Oncolyis by Clostridia

V. Transplanted Tumors of the Hamster

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SUMMARY

Treatment of transplanted tumors of the hamster with nonpathogenic clostridia were reported. The intravenously injected spores germinated in the tumors and produced within very few days an extensive or complete lysis of the tumor. A cure or increase in survival time has not been obtained so far. The possibilities and limitations of this novel biological tumor treatment have been discussed.

MATERIALS AND METHODS

Tumors.—The tumor material used was derived from the hamster tumors which have been described by Fortner et al. (1, 2) and had been carried for about a year in this laboratory. The following lines were used: amelanotic melanoma #3 (A. Mel. #3), amelanotic melanoma #4 (A. Mel. #4), renal adenocarcinoma #1 (R. Ca. #1), adrenal cortical carcinoma #4 (Ad. Ca. #4), cystadenocarcinoma of the liver #1 (Hep. #1).

A suspension of tumor in physiological saline (Hanks BSS) was prepared (1:2 or 1:5), and 0.5 ml. of the suspension was transplanted subcutaneously into the left flank of the animals. The period for the development of nodules of optimal size varied from 8 to 14 days, depending on the type of tumor. The A. Mel. #3 grew slowly, but the A. Mel. #4 showed pronounced formation of metastases even while the primary tumor was still small, and the animals died within 8 days (for further details see Fortner et al. [1, 2]).

Therapy.—Animals with tumors which were growing well received single intravenous injections of 100–200 million spores of Cl. butyricum (M-55). The time of injection was chosen in such a manner that optimal oncolysis could be expected to occur within the normal survival time of the tumor-bearing animals. The optimal times of treatment after transplantation were on the following days: For the A. Mel. #3, on days 10–11; for the A. Mel. #4, on day 3; for the R. Ca. #1 and the Ad. Ca. #4, on days 4–7; and for Hep. #1 on about day 7.

In survival time experiments the experimental results were evaluated by recording the average survival time, the number of tumors lysed, and the percentage of oncolysis occurring in the primary tumor. Smears of the lysed tumors were prepared in each instance and stained with methylene blue for the demonstration of vegetative forms of clostridia. The histological examinations were primarily done on the A. Mel. #3.

Combination therapy.—In a few experiments additional treatments were given with aerobic spore formers, ethyleniminobenzoquinones, tumor extracts, iron dextran complexes, lipopolysaccharides, anti-histaminics, and high doses of vitamin C.

Behavior of clostridia in normal hamsters.—To establish whether normal hamsters can tolerate therapeutic doses of clostridia without side effects, twenty animals of either sex weighing between 50 and 70 gm. were given injections intravenously of 100 million spores in 0.5 ml. physiological saline. The animals were killed by exsanguination under light anesthesia at 1, 3, 5, 9, 11, and 14 days, and at 3, 5, and 10 weeks after the injection. Six animals served as controls and were killed at the end of the experiment.

Bacteriological and histological procedures.—To demonstrate the presence of clostridia in heart, liver, spleen, or kidney, organ pieces were incubated in an anaerobic medium which was made up of equal parts of trypticase soy broth and anaerobic agar. Any turbidity which developed was graded from + to ++++ after 2–3 days' incubation, and smears from the media were stained according to Gram. Frozen sections and paraffin sections were prepared from the same organs and stained with hematoxylin-eosin, Sudan Black B, and Gram. The Gram stain was used according to the method recommended by Pearse (7) (slightly modified)—which is essentially the Eosin-Gram method of Weigert.

Behavior of clostridia in tumor-bearing hamsters.—These experiments were designed to demonstrate whether organ lesions occur during the course of lysis of the primary tumor, which would point to a toxic influence of the tumor lysate. Twenty golden hamsters bearing the A. Mel. #3, measuring 12–18 mm., received 100 million spores intravenously 12 days after subcutaneous transplantation.
Ten animals served as untreated controls. The animals were killed by exsanguination, under light anesthesia, 1, 2, 3, 4, 5, 8, and 11 days afterwards. Smears of the lysed tumors were stained according to Gram, and frozen and paraffin sections of macroscopically visible metastases as well as pieces of heart, lung, liver, spleen, and kidney were stained with hematoxylin-eosin and with Sudan Black B. Aliquot pieces of the same organs were put on an anaerobic culture medium, and turbidity and smears were evaluated in the same manner as previously stated for normal animals.

RESULTS

Normal hamsters.—The tolerance of normal hamsters for clostridia was high; clinically no disease symptoms of any sort were observed during the course of the experiment. Within 14 days following injection, all organ pieces regularly showed germination of the spores in culture, with considerable turbidity of the medium within 2–3 days. At later periods clostridia could be obtained only irregularly, with organisms being demonstrable in liver and kidney for a maximal period of 10 weeks. In smears of the culture medium spores, sporangia and rods were found in large amounts.

Histological investigations on heart, liver, spleen, and kidney did not show any pathological finding which could be ascribed to a specific effect of the spores on these tissues. In none of the sections could any forms of clostridia be demonstrated by Gram staining. Since spores are frequently gram-negative, only germination and presence of vegetative forms could be excluded with certainty.

Experiments with clostridia on tumor-bearing hamsters.—The tumors used showed a variable but on the average considerable turbidity of the medium within 2-3 days. In smear preparations of the lysed tumors large numbers of rods were found in each case besides spores and sporangia.

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TABLE 1

Oncolysis of Transplanted Hamster Tumors by Spores of *Clostridium Butyricum* (M-55)*

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Time after transplantation (days)</th>
<th>Tumor diameter (mm.)</th>
<th>Spore dose in millions</th>
<th>No. with lysis</th>
<th>Extent of lysis (per cent)</th>
<th>Av. survival time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Mel. #3</td>
<td>11</td>
<td>18-22</td>
<td>1 × 200</td>
<td>10/10</td>
<td>95</td>
<td>10.6</td>
</tr>
<tr>
<td>A. Mel. #4</td>
<td>3</td>
<td>2-3</td>
<td>1 × 200</td>
<td>7/10</td>
<td>13</td>
<td>6.9</td>
</tr>
<tr>
<td>R. Ca. #1</td>
<td>4</td>
<td>8-12</td>
<td>1 × 200</td>
<td>10/10</td>
<td>67</td>
<td>6.5</td>
</tr>
<tr>
<td>Ad. Ca. #4</td>
<td>4</td>
<td>8-12</td>
<td>1 × 100</td>
<td>10/10</td>
<td>76</td>
<td>9.5</td>
</tr>
<tr>
<td>Hep. #1</td>
<td>7</td>
<td>12-18</td>
<td>1 × 200</td>
<td>10/10</td>
<td>95</td>
<td>9.4</td>
</tr>
</tbody>
</table>

* These experiments were reproducible with different spore batches in each tumor and with good agreement with the results in this table.

The clinical course of the oncolysis of the tumors was rather characteristic. In tumors which responded well, already within the 1st or 2nd day a circumscribed softening and liquefaction occurred that progressed relatively rapidly and reached its maximum in a very short time. At this point, or sometimes even a little earlier, destruction of superficial cutaneous musculature, subcutaneous tissue, and the epidermis occurred. Any marginal areas which still remained were as a rule not further attacked. An extension of the oncolysis to the abdominal musculature with breakthrough into the peritoneum was, however, never observed. The regional lymph node metastases in the axilla were frequently included in the oncolytic process and were partially or completely lysed. The whole process occurred somewhat more slowly in less sensitive tumors.

An example of the extent of oncolysis that can be obtained for instance in the A. Mel. #3 is given by Figures 3 and 4, showing a saline control and a spore-treated animal.

The experimental results are summarized in Table 1. Although the values for the per cent lysis refer solely to animals in which tumor lysis occurred, the data on survival time refer to all animals in the experimental group and are calculated from the day of the start of treatment.

In smear preparations of the lysed tumors large numbers of rods were found in each case besides spores and sporangia. The essentially negative results obtained with the A. Mel. #4 were primarily due to the fact that the clinical course leading to rapid death of the animals did not permit the optimal lytic activity of the clostridia to occur.

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At the height of oncolysis sudden death of the animals occurred in most cases. Since death was dependent on the extent and the rapidity of the lytic process, it was presumable due to intoxication of the organisms by lysis products which were derived either from the clostridia or from the tumor cells. The intoxication was the reason why the survival time of the treated animals was generally less and never longer than that of the untreated controls.

Only an occasional animal survived the clinically complete lysis of the primary tumor. These usually died later as a result of local recurrence or macroscopically visible metastases to the lymph nodes in the peritoneal area. Recurrences were generally not influenced by further treatment with spores; larger lymph node metastases showed, however, frequently incomplete oncolysis with perforation into the intestinal canal or into the peritoneal cavity with the formation of a diffuse peritonitis. Efforts to compensate or alleviate the toxic effect on the
organisms as a whole with antihistaminic preparations and with high doses of vitamin C have failed so far. Similarly, reduction of the spore doses did not lead to an improvement in the final results. In the case of the A. Mel. #3, even when the dose was lowered to 3 million spores, almost no loss in effectiveness was observed, and the clinical course was the same as with higher doses. At 1.5 million spores, however, a clear decrease in effectiveness was observed.

The histological examination of organs of spore-treated hamsters bearing the A. Mel. #3 did not give any indication that any damage was produced either by clostridia or their lytic products during the course of oncolysis. In control as well as in experimental animals, liver, heart muscle, and kidney showed a more or less variable lipide infiltration which was independent of the duration of the experiment. A slight hyaline swelling of the kidney epithelium was also unrelated to treatment. There were no pathological findings in the lungs. Sections of the spleen showed a nonspecific inflammatory reaction with hyperemia, increased granulocytes, as well as a reticular hyperplasia. Although vegetative forms could not be demonstrated histologically, clostridia could be grown by culture from all organs during the indicated experimental period.

Again independent of the duration of the experiment or therapy with clostridia, microscopic metastases were found sometimes to a considerable extent in lungs, liver, heart, spleen, and kidneys. An increase in the extent of metastases which was originally suspected to be due to oncolysis was not confirmed by more careful examination of all the histological preparations. Rods could not be found in the metastases in the organs, and the rods which were found in the regional lymph node metastases probably reached there by direct contact with the lysed primary tumor.

Three of the hamsters which were killed on the 11th day after spore treatment showed rapidly growing recurrences from the remnants of the primary tumor, as well as metastases in the regional lymph node, although, clinically, oncolysis had previously been very extensive. This, and similar observations, suggest that an improved effect might be obtained by combined or additional therapy with cytostatic substances. Experiments in this direction have so far not given an improvement in the results in hamsters. Thiele, Arison, and Boxer (8), however, reported that synergistic effects of alkylating agents with spores could be obtained in mouse tumors.

The histological examination of the tumors that underwent lysis has not indicated so far that the clostridia act directly for the formation of granulation tissue. In each case vegetative forms of clostridia were histologically demonstrated in large amounts in the necrotic areas but never in appreciable numbers within the intact tumor tissue (Fig. 2).

DISCUSSION

The experimental results so far indicate that four out of the five hamster tumors responded well to therapy with clostridia. The process began within a few days and embraced more or less large parts of the tumors up to complete liquefaction and resorption.

The animals died at the height of the oncolysis due to intoxication of the organisms as a whole by degradation products or due to metastases. A morphological explanation for the toxic death of the animals has not been found so far, which makes it probable that humoral processes were primarily responsible. These processes occur so rapidly that no morphological expression is noticeable.

On the other hand, the findings in the case of the A. Mel. #3 showed that, at the time of the oncolysis, there were already extensive metastases in heart, lung, liver, and frequently also in spleen and kidney, which in their turn could be responsible for the death of the animals.

With the other tumors similar conditions were probably occurring. The life span of the control animals, in turn, was limited by the occurrence of the metastases and necrotic degradation products from the tumors. Therefore, in principle, the process which led to death was probably the same for control as well as experimental animals. In the animals with the lysed tumors, the toxic effects occurred in a much shorter time simply because the necrotic material is formed precipitously.

It must be emphasized that, in spite of the excellent influence of the treatment on the primary tumor in the beginning, the animals were not cured by this therapy and that a prolongation of life compared with control animals has not been obtained so far. Combination experiments with cytostatic compounds or with detoxifying preparations are under way, but so far no improvement in the effect has been observed.

Metastases in organs or lymph nodes, in contrast to those in primary tumors, were generally not affected by the spores unless they had reached a considerable size. These findings raise the question whether the tumor tissue itself can be considered as the specific place for germination of the anaerobic spores. The results so far point toward microscopic and macroscopic necroses as the place where truly anaerobic conditions prevail and germination of spores occurs. This was the case with primary tumors on the day of treatment and could be demonstrated histologically during the course of the experiment. In metastases, vegetative forms of clostridia and signs of oncolysis were found only occasionally when they reached a certain size, with consequent development of central necroses.

Aside from the necroses, the nonspecific defense mechanisms of the host and, particularly, of the host species play a major role, as demonstrated by experiments on rat tumors where clostridia in general do not germinate in the necroses (3).
The available results and experiences on hamster tumors lead to the conclusion that this new type of tumor treatment has not fulfilled the original hopes, particularly with respect to complete cures.

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REFERENCES

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