Occurrence of Desmosterol in Tumors of the Nervous System Induced in the Rat by Nitrosourea Derivatives

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SUMMARY

Desmosterol (24-dehydrocholesterol), a precursor of cholesterol, represents 5 to 20% of the total sterols of the brain during maturation, although it is practically absent in normal adult brain. The desmosterol content is increased to a small extent in human glial tumors and in some transplantable experimental tumors.

In the present experiments it has been shown that desmosterol accumulates in considerable amounts in experimental tumors of the nervous system obtained in Long-Evans rats either by i.v. administration of methylnitrosourea or by transplacental induction with ethylnitrosourea. The significance of this finding is discussed.

INTRODUCTION

Previous data from our group have shown the presence of desmosterol (24-dehydrocholesterol) in brain tumors (3). This sterol, which is the last precursor of cholesterol in one of its biosynthetic routes, does not normally accumulate in tissues except in brain during maturation (10). For example, in developing mouse brain the largest amount of desmosterol (24% of the total sterols) was detected at 5 days after birth, while desmosterol represents 7% of the total sterols in the brain of a human fetus at 10 weeks. When maturation is reached and cholesterol synthesis is markedly reduced (7), the desmosterol content of both human and animal brain declines to levels usually undetectable by gas chromatographic analysis (10, 12). Increased desmosterol concentrations occur in human glioblastomas (3) and in some transplantable and spontaneous animal brain tumors (5). The concentration of this sterol in the tumors examined has never exceeded 5% of the total sterols.

A new type of experimental tumor, induced in rats by administration of nitrosourea derivatives, has been obtained by Druckrey et al. (1). These authors have demonstrated that MNU3 exerts a carcinogenic action with a high selectivity for the nervous system (1). The tumors, obtained in brain and spinal cord, were neurinomas, ependymomas, oligodendrogliomas, glioblastomas, etc. In other experiments, Druckrey et al. (2) have shown that a single injection of ENU to a pregnant rat induces tumors of the nervous system in the majority of the offspring.

After administering MNU to rats of the Long-Evans strain once a month for 8 months, we obtained a large series of tumors of the nervous system (11), confirming the observation of Druckrey et al. (1). With a single injection of ENU to pregnant rats, we also induced tumors of the central and peripheral nervous system in 90% of the offspring (8). The histological and histochemical appearance of these tumors was very similar to those of human tumors (8, 11). The possibility that these experimental tumors might contain desmosterol was considered. Our results are presented below.

MATERIALS AND METHODS

Tumor Induction. NaCl solutions (0.9%) of either MNU or ENU were used within 1 hr of preparation. Long-Evans male rats, weighing 150 g at the beginning of the experiments, were given injections of 25 mg/kg MNU (kindly supplied by Prof. H. Druckrey, Max-Planck-Institut für Immunobiologie, Freiburg, Germany) in the tail vein once a month. The animals received a total of 8 injections. The tumoral symptoms have been previously described (11).

Pregnant Long-Evans rats were given injections in the tail vein with 10 mg/kg ENU (kindly supplied by Prof. H. Druckrey, Max-Planck-Institut für Immunobiologie, Freiburg, Germany) on the 17th day of pregnancy. After delivery and lactation, the litters were separated, and the animals were observed up to the onset of clinical symptoms (8).

Sterol Analysis. Tumor samples were weighed and saponified with NaOH in 95% ethanol for 1.5 hr at 60°. A volume of water equivalent to that of the ethanol was added, and the unsaponifiable material was extracted with petroleum ether (b.p., 60–80°). The extracts were washed...
with distilled water, dried over anhydrous Na₂SO₄, and evaporated in a nitrogen atmosphere. The dried unsaponifiable material, containing the sterols, was acetylated with acetic anhydride and pyridine (6).

The acetylated sterols were analyzed with a Fractovap Model C gas chromatograph equipped with a hydrogen flame detector and a glass column (2 m x 3 mm) packed with 1% phenyl silicone (General Electric Co., Schenectady, N. Y.). The carrier gas was nitrogen, and the column temperature was 220°. The amounts of cholesterol and desmosterol were calculated with 5-androsten-3β-ol-17-one acetate as an internal standard. The identity of desmosterol was confirmed by mass spectrometry (12).

RESULTS AND DISCUSSION

The results obtained from analyzing the tumors induced with MNU are summarized in Table 1. In this series, there was a remarkable concentration of desmosterol (from 6.4 to 16.3% of the total sterols) in the oligodendrogliomas. The other gliomas, both isomorphous and polymorphous, exhibited a larger variability in desmosterol content. Desmosterol was also found in a glial focus (precancerous). Neurinomas were rare in this series, and only the spinal one contained appreciable amounts of desmosterol. A spinocellular carcinoma also had a high percentage of desmosterol. Desmosterol was not detected in samples of normal brain and spinal cord obtained from the same animals.

Desmosterol was also detected (2 to 16% of the total sterols) in 4 glioblastomas of the rabbit (kindly supplied by Dr. Osske, Erfurt, Germany) obtained by chronic administration of MNU (9). One sarcoma of the same series contained less than 1% desmosterol.

Table 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Desmosterol (%) total sterols</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 oligodendrogliomas</td>
<td>12.5, 16.3, 8.0, 12.8, 6.4</td>
</tr>
<tr>
<td>2 isomorphous gliomas</td>
<td>2.0, 13.7</td>
</tr>
<tr>
<td>4 polymorphous gliomas</td>
<td>2.8, 8.0, 7.9, 15.7</td>
</tr>
<tr>
<td>3 glioblastomas</td>
<td>4.9</td>
</tr>
<tr>
<td>1 spinal neurinoma</td>
<td>4.3</td>
</tr>
<tr>
<td>2 Gasserian neurinomas</td>
<td>n.d.</td>
</tr>
<tr>
<td>1 spinocellular carcinoma</td>
<td>14.3</td>
</tr>
<tr>
<td>Normal brain samples</td>
<td>n.d.</td>
</tr>
<tr>
<td>Normal spinal cord samples</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

except for 3 cases out of 23, contained large amounts of desmosterol.

The presence of desmosterol in many of the analyzed tumors induced by nitrosourea derivatives confirms our previous finding that this sterol is a biochemical constant of tumors of the nervous system (4). This is particularly true in the case of the experimental oligodendrogliomas, which consistently showed a high desmosterol content. The large variability in desmosterol content in the other types of gliomas may be due to the difficulty in obtaining adequate samples for biochemical analysis, since these tumors are often mixed with infiltrated brain tissue and kill the animal in a very short time, before the tumors reach a consistent size. The presence of large amounts of desmosterol in the experimental neurinomas is in contrast to the absence of this sterol in the samples of human neurinomas which have been analyzed. Human neurinomas are, in general, slow growing and rarely malignant, while the animal neurinomas induced by MNU and ENU are very rapidly proliferating tissues, indicating that the presence of desmosterol may be related to the immaturity and growth velocity of the tumors.

In general, the histological and biochemical similarities between these experimental tumors and human tumors of the nervous system, associated with rapid growth, indicate that tumors induced with nitrosourea derivatives are suitable experimental models for studying the neurochemistry of brain tumors.

ACKNOWLEDGMENTS

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Desmosterol in Induced Brain Tumors


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