FOREIGN-BODY TUMORIGENESIS IN RATS
BY VARIOUS KINDS OF PLASTICS - INDUCTION
OF MALIGNANT FIBROUS HISTIOCYTOMAS

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SUMMARY

Five kinds of plastics (3 polyvinyl chlorides, 1 polyhydroxyethyl metacrylate
and 1 dimethyl polysiloxane) were implanted into subcutaneous tissues of Wistar
rats of both sexes. Subcutaneous tumors developed in all experimental groups.
The incidences of the tumors, however, differed from each other, although
these materials were tested on the same experimental condition. This result
indicates that chemical characters of the materials may influence the incidence
of subcutaneous tumors. Histologically, most of these subcutaneous tumors
were mesenchymal tumors with spindle cells arranged in a storiform pattern,
with sheets of histiocyte-like cells or pleomorphic giant cells. Electron micro-
scopy showed mixture of fibroblastic cells, histiocytic cells and undifferentiated
cells in these tumors. From these histological and electron microscopical
findings, many of the tumors were diagnosed as malignant fibrous histio-
cytomas.

Key words: Foreign-body tumorigenesis, Plastics, Malignant fibrous histio-
cytoma, Rat
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Turner (1941) first reported foreign-body tumorigenesis and there are many subsequent reports on this subject (Bischoff and Bryson 1964; Brand et al. 1975a, 1975b, 1977; Carter et al. 1971; Druckrey and Schmähl 1952; Hueper 1964; Nothdurft 1955, 1958; Oppenheimer et al. 1948, 1955; Stinson 1964). These workers reported that the size and/or shape of implanted materials was the most important factor in induction of tumors. On the other hand, concerning the histology of foreign-body tumors, Oppenheimer et al. (1955) reported earlier that almost all were fibrosarcomas. Thereafter, Johnson et al. (1973) suggested that these tumors arose from pluripotential mesenchymal cells.

In the present study, we compared the tumorigenic activities of five kinds of plastics (3 polyvinyl chlorides, 1 polyhydroxyethyl methacrylate and 1 dimethyl polysiloxane), that are used clinically, by implanting them into the subcutaneous tissue of rats and investigated the morphological findings of the induced subcutaneous tumors by light and electron microscopy. A short report on this subject is to be published (Maekawa et al. 1984).

MATERIALS AND METHODS

Five kinds of sterilized sheet materials of 0.3-0.5 mm in thickness; 3 plasticized polyvinyl chlorides (PVC), 1 polyhydroxyethyl methacrylate (HEMA), and 1 dimethyl polysiloxane (silicone), were provided by Dr. T. Ooba, Department of Medical Supplies, National Institute of Hygienic Sciences, Tokyo. These sheet materials were cut into 10 × 20 mm pieces with scissors before use.

Eleven weeks old Slc: Wistar rats, purchased from Shizuoka Laboratory Animal Center (Hamamatsu), were divided into five experimental groups each consisting of 25 males and 25 females, and 1 control group consisting of 15 males and 15 females: Group 1, PVC-1; Group 2, PVC-2; Group 3, PVC-3; Group 4, HEMA; Group 5, silicone and Group 6, control. The test materials were implanted in the subcutaneous tissues (the interscapular region) of animals in each experimental group. All animals were maintained on CE-2 basal diet (CLEA Japan Inc., Tokyo) and tap water.

For observation of early histological changes in the subcutaneous tissues in which materials were implanted, 5 males and 5 females in each experimental group, and 3 males and 3 females in the control group were sacrificed at week 13 after implantation. The remaining animals were allowed to live for 2 years, and then all survivors were sacrificed. Dead or moribund animals were autopsied completely, and examined for development of tumors in the subcutaneous tissue and other organs. Tumors and all organs were fixed in buffered 10% formalin and the sections were routinely stained with hematoxylin and eosin. Some of subcutaneous tumors were stained with PAS, PTAH, Azan, van-Gieson and silver stains, in addition to hematoxylin and eosin. Several samples of the tumors were also examined by electron microscopy.
RESULTS

At week 13, all implanted materials were covered with thin capsules. Histologically, these capsules were composed of hyalinized connective tissues. Slight infiltration of small round cells and macrophages, and proliferation of capillary vessels and fibroblast-like cells were observed, but no atypical cell growth was detected in any rats. The maximum thickness of these capsules in each group was calculated and it was about 0.21-0.30 mm, and there was no difference on the mean thickness among the experimental groups.

The incidences of total and subcutaneous tumors and mean survival times in each group are shown in Table 1. The first tumor was seen in a rat autopsied in week 32 after implantation. All rats surviving beyond this time were counted in effective numbers, except several rats in which autolysis was too advanced to allow histological examination. In males, there was no significant difference in the incidences of total tumors between each experimental and control group. On the other hand, in females, the incidences of total tumors in all experimental groups except Group 5 were higher than that in the control group, and the incidences in Group 3 and 4 were significantly higher than that in the control group ($\chi^2$ test, $p<0.05$). Subcutaneous tumors were detected in all groups except the control group, although the incidences differed in each

Table 1  Incidences of Subcutaneous Tumors in Rats After Implantation of Various Kinds of Plastics

<table>
<thead>
<tr>
<th>Group</th>
<th>Material</th>
<th>Sex</th>
<th>No. of rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>initial effective</td>
</tr>
<tr>
<td>1</td>
<td>PVC-1</td>
<td>M</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>PVC-2</td>
<td>M</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>PVC-3</td>
<td>M</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>HEMA</td>
<td>M</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>silicone</td>
<td>M</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>control</td>
<td>M</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>12</td>
</tr>
</tbody>
</table>

* Significant difference from females in Group 6 ($\chi^2$ test, $p<0.05$).
** Significant difference from each sex in Group 5 ($p<0.05$).
*** Significant difference from females in Group 5 ($p<0.01$) and in Group 1 ($p<0.05$).
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group. The incidence was highest in Group 3, followed in order by those in Group 4, Group 2, Group 1 and Group 5. The incidences in males of Group 3 and both sexes of Group 4 were significantly higher than those in each sex of Group 5 ($\chi^2$ test, $p<0.05$), and the incidence in females of Group 3 was higher than those in females of Group 5 ($p<0.01$) and Group 1 ($p<0.05$).

All subcutaneous tumors were detected in the back where samples were implanted. Almost all implanted samples were involved in the tumors. Samples were sometimes found folded or rolled in the tumors and many films of the materials other than silicone were brittle. Tumors were round or oval in shape and white or brown in color. The tumors differed in size: the largest weighing 150 g measured $87 \times 67 \times 68$ mm, and the smallest was the size of a soy-bean. Histologically, these subcutaneous tumors were variegated, but there was no histological differences in the groups. Almost all were mesenchymal tumors with spindle cells arranged in a storiform pattern, with sheets of histiocyte-like cells, or pleomorphic giant cells (Photos 1-3). In some cases, matrix of the tumors showed myxoid or osteoid-like and in 2 cases ossification was marked. Many mitotic figures were observed. No cross striation was detected in any tumors. Nine samples of a total of 59 subcutaneous tumors were examined by electron microscopy. Electron microscopy showed a mixture of fibroblast-like cells, histiocyte-like cells and undifferentiated cells in these tumors. Fibroblast-like cells were mostly elongated or polygonal in shape and had smooth or slightly lobulated nuclei. Their cytoplasm contained abundant rough-surfaced endoplasmic reticulum and also other cytoplasmic organelles in various developmental stages. On the other hand, histiocyte-like cells varied in shape and had round or reniform nuclei. The cytoplasm contained abundant ribosomes and moderate amounts of smooth or rough surfaced endoplasmic reticulum. Dilated endoplasmic reticulum and intracytoplasmic actin-like filaments were observed in some cases. From these histological and electron microscopical findings, 53 out of 59 subcutaneous tumors were diagnosed as malignant fibrous histiocytomas, as shown in Table 2. As other subcutaneous tumors, 5 fibrosarcomas and 1 fibroma were detected. Only 7 of 59 tumors metastasized to the remote organs such as the lung, and 6 of these 7 tumors were malignant fibrous histiocytomas.

In addition to subcutaneous tumors, many other tumors were observed in all groups including the control group. Tumors of the testis/uterus and leukemias were the most common and were detected in all groups. Tumors were also detected in various organs, but at low incidences. In any experimental groups, no significant increase in the incidence of these tumors was observed. Histological findings of these tumors were quite similar to those of spontaneous tumors observed in this strain of rats, as reported previously (Maekawa et al. 1983).

In the back of rats without subcutaneous tumors, implanted samples were enclosed in capsules. The thickness of these capsules were the same or slightly more than that of capsules at week 13. In some cases, however, atypical cell proliferation was detected in the inner layer of the capsules (Photo 4) and some of these lesions were diagnosed as
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pre-neoplastic or early neoplastic changes. These lesions were observed in all experimental groups, although the incidences were low and there was no difference in each group.

Photo. 1. Malignant fibrous histiocytoma (fibroblastic type) with prominent storiform pattern, observed in a female rat in PVC-1 group killed at the 86th week. Hematoxylin and Eosin. (×100)

Photo. 2. Malignant fibrous histiocytoma (histiocytic type) of a male rat in HEMA group killed at the 40th week. Diffuse proliferation of histiocytic cells with glassy cytoplasm is prominent. H & E. (×100)
Photo. 3. Malignant fibrous histiocytoma (pleomorphic type) of a male rat in PVC-3 group killed at the 46th week. Pleomorphic giant cells with bizarre nuclei admixed with histiocyte-like cells are shown. H & E. (×200)

Photo. 4. A capsule of a female rat without subcutaneous tumors in PVC-2 group killed at the 89th week. Atypical cell proliferation is observed at the inner layer of the capsule. H & E. (×200)
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**Table 2** Histological Classification of Subcutaneous Tumors Induced by Various Kinds of Plastics

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Number</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant fibrous histiocytoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fibroblastic type</td>
<td>28 (4)*</td>
<td></td>
</tr>
<tr>
<td>histiocytic type</td>
<td>15 (2)*</td>
<td></td>
</tr>
<tr>
<td>pleomorphic type</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Other tumors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibroma</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Fibrosarcoma</td>
<td>5 (1)*</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>59 (7)*</td>
<td></td>
</tr>
</tbody>
</table>

* No. of rats with metastasis to remote organs

**DISCUSSION**

In this study, many tumors including subcutaneous tumors were observed. Except subcutaneous tumors, other tumors such as tumors of the testis, uterus, hematopoietic organs and endocrine organs are observed spontaneously at relatively high incidences in this strain of rats (Maekawa et al. 1983). All subcutaneous tumors, however, were detected in the back where samples were implanted and almost all implanted samples were detected in the tumors. Therefore, all subcutaneous tumors observed in this study were considered as induced tumors by implantation of polymers.

Earlier studies by Oppenheimer et al. (1948, 1955) and others (Bischoff and Bryson 1964; Brand et al. 1975a, 1975b, 1977; Carter et al. 1971; Druckrey and Schmähl 1952; Hueper 1964; Imai and Masuhara 1979; Nothdurft 1955, 1958; Stinson 1964; Turner 1941) showed that subcutaneous sarcomas could be induced in rats and mice by implantation of many kinds of foreign bodies, and the physical presence and nature of the foreign body, not its chemical reactivity, were responsible for tumor development. These workers also reported that the size and/or shape of implanted materials was important factors in induction of tumors, and all kinds of sheet materials of more 10 mm in diameter produced tumors. In this study, test materials as sheets of 10×20 mm induced tumors in all experimental groups. This result was consistent with the data of others.

In this study, however, the incidences of the tumors were different in groups, although these materials were tested on the same experimental condition. The reason for the difference in the tumor-incidence is obscure. But medical grade PVC materials are known to contain many plasticizers, and the types and amounts of plasticizers in PVC differ with the manufacturer (Watanabe et al. 1978). HEMA is reported to be degraded in subcutaneous tissues and it seem rather active and unstable in the animal body (Kojima et al. 1974). On the other hand, silicone has been said to be stable. These
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chemical characters of the materials may influence the incidence of subcutaneous tumors, although the chromosomal aberration tests in mammalian cells and the mutation tests in microorganisms with extract from the same materials used in this study were all negative.

Johnson et al. (1973) suggested that subcutaneous tumors induced by implantation of foreign-bodies arose more often from the non-fibroblastic pluripotential mesenchymal cells than fibroblasts, although Oppenheimer et al. (1955) reported earlier that almost all of these foreign-body sarcomas were fibrosarcomas, and Carter (1973) reported also that fibrosarcomas are the most frequent soft-tissue tumors in rats. On the other hand, it has been suggested during the last 2 decades that malignant fibrous histiocytomas are the most common type of soft-tissue tumors in humans. In our study, many subcutaneous tumors had the same characteristics to histological and electron microscopical findings of malignant fibrous histiocytomas in humans, and also those of the tumors induced by subcutaneous injections of chemical carcinogens in rats (Konishi et al. 1982; Maekawa et al. 1982; Takahashi et al. 1982). These findings indicate that malignant fibrous histiocytomas are the most common subcutaneous tumors not only in humans but also in rats.

Karp et al. (1973) reported that tissue anoxia was not so important in foreign-body tumorigenesis. Brand et al. (1975a) reported several stages in foreign-body tumorigenesis: preneoplastic cells accumulate in the tissue outside of the capsule at an early stage and then they migrate into the inner layer of the capsule, attach to the foreign-body and proliferate. Imai and Masuhara (1979) reported that the thickness of the capsule is very important in foreign-body tumorigenesis. In this study, histological observation of subcutaneous tissues in rats without subcutaneous tumors indicate that atypical cell growth or preneoplastic changes occurred first not in the tissue outside of the capsule, but in the inner layer of the capsules. This finding suggests that the capsule produced around the foreign-body has the most important role in foreign-body tumorigenesis, as reported by others.

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REFERENCES


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