Blastomogenic Activity of \( p \)-Hydroxyphenyllactic Acid in Mice

M. O. Rauschenbach, E. I. Zharova, T. I. Sergeeva, V. D. Ivanova, and N. A. Probatova

Laboratory of System Blood Diseases and Division of Pathological Anatomy, Institute of Experimental and Clinical Oncology, Kashirskoye Shosse, 6, Moscow V-409, U. S. S. R.

SUMMARY

Blastomogenic activity of \( p \)-hydroxyphenyllactic acid was established by prolonged s.c. administration into C57BL/6 and CC57BR mice. Phenyllactic acid was also tested and was not blastomogenic under the same conditions. The preparations in aqueous solutions were injected into the dorsal region twice weekly in 2.5-mg doses. The total dose per mouse was 42 mg \( p \)-hydroxyphenyllactic acid or 50 mg phenyllactic acid.

The resorptive character and many forms of blastomogenic activity of \( p \)-hydroxyphenyllactic acid were shown. It induced neoplasms of varied histogenesis (leukemias, adenomas, hepatomas, and tumors of the vascular tissue), including benign and malignant tumors and precancerous conditions of the urinary bladder.

INTRODUCTION

It has been established that patients with various malignant neoplasms suffer from disturbances in tryptophan metabolism (Refs. 7, 8, 19, 25, 27—29, 31, and 32; W. S. Popnicolov, unpublished observation). A number of studies have provided evidence for blastomogenic activity of the tryptophan metabolites that appear in large amounts in the urine of leukemic patients (2, 9—11, 14, 15, 33, 38, 39).

Investigation of blastomogenic activity of tyrosine metabolites was considered to be of interest since tyrosine metabolism is known to be closely associated with that of tryptophan and since tyrosine breakdown involves formation of phenol and indole precursors of melanogens. As revealed from their chemical structure, a number of tyrosine and tryptophan metabolites bear a certain resemblance to carcinogenic aromatic amines. Moreover, some diseases affect, simultaneously, the metabolism of tryptophan, tyrosine, and phenylalanine.

Previously, Ivanova and Kaverzneva (20) reported on the excretion of phenolic acid metabolites of tyrosine in 21 patients with various forms of leukemia and demonstrated marked changes in the excretion of phenolic acids. Of special interest were studies on the excretion of \( p \)-hydroxyphenyllactic acid, since its concentration in the urine of all the patients examined was increased to a range of 3.2 to 29 mg/24 hr. In healthy patients these metabolites of tyrosine were rarely detectable; in 4 out of 18 cases they appeared in the range of 1.2 to 5.0 mg/24 hr.

The present study documents that \( p \)-hydroxyphenyllactic acid is characterized by blastomogenic activity.

MATERIALS AND METHODS

Analytical \( p \)-hydroxyphenyllactic acid was purchased from the Department of Organic Chemistry, Mendeleev Moscow Chemico-Technological Institute, Moscow, U. S. S. R. It was obtained by reduction of \( p \)-hydroxyphenylpyruvic acid by amalgam sodium produced electrolytically (24). \( p \)-Hydroxyphenyllactic acid melted at 143—144°. Chromatographic data coincided with the data of Armstrong et al. (3).

Six-week-old female and male noninbred stock of C57BL/6 and CC57BR mice were used. They were obtained from the vivarium under quarantine regulation. Ten animals were housed in each metal cage. All experimental and control animals were kept on a standard diet and given water ad libitum.

The metabolites were dissolved in distilled water in 1 mg/0.1 ml concentration. A dose of 2.5 mg metabolites in 0.25 ml water per mouse was injected s.c. into the dorsal region twice a week. For \( p \)-hydroxyphenyllactic acid the total dose was 42 mg (233 \( \mu \)moles) per mouse and for phenyllactic acid it was 50 mg (301 \( \mu \)moles).

The control mice were untreated and had been studied by us a few months before.

All animals were killed by cervical dislocation, and postmortem examination was carried out by a pathologist on every animal. Tumor tissues from salivary glands, lungs, heart, thymus, peripheral and mesenteric lymph glands, liver, spleen, kidney and adrenals, urinary bladder, ovaries, and uterus were fixed in 10% formalin, embedded in paraffin blocks, sectioned, and stained with hematoxylin and eosin. All tissues were examined histologically, using the criteria described elsewhere (1, 6, 12, 13, 23, 35).

RESULTS

The 1st neoplasm (reticulosis) recorded was in a control mouse at the age of 17 months. During the subsequent 5 months, all control animals were killed, and neoplasms were detected in 14 and 38% of C57BL/6 and CC57BR mice, respectively.
As shown in Table 1, 45% of the animals treated with phenyllactic acid developed neoplasms. We cannot, however, characterize this metabolite as a substance possessing blastomogenic activity, since the index of its blastomogenic activity is not significant and the histogenesis of the neoplasms that developed in this group of animals generally resembles the histogenetic profile of spontaneous neoplasms. It should also be taken into account that the experiments were performed on CC57BR mice, which are notable for a high sensitivity to induced blastomogenesis, and that the animals were given a somewhat higher dose of the preparation than of \( p \)-hydroxyphenyllactic acid.

A considerable number of neoplasms were found in mice of both strains treated with \( p \)-hydroxyphenyllactic acid. The 1st neoplasm (reticulosis) developed at 3.5 months, and the last developed at 17 months after termination of treatment. It should be especially noted that the treated animals developed tumors and leukemia more readily than the controls. CC57BR mice developed a greater number of neoplasms than did C57BL/6 mice (82 and 64%, respectively).

Some features of blastomogenesis induced by \( p \)-hydroxyphenyllactic acid should be noted. Reticulo- and lymphosarcomatosis accounted for the most cases of leukemia in mice of both strains, while only a small number of animals developed lympholeukemia. Reticulo- and lymphosarcomatosis were both noted for tumor-like proliferations in hematopoietic and nonhematopoietic organs with expressed splenomegaly and invasion of surrounding tissues (Figs. 1 to 10).

Leukemias observed in this study were characterized by a simultaneous development of tumors of varied histogenesis. Thus, of the total of 91 cases of leukemia in mice of both strains given \( p \)-hydroxyphenyllactic acid, 32 cases of leukemia were combined with the development of 1 or more tumors. For example, the following combinations were found: (a) reticulosis, ovarian hemangioma, and papilloma of the urinary bladder; (b) reticulosis, adenoma of the lung, and capillary hemangioma of the skin. Most often, combined development of leukemia with tumors was observed in CC57BR mice.

The tumors varied in histogenesis. Hepatomas and papillomas of the urinary bladder were found most frequently. Tumor nodules were situated as a rule on the front surface of the liver. In tumor tissue of some hepatomas malignancy was manifested by a strongly basophilic cytoplasm, considerable decrease in the cell size, hyperchromic nuclei, and an increase in the number of dividing cells.

While there were no metastatic tumors, hepatoma cells were sometimes found in the blood vessels of the kidneys. Some animals developed hepatoma in combination with capillary hemangioma. Simultaneously with the appearance of hepatoma, the tumor node showed hemorrhagic cysts and vast fields of proliferating vascular endothelium (Fig. 11).

A considerable number of animals developed adenoma of the lung and hemangioma localized in various sites.

As in the case of blastomogenesis induced by tryptophan metabolites, a marked sensitivity to blastomogenic action of \( p \)-hydroxyphenyllactic acid was shown by transitional epithelium of the urinary bladder. Singular or multiple papillomas developed in the urinary bladder in 30% of the cases, while in 4 cases planocellular cancer was found (Fig. 12), and in 13 cases squamous metaplasia (Fig. 13), which is considered a precancerous state (5), was found.

**DISCUSSION**

The results of this study provide evidence that \( p \)-hydroxyphenyllactic acid has pronounced leukemogenic and blastomogenic effects, while phenyllactic acid, a product of phenylalanine metabolism, under the same conditions is inactive.

Compared with tryptophan metabolites, \( p \)-hydroxyphenyllactic acid is a more active blastomogen. Thus even a more active tryptophan metabolite, 3-hydroxyanthranilic acid, induces neoplasms in only 33 or 64% of C57BL/6 and CC57BR mice, respectively, compared with 64 and 82% for \( p \)-hydroxyphenyllactic acid.

Development of hepatoma is new for this type of endogenic blastomogenesis. Hepatoma developed more often in male CC57BR mice, as reported elsewhere (16, 22, 30). The reason for this phenomenon is unclear. An attempt to elucidate the role of androgens in hepatoma development was unsuccessful (30).

In this study the animals were given a smaller dose of the preparation in contrast to the well-known hepatocarcino-

### Table 1

**Results of investigation of blastomogenic activity of phenolic acids**

<table>
<thead>
<tr>
<th>Phenolic acids</th>
<th>Strain</th>
<th>No. of mice with neoplasms/no. of mice surviving the time of development of the primary tumor</th>
<th>%</th>
<th>Leukemic</th>
<th>Sarcoma</th>
<th>Hepatoma</th>
<th>Cancer of the urinary bladder</th>
<th>Papilloma of the urinary bladder</th>
<th>Adenoma</th>
<th>Hemangioma</th>
</tr>
</thead>
<tbody>
<tr>
<td>( p )-Hydroxyphenyllactic acid</td>
<td>C57BL/6</td>
<td>54/84</td>
<td>64</td>
<td>47</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CC57BR</td>
<td>61/74</td>
<td>82</td>
<td>43</td>
<td>14</td>
<td>23</td>
<td>16</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenyllactic acid</td>
<td>CC57BR</td>
<td>19/42</td>
<td>45</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>C57BL/6</td>
<td>4/29</td>
<td>14</td>
<td>3</td>
<td></td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CC57BR</td>
<td>10/26</td>
<td>38</td>
<td>5</td>
<td></td>
<td></td>
<td>3</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
gens. Thus, a 2- to 3.5-g dose of auramine induced hepatoma in 57 to 73% of CBA mice (37). In 60 to 70% of mice of the same strain, hepatoma was induced by hydrazine administration at a total dose of 283 mg (26). It can be assumed that an increase in a dosage of \( p \)-hydroxyphenyllactic acid might more readily induce development of hepatoma.

Hepatomas observed in this study share some features which are characteristic of hepatomas induced by well-known hepatocarcinogens. Thus, a low metastatic spread of hepatomas is corroborated by published data (4, 18, 26, 34). As mentioned above, hepatomas were combined with capillary hemangiomas of the liver. No similar combination is found in the literature, although there are reports that show evidence of hemorrhagic cysts and proliferating spindle-shaped cells within the tumor (16, 26). It is possible that the above reports have bearing on capillary hemangiom.

Finally, we found the appearance of vast nodule-like proliferations of hematopoietic elements around the blood vessels in the tumor stroma (Fig. 14) and in the surrounding liver tissue of interest. Some animals without leukemia can also develop similar tumors, as reported elsewhere (36, 37).

In summary, bearing in mind that we are still far from understanding the mechanisms of blastomogenic action of chemical carcinogens, it may, nevertheless, be suggested that \( p \)-hydroxyphenyllactic acid is one of the end factors of endogenic blastomogenesis induced by tryptophan metabolites. This hypothesis is supported indirectly by evidence that tryptophan and its metabolites and, in particular, the metabolites exerting blastomogenic effect (3-hydroxyanthranilic acid, quinoline acid, and xanthurenic acid) enhance activity of tyrosine aminotransferase (17, 21). This can be followed by an excessive accumulation in the organism of \( p \)-hydroxyphenylpyruvic and \( p \)-hydroxyphenyllactic acids. Our contention is also supported by the markedly enhanced excretion of \( p \)-hydroxyphenyllactic acid by all leukemic patients and its more pronounced blastomogenic activity compared with tryptophan metabolites.

REFERENCES

M. O. Rauschenbach et al.


Figs. 1—14. Tissues from treated animals.

Fig. 1. Peribronchial leukemic infiltration. H & E, a, x 120; b, x 450.

Fig. 2. Leukemic infiltration in the endocardium. H & E, x 120.

Fig. 3. Nodular leukemic infiltration in the kidney. H & E, a, x 120; b, and c, x 450.

Fig. 4. Nodular leukemic infiltration in the ovary. H & E, x 120.

Fig. 5. Massive leukemic infiltrations in liver tissue. H & E, a, x 120; b, x 450.

Fig. 6. Leukemic infiltration along a blood vessel in liver. H & E, a, x 120; b, x 450.

Fig. 7. Nodular proliferation of reticular cells in the spleen. H & E, x 120.

Fig. 8. Reticulum cell sarcoma in the lymph node. Leukemic infiltration of the capsule and surrounding cellular tissue. H & E, a, x 120; b, x 450.

Fig. 9. Leukemic infiltration of submucosa in urinary bladder. H & E, x 120.

Fig. 10. Circular leukemic infiltration in the urethra. H & E, a, x 120; b, x 450.

Fig. 11. Hepatoma and capillary hemangiomata. H & E, a, x 120 b, x 250; c, same as a, proliferation of endothelium, x 450.

Fig. 12. Urinary bladder cancer. H & E, x 120.

Fig. 13. Squamous metaplasia in the urinary bladder. H & E, x 250.

Fig. 14. Hepatoma. Proliferation of hematopoietic cells. H & E, a, x 120; b, x 250.
Blastomogenic Activity of p-Hydroxyphenyllactic Acid
Blastomogenic Activity of \( p \)-Hydroxyphenyllactic Acid
Blastomogenic Activity of \( p \)-Hydroxyphenyllactic Acid in Mice
