The kidney weight increased markedly in rats treated with dimethylnitrosoamine and N-(3,5-dichlorophenyl)succinimide. However, no tumors were found in any organs other than the kidneys.

**Kidney Tumors**

Histopathological changes and the incidence of kidney tumors are summarized in Tables II and III. Histologically, kidney tumors were classified as renal cell tumors, embryonal cell tumors, and hemangioendotheliomas, as described previously.15) Group 1: Eight of the 14 rats (57.1%) developed kidney tumors. Two of the lesions were renal cell tumors, 8 were embryonal cell tumors, and 1 was a hemangioendothelioma. Two rats had both embryonal cell and renal cell tumors in the kidney. No adenoma, dark cell carcinoma, nephroblastoma, or undifferentiated nephroblastoma was seen. In non-neoplastic areas of the kidneys in this group, interstitial cell infiltration and proliferation or tubular epithelial cells were seen, but no cystic changes or colloidal casts in the tubules were observed.

Group 2: All the rats in this group developed kidney tumors. Ten (76.9%) were renal cell tumors, 10 (76.9%) were embryonal cell tumors, and 4 (30.8%) were hemangioendotheliomas.

| Table I. Changes in Body, Liver, and Kidney Weights of Rats Given Diet With or Without Dimethylnitrosoamine and/or N-(3,5-Dichlorophenyl)succinimide |
|---|---|---|---|---|---|---|
| Group | Treatment<sup>2)</sup> | No. of rats | Body weight (g) | Liver weight (g) | Kidney weight (g) |
| | | | Initial | Final | (%) of body wt. | Right | Left |
| 1 | DMN | 14 | 176.4 | 402.3 | 11.7 | 2.9 | 1.7 | 1.4 |
| 2 | DMN→NDPS | 13 | 194.2 | 427.4 | 10.2 | 2.4 | 2.9 | 1.2 |
| 3 | BD→NDPS | 10 | 167.0 | 420.1 | 9.4 | 2.2 | 2.9 | 1.1 |
| 4 | NDPS→DMN | 11 | 185.8 | 468.2 | 14.5 | 3.1 | 1.5 | 1.4 |
| 5 | NDPS→BD | 11 | 215.1 | 429.3 | 12.0 | 2.8 | 2.9 | 1.7 |
| 6 | BD | 6 | 174.7 | 443.5 | 10.9 | 2.2 | 2.9 | 1.2 |

Rats were killed 24 weeks after the start of dimethylnitrosoamine diet or basal diet.

<sup>2</sup> DMN=diet containing 500 ppm dimethylnitrosoamine, NDPS=diet containing 5000 ppm N-(3,5-dichlorophenyl)succinimide, BD=basal diet.
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Table II. Histological Changes in Non-tumorous Areas of Kidneys in Rats on Diet With or Without Dimethylnitrosoamine and/or N-(3,5-Dichlorophenyl)succinimide

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Glomerular Fibrosis</th>
<th>Interstitial cell infiltration</th>
<th>Cystic changes of tubules</th>
<th>Colloidal casts</th>
<th>Tubular epithelial proliferation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DMN</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>DMN→NDPS</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>3</td>
<td>BD→NDPS</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>4</td>
<td>NDPS→DMN</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>NDPS→BD</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>6</td>
<td>BD</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a) Abbreviations as in Table I
b) - no change, + trace, + slight, ++ moderate, +++ marked.

Table III. Histological Types of Kidney Tumors in Rats Induced by Dimethylnitrosoamine and/or N-(3,5-Dichlorophenyl)succinimide

<table>
<thead>
<tr>
<th>Histological type</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal cell tumor</td>
<td>2</td>
<td>10</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Adenoma</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Clear cell carcinoma</td>
<td>2</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dark cell carcinoma</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Embryonal cell tumor</td>
<td>8</td>
<td>10</td>
<td>75</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Teratoid nephroblastoma</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hyalinized nephroblastoma</td>
<td>7</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nephroblastoma</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Undifferentiated nephroblastoma</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hemangioendothelioma</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total No. of tumors/No. of animals (percentage incidence)</td>
<td>8/14(57.1)</td>
<td>13/13(100.0)</td>
<td>0/10</td>
<td>3/11(27.3)</td>
<td>0/11</td>
<td>0/6</td>
</tr>
</tbody>
</table>

a) Two rats had both renal cell and embryonal cell tumors.
b) Five rats had both renal cell and embryonal cell tumors, 2 had both renal cell tumors and hemangioendotheliomas, and one had 3 different tumors or had both embryonal cell tumor and hemangioendothelioma.

Five rats had both renal cell and embryonal cell tumors in the same kidney (Photos 1～4). Two of the 4 hemangioendotheliomas showed especially numerous mitotic figures in proliferating endothelial cells. No dark cell carcinoma was seen. Two rats had both renal cell tumors and hemangioendotheliomas, and 1 had 3 different histological tumors or had both embryonal cell tumors and hemangioendotheliomas. In non-neoplastic areas of the kidneys in this group, interstitial cell infiltration, mainly consisting of lymphocytes and proliferating tubular epithelial cells, was marked. Colloidal casts in tubular lumina and cystic changes of tubules were also frequently seen. Slight proliferative growth of connective tissue elements was observed. However, no remarkable changes were seen in the glomeruli or vascular system of the kidneys.

Group 3: No kidney tumors were seen in any of the 10 rats. However, slight proliferation of tubular epithelial cells and colloidal casts in tubular lumina were noticed. A few animals showed cystic changes of the tubular lumina.

Group 4: Three of the 11 rats (27.3%) developed adenoma (Photo 5) but no embryonal cell tumors or hemangioendotheliomas were seen. In non-neoplastic areas of the kidneys, there were moderate interstitial cell infiltration and proliferation of tubular epithelium.
Occasionally, fibrosis of the interstitial tissue, cystic changes of tubules, and colloidal casts in the tubular lumina were seen.

Group 5: No kidney tumors were found in any of the 11 rats in this group. Cystic changes of the tubules and colloidal casts in the tubular lumina were frequent. Epithelial proliferation of tubules was rare but fibrosis of interstitial tissue with lymphocytic infiltration was noticed. No changes were seen in the glomeruli or vascular system of the kidneys (Photos 6 and 7).

Group 6: No remarkable change was seen in the kidneys of any rats in this group.

Other Organs Apart from the kidneys only changes of the liver were examined histologically in the present studies. The liver of animals in group 1, which received dimethylnitrosoamine alone, showed a slight oval cell infiltration and bile duct proliferation, but no liver cancer was seen. In group 2, which received dimethylnitrosoamine and then N-(3,5-dichlorophenyl)succinimide, the liver showed a marked oval cell infiltration and bile duct proliferation. One of the 13 animals developed a hemangioendothelioma or nodular hyperplastic changes. Cystic changes of the bile duct were occasionally seen. In contrast, treatment with dimethylnitrosoamine after pretreatment with N-(3,5-dichlorophenyl)succinimide, caused no marked changes in the liver. In groups 3, 5, and 6, no pathological changes were seen in the liver, and no changes in other organs.

DISCUSSION

In many previous studies on kidney tumors in rats induced by dimethylnitrosoamine, incidence of mesenchymal tumors was reported to be higher than that of epithelial tumors, but our present results showed that the histological pattern and incidence of kidney tumors in rats induced by dimethylnitrosoamine changed by treatment with N-(3,5-dichlorophenyl)succinimide. Previously, in histopathological analyses of experimental kidney tumors in rats induced by various chemical carcinogens, we observed about the same results. In the present investigation also 3 different kinds of kidney tumors were induced in rats by dimethylnitrosoamine alone or in combination with N-(3,5-dichlorophenyl)succinimide. N-(3,5-Dichlorophenyl)succinimide is reported to be nephrotoxic in rats and mice and, therefore, the effect of this nephrotoxic chemical substance on kidney tumors in rats induced by dimethylnitrosoamine was investigated. In groups 3 and 5, rats treated with N-(3,5-dichlorophenyl)succinimide only developed severe interstitial nephritis, such as interstitial cell infiltration, fibrosis, cystic changes of the tubules, and colloidal casts in the tubules. On treatment with dimethylnitrosoamine alone, only slight cystic changes of the tubular lumina and colloidal casts in tubules were seen. Induction of interstitial nephritis by chemical substances such as phenacetin or its derivatives was reported by several observers. The present results show that experimental interstitial nephritis can be induced in rats by oral administration of N-(3,5-dichlorophenyl)succinimide.

In the groups which received dimethylnitrosoamine and N-(3,5-dichlorophenyl)succinimide, interstitial cell infiltration and tubular epithelial proliferation in non-tumorous areas of the kidney were more marked than in rats which received N-(3,5-dichlorophenyl)succinimide or dimethylnitrosoamine alone. In the rats treated with N-(3,5-dichlorophenyl)succinimide after administration of dimethylnitrosoamine the incidence of kidney tumors, especially renal cell tumors, as epithelial kidney tumors, was higher than that after administration of dimethylnitrosoamine alone. Also, the incidence of embryonal cell tumors and hemangioendotheliomas slightly increased by treatment with N-(3,5-dichlorophenyl)succinimide. In contrast, pre-treatment with N-(3,5-dichlorophenyl)succinimide clearly inhibited the induction of kidney
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tumors by dimethylnitrosoamine, and especially, of embryonal cell tumors and hemangioendotheliomas as mesenchymal kidney tumors.

The present results suggest that N-(3,5-dichlorophenyl)succinimide changes the histological pattern of kidney tumors induced by dimethylnitrosoamine. The most important finding was a marked increase in the production of renal cell tumors on administration of the nephrotoxic chemical. The reason for this is unknown but it seems possible that changes in the tubular epithelium induced by dimethylnitrosoamine might be accelerated by the administration of N-(3,5-dichlorophenyl)succinimide, but pretreatment with N-(3,5-dichlorophenyl)succinimide showed results reverse of that by a post-treatment with the nephrotoxic chemical. Thus it changed the susceptibility of epithelial and mesenchymal cell elements of the kidney to induction of tumors by dimethylnitrosoamine. Pretreatment with N-(3,5-dichlorophenyl)succinimide also inhibited liver changes in rats induced with dimethylnitrosoamine. It is interesting in this connection that it has been reported that the induction of liver or bladder tumors by nitrosocompounds is inhibited by treatment with 1-naphthyl isothiocyanate.\(^{17,23}\) Induction of kidney tumors by dimethylnitrosoamine may be due to the same mechanism and be inhibited in the same way but the relationship between changes in the histological pattern of kidney tumors and in the incidence of tumors in rat kidney of administration of N-(3,5-dichlorophenyl)succinimide requires further biochemical and histopathological studies.

This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education (No. 90319/1972 & No. 801075/1973) and by a grant from the Experimental Pathological Research Association (1973), which are gratefully acknowledged.

(Received October 15, 1973)

REFERENCES

18) Ito, N., Sugihara, S., Kadota, T., Miyamoto, J., unpublished results.
EXPLANATION OF PLATES

Photo 1. Clear cell carcinoma in the kidney of a rat treated with dimethylnitrosoamine and then N-(3,5-dichlorophenyl)succinimide (Group 2). Cancer cells are arranged in a delicate stroma. H-E. x100.

Photo 2. Hyalinized nephroblastoma in the kidney of a rat treated with dimethylnitrosoamine and then N-(3,5-dichlorophenyl)succinimide (Group 2). H-E. x200.

Photo 3. Undifferentiated nephroblastoma in the kidney of a rat treated with dimethylnitrosoamine and then N-(3,5-dichlorophenyl)succinimide (Group 2), showing aggregates of tumor cells and nuclear irregularities. H-E. x200.

Photo 4. Hemangioendothelioma in the kidney of a rat treated with dimethylnitrosoamine and then N-(3,5-dichlorophenyl)succinimide (Group 2), showing tumor tissue surrounding a glomerulus. H-E. x100.

Photo 5. Adenoma in the kidney of a rat treated with N-(3,5-dichlorophenyl)succinimide and then dimethylnitrosoamine (Group 4). H-E. x200.

Photo 6. Cystic changes of tubule and many colloidal casts in the tubular lumina in the kidney of a rat treated with N-(3,5-dichlorophenyl)succinimide only (Group 5). H-E. x100.

Photo 7. Diffuse fibrosis and lymphocytic infiltration into the interstitial tissue of the kidney of a rat treated with N-(3,5-dichlorophenyl)succinimide only (Group 5). H-E. x100.

H-E = Hematoxylin and Eosin stain.
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