The production of ocular and orbital neoplasms by intra-arterial inoculation of tumor cells from the Walker carcinosarcoma 256

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The behavior of tumor cells in the ocular and orbital tissues was studied by injecting cell suspensions from Walker carcinosarcoma 256 into the right common carotid artery of Wistar rats. The animals were sacrificed at different postoperative periods up to 12 days, and the intra- and extracellular tissues were examined microscopically. Tumors developed in the ocular and orbital tissues in about one third of the animals, but only after a latent period of 3 to 5 days. During this time isolated tumor cells or clumps of 2 to 4 cells were detected but never any larger cell collections. Most of the ocular and orbital structures excepting the cornea, lens, and vitreous were involved. The right orbit, right eye, left orbit, and left eye were affected in that order of frequency. The low incidence of tumors on the left side probably resulted from most of the circulating tumor cells being trapped in the lung tissues.

The purpose of the present experiment was to study the spread, behavior, and fate of tumor cell emboli in the eye and orbital tissues.

In a previous experiment it was not possible to produce ocular or orbital metastasis in Wistar rats by intramuscular injections of a suspension of tumor cells from Walker carcinosarcoma 256, although, when the tumor cells were injected directly into the eye and orbital tissues, malignant tumors developed in every instance at the site of inoculation. It is quite probable that the failure of metastasis was due to most of the cells being trapped in the lungs. In the present experiment, we proposed to inject the tumor cells directly into the carotid system in order to give them the shortest possible route to the eye and orbital tissues.

Unlike that in man, in the rat the main blood supply to the eye and orbit comes from the external carotid artery by way of the internal maxillary from which arises the external ophthalmic artery. The latter divides into several branches to supply the eye muscles and orbital contents, and, via the long and short ciliary arteries, supplies the globe. The internal ophthalmic artery derives from the circle of Willis, i.e., ultimately from the internal carotid artery, and supplies the retina as the central retinal artery and also anastomoses with the nasal long ciliary artery.2

A tumor embolus may consist of a large number of tumor cells, a clump of a few or even a single cell. It may die or remain sterile in the site of arrest. A metastatic
growth develops only if the transported cells survive, multiply, and effect extravascular extension. The most critical phase in the life of a blood-borne embolus is the period immediately following its arrest.³

Methods and materials

A tumor cell suspension was made by the following technique: A Wistar rat bearing a 10-day-old Walker carcinosarcoma 256 in the gluteal muscle was obtained from the Banting and Best Department of Medical Research. The animal was sacrificed and the tumor exposed by dissection. The capsule of the growth was opened and a piece of tumor, about 3 cm., was removed. This was then finely broken down in a tissue grinder in 2 ml. of physiologic saline containing 1,000 units of penicillin. To exclude any coarse particles, the cell suspension was filtered through 4 layers of surgical gauze. The filtrate was then diluted with saline so that 1 ml. of the suspension contained about 700 cells. All the procedures were carried out under sterile conditions.

Thirty-five Wistar rats, each weighing approximately 250 grams, were used in this experiment. The right common carotid artery of each animal was exposed, and 0.5 ml. of the tumor cell suspension was injected into it with a 30 gauge needle. A preplaced ligature tied proximal to the site of injection prevented transmission of any tumor cells toward the heart.

The animals were divided into 5 equal groups to be sacrificed in turn on the second, fourth, sixth, ninth, and twelfth days after injection. At autopsy both eyes, the contents and bony walls of both orbits and other tissues including the heart, lungs, kidney, liver, spleen, meninges, and the brain of each rat were removed, and fixed in Bouin’s solution. Paraffin sections of the tissues were made and stained with hematoxylin and eosin. At least eight different regions of each specimen were examined microscopically.

Results

After intra-arterial injections of cancer cells, tumors developed in various parts of
Table 1. Incidence of tumor cell and tumor formation in right eye and orbit on day of sacrifice

<table>
<thead>
<tr>
<th>Tumor cell</th>
<th>Tumor formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 day</td>
<td>4 day</td>
</tr>
<tr>
<td>A.C.</td>
<td>0</td>
</tr>
<tr>
<td>P.C.</td>
<td>0</td>
</tr>
<tr>
<td>Iris</td>
<td>0</td>
</tr>
<tr>
<td>Ciliary body</td>
<td>2</td>
</tr>
<tr>
<td>choroid</td>
<td>3</td>
</tr>
<tr>
<td>retina</td>
<td>0</td>
</tr>
<tr>
<td>sclera</td>
<td>0</td>
</tr>
<tr>
<td>cornea</td>
<td>0</td>
</tr>
<tr>
<td>lens</td>
<td>0</td>
</tr>
<tr>
<td>vitreous</td>
<td>0</td>
</tr>
<tr>
<td>lacrimal glands</td>
<td>0</td>
</tr>
<tr>
<td>Deep orbital tissues</td>
<td>0</td>
</tr>
<tr>
<td>extraocular muscles</td>
<td>0</td>
</tr>
<tr>
<td>Subconjunctival tissue</td>
<td>1</td>
</tr>
<tr>
<td>Orbital bone marrow</td>
<td>0</td>
</tr>
<tr>
<td>Blood vessels</td>
<td>0</td>
</tr>
</tbody>
</table>

The body including the eye and the orbit. A latent period which varied from 3 to 5 days preceded the development of a tumor. Ninety-five per cent of the animals developed growths in the lungs, 40 per cent in the brain and meninges, and 40 per cent in the eye and orbital tissues. In the majority of the animals, multiple structures were involved. Most of the animals with eye and orbital tumors had brain and meningeal lesions. The right orbit, right eye, left orbit, and left eye were affected in that order of frequency. The nature of the lesions in some of the eye and orbital tissues has been shown in the photomicrographs (Figs. 1 to 10).

The results obtained in the right eye and orbital tissues have been summarized in Table I. The numbers in the table show how many animals in one particular group were affected by tumor cells or by tumor formations. A tumor cell means either a single isolated cell or a clump consisting of not more than 4 cells. Any collection of more than 4 tumor cells has been considered a tumor formation. The table also

Fig. 5. Isolated tumor cells in the choroid. (Hematoxylin and eosin, x550; reduced %.)

Fig. 6. A single tumor cell in the retina. (Hematoxylin and eosin, x350; reduced %.)
shows which of the different tissues of the eye and orbit were involved. The ciphers in the table do not necessarily indicate total immunity from tumor cells or tumor formations, as the specimens were studied in random sections.

As shown in Table I, tumor cells were detected in the right intraocular structures as early as the second day, but tumor formations were not observed before the sixth day. The choroid and ciliary body were the favorite sites for the lodgment of tumor cells. Most of the intraocular tissues were involved except the cornea, lens, and vitreous. In the right orbital tissues the tumor cells were detected as early as the second day, but the tumor formations were first observed on the fourth day. Most of the different structures of the orbit were involved, including the lacrimal glands and extraocular muscles.

On the left side, the earliest appearance of the tumor cells in the intraocular tissues was on the second day. The ciliary body, choroid, retina, and sclera were involved. Definite tumor formations were not observed in any of the intraocular specimens. In the left orbital tissues, both tumor cells and tumor formations were first detected on the sixth day. The structures involved were generally the same as those in the right orbit.

Discussion

Two hypotheses have been advanced for explaining the distribution of metastatic tumors in different anatomic sites and especially for the scarcity of metastasis in

Fig. 7. A tumor formation in the subconjunctival tissue near the fornix. (Hematoxylin and eosin. ×350; reduced %.)

Fig. 8. A tumor formation in an orbital blood vessel. (Hematoxylin and eosin. ×450; reduced %.)

Fig. 9. A tumor formation in an extraocular muscle. (Hematoxylin and eosin. ×450; reduced %.)

Fig. 10. A tumor formation in an orbital bone. (Hematoxylin and eosin. ×450; reduced %.)
Tumor cells in ocular and orbital tissues

Certain organs. According to the first hypothesis, the establishment of tumor emboli in a particular location and their subsequent development into metastatic tumors are determined mainly by the local biochemical factors. According to the other hypothesis, the distribution of metastatic tumors depends chiefly on the circulation, i.e., on the number of tumor emboli reaching and lodging in a particular organ.

Observations in our present experiment give support to the second view. The ocular and orbital tissues in man are generally considered unfavorable sites for tumor metastasis. It has been assumed that the relatively low frequency of metastasis in these tissues is due to the peculiar origin of the ophthalmic artery. By injecting tumor cells directly into the carotid system, it has been possible to demonstrate that the intra- and extraocular tissues are quite susceptible to tumor formation. The majority of the animals with eye and orbital tumors had lesions in the brain and meninges. This may indicate that there is some anatomic similarity in the blood vessels beyond the site of injection.

There is a specific difference in the distribution of tumor metastasis which could be explained by differences in vascular anatomy. In man, metastatic tumors of the ciliary body, lacrimal gland, and orbital muscle are rare. The high frequency of tumor formations in these tissues in the Wistar rats is most likely to be due to peculiarities of the rat's vascular system.

The low incidence of tumors in the left ocular and orbital tissues probably resulted from the failure of tumor cell emboli to reach the capillaries of these structures in sufficient numbers. It is quite probable that following intra-arterial injection on the right side most of the circulating tumor cells were trapped in the lung. It is hard to explain the low frequency of tumors on the left side on the assumption of some biochemical difference between the two eyes.

Some controversy exists regarding the possibility of the development of a tumor from a very small number of cells. According to one school, metastatic tumors can derive only from emboli consisting of a large number of cells, whereas according to the others, a clump of 2 to 4 cells or even a single cell is sufficient to give rise to a tumor.

The findings in this experiment tend to support the latter view. Before the detection of a definite tumor formation, a latent period varying from 3 to 5 days was observed. During this period only isolated single cells or clumps of 2 to 4 cells were noted, but never any larger cell collections.

According to Reese, in the terminal stage of malignant diseases subclinical metastasis of carcinoma in the uvea is common. Viable cancer cells have been detected in blood obtained from the chambers of the heart at autopsy.

In view of this and of the possibility that eye tumors may develop from a very small number of cells, one might well hesitate to use ocular donor material from cancerous individuals. At least one case is on record in which an ocular cancer was transmitted via corneal graft.

Long-term, careful, and systematic studies on eye patients who have received material from cancerous donors could clarify the doubt. But until adequate, properly controlled data are available conclusions have to be drawn, to a large extent, from investigations performed on animals.

REFERENCES

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