Intratumor Therapy in Rodents with Aqueous Clam Extracts

C. P. Li, N. M. Tauraso, B. Prescott, B. E. Eddy, R. C. Hoye, E. C. Martino, G. Caldes, and C. Gorschboth

SUMMARY

A therapeutic effect was observed when an aqueous extract of the common clam (Mercenaria mercenaria) was injected directly into and around small, superficial, and slowly growing solid s.c. adenovirus 12 and SV40 virus-induced tumors in hamsters. The treatment was given daily or at selected intervals. Half of 74 treated hamsters showed complete tumor regression within 4 weeks after the 1st injection. No systemic toxicity was evident. The hamsters were apparently normal during an observation period of 4 to 9 months following tumor regression. The remaining treated tumors grew after partial regression and killed the hosts. In parallel control studies, 75 tumor-bearing hamsters either were untreated or were treated with various placebos, including 8.5% NaCl solution and heated clam extract. All these control tumors grew to a large size and killed the hamsters. A limited number of melanomas in CDF1 mice were similarly treated with clam extract, and one-third showed complete regression.

INTRODUCTION

Cancer chemotherapy may be subject to a number of difficult problems. After administration, the drug may be deactivated by the liver or removed by binding to plasma proteins or by rapid excretion. Moreover, in general, tumors have a relatively poor blood supply, and drugs can reach the inner area of the tumor only by diffusion. Thus, although a drug may be a highly selective tumor inhibitor, it may not reach all the tumor cells in a high concentration. We therefore conducted experiments to determine the effect of intratumor therapy in hamsters with aqueous clam (Mercenaria mercenaria) extracts (3, 10). Preliminary results were reported previously (5). The extended studies are presented here. Preliminary results on similar treatment of melanoma in CDF1 mice are also included.

MATERIALS AND METHODS

Clam extracts. The clams from Chesapeake Bay were obtained in the summer months from a local market in Washington, D. C. The shucked clams, fresh or frozen, were homogenized in a Waring Blendor with an equal volume of cold, distilled water. The homogenate was stirred for 1 to 2 hr at 4°C and then centrifuged at 600 X g for 40 min at the same temperature. The supernatant fluid was dialyzed against distilled water (pH 7.0) for 72 hr at 4°C and then lyophilized. The yield usually varied from 2 to 5% of the wet weight of the clam and was brownish powder, forming a colloidal suspension in water. Its activity was lost or greatly reduced when heated for 30 min at 70°C or at a higher temperature. The active principle was nondialyzable and appeared to be either a glycoprotein or associated with glycoproteins, with a molecular weight estimated to be >10,000 (3). The lyophilized powder was stored at —20°C before use.

Primary Adenovirus 12 Tumors. Newborn or 1-day-old Syrian hamsters were inoculated s.c. on the upper back with 0.2 ml of cell culture virus diluted 1:2 or 1:3 in Hanks’ balanced salt solution. The Huie or Hollinshead strain of adenovirus 12 was used. Solid s.c. tumors developed usually within 4 to 10 weeks after virus inoculation (3, 4).

Implanted Adenovirus 12 Tumors. Young adult hamsters were inoculated s.c. with 10⁷ adenovirus 12 tumor cells. Tumors appeared usually within 10 days.

Implanted SV40 Tumors. Hamsters, approximately 30 days old, were inoculated s.c. on the upper back with 10⁷ adenovirus 12 tumor cells. Solid s.c. tumors developed usually within 40 days.

Melanoma. The CDF1 mice, 6 to 7 weeks old, were implanted s.c. in the lower back with approximately 350,000 melanoma (S-91) cells. Tumors appeared within 20 days.

Method of Treatment. Tumors of 0.5 to 1.0 sq cm were selected for treatment. Larger and fast-growing tumors were not used because preliminary studies revealed that such tumors did not respond to the treatment. The treatment consisted of a series of 4 to 12 injections. Unless otherwise stated, each injection contained 50 to 100 mg of the clam extract suspended in 0.5 or 1.0 ml of distilled water of 8.5% NaCl solution. In later experiments, 3 consecutive daily injections, 100 mg each, of the extract were found to be sufficient.

Peanut oil, protein suspensions such as milk, proteolytic enzymes such as trypsin, and clam extract heated to 70°C for 30 min or autoclaved at 121°C for 15 min were included in treating the controls.

Melanomas in CDF1 mice were similarly treated, the dosage of clam extract being stated in Fig. 3.

RESULTS

Adenovirus 12 and SV40 virus tumors. A total of 149 tumor-bearing hamsters were used in 25 experiments: 17 had primary adenovirus 12 tumors, 76 had transplanted adenovirus 12 tumors, and 56 had transplanted SV40 tumors. Among these 149 tumors, 74 were treated with clam extract, 44 were...
Local treatment of s.c. tumors in hamsters with clam extracts

Primary or transplanted s.c. solid adenovirus 12 and SV40 tumors were used for these experiments. The powdered clam extract resuspended in water or 8.5% NaCl solution was injected directly into and around the tumor. After a series of 3 to 10 injections given daily or at selected intervals, 50% of the tumors regressed through a process of ulceration (see Fig. 3) and the hosts survived. All control animals, untreated or treated with 8.5% NaCl solution or other placebos, died.

Table 1

<table>
<thead>
<tr>
<th>Tumor</th>
<th>No. of animals</th>
<th>Treated No.</th>
<th>Treated %</th>
<th>No. treatment No.</th>
<th>No. treatment %</th>
<th>Treated with placebo No.</th>
<th>Treated with placebo %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary adenovirus 12</td>
<td>17</td>
<td>6/12</td>
<td>50</td>
<td>0/5</td>
<td>0</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Transplanted adenovirus 12</td>
<td>76</td>
<td>20/38</td>
<td>53</td>
<td>0/23</td>
<td>0</td>
<td>0/15</td>
<td>0</td>
</tr>
<tr>
<td>Transplanted SV40</td>
<td>56</td>
<td>11/24</td>
<td>46</td>
<td>0/16</td>
<td>0</td>
<td>0/16</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>149</td>
<td>37/74</td>
<td>50</td>
<td>0/44</td>
<td>0</td>
<td>0/31</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of animals with regressed tumor/number of animals treated.

<sup>b</sup> ND, not done.

Fig. 1. Transplanted SV40 virus-induced tumors in 2 hamsters, 1 treated (above) and 1 untreated (below). Treatment started on Day 0. The 2 hamsters were photographed simultaneously on the indicated days after the 1st treatment. On Day 3, the treated tumor was somewhat swollen; it then showed a local reaction with ulceration and necrosis as described in the text. By Day 21, most of the ulcer was healed, the hair grew back, and the animal was apparently normal. There was no recurrence of tumor in the following 2 years. The untreated tumor grew progressively larger and finally killed the host on Day 45.

The tumor became swollen but soft within 24 hr after receiving the 1st injection of the clam extract. Occasionally, the solid tumor was completely dissolved, leaving a sac partially filled with fluid. Later, the overlying skin became moist, evidently due to oozing of fluid from within. With continuing injections, the tumors shrank to a dry black eschar that eventually separated, leaving a clean, shallow ulcer.

Although all of the 74 treated tumors showed the initial stage of the above-described reaction, in only 28 hamsters did the ulcer heal in 3 to 4 weeks, and complete recovery resulted. No tumor recurred during the following 4 to 9 or more months. A typical example is illustrated in Fig. 1.

In 29 treated hamsters, the tumors regressed for awhile and then grew again 14 to 75 days after the 1st treatment, usually at the original site and rarely in some distant area. These recurring tumors were then retreated with a course of 1 to 5 injections of 50 to 100 mg of the clam extract; 9 of these 29 retreated tumors regressed completely, while the other 20 animals did not respond and died. The remaining 17 treated hamsters showed no regression and died. Thus, of 74 treated hamsters, 37 or 50% were completely cured (Table 1).

In the control animals (44 untreated and 31 treated with placebos), the tumor grew to an enormous size, finally killing the hosts. Moreover, we have never observed spontaneous regression in hundreds of hamsters bearing these types of viral tumors.

Tumor Growth Curve. A growth curve of primary adenovirus 12 tumors in 6 hamsters, untreated or treated with
heated clam extracts, is plotted in Chart 1. There was no difference in the tumor growth curve whether the tumors were not treated or were treated with heated clam extracts, which evidently acted as placebos. Of the hamsters included in this experiment, 3 were treated with unheated clam extract, and their tumors all regressed completely.

**Cheek Pouch Tumor.** Occasionally, after s.c. injection of adenovirus 12 in the upper back region of the hamsters, a cheek pouch tumor developed, hanging out from the side of the mouth. Such tumors, like the s.c. ones, always killed the host. It was therefore of interest to observe a complete cure of such a tumor, as shown in Fig. 2. Another cheek pouch tumor was similarly treated without success.

**Body Weight of the Treated Hamsters.** It was previously reported (4) that treatment of tumor-bearing hamsters with clam extracts retarded their body growth to 20 to 25% below normal. This growth retardation did not happen in the present experiments. During the 10 days following the 1st treatment, the hamster often lost about 5% of its weight; thereafter, the animal grew normally.

The intratumor treatment did not prolong the survival time of those animals that showed no regression or only temporary regression. Sex did not seem to play any significant role in response to treatment. Primary and transplantable tumors seemed equally sensitive.

All the recovered animals have been apparently normal during the 4 to 9 or more months after treatment.

**Melanoma.** In preliminary experiments, 12 CDF1 mice bearing melanoma were treated with unheated clam extract. Local reactions similar to those observed in hamster tumors occurred. Of the 12 treated mice, 4 showed complete tumor regression; 1 example is shown in Fig. 3. These recovered mice were apparently normal during the entire observation period (180 days). All the remaining 8 treated mice died with large tumors (1 of them showed partial regression in the beginning). All 30 untreated tumor-bearing mice died within 20 to 50 days with large neoplasms. We have not observed spontaneous regression of melanoma in CDF1 mice in any of our experiments.

**DISCUSSION**

Since we reported intratumor therapy with clam extracts in hamsters in 1966 (5), several investigators have used similar techniques for immunotherapy of human or animal tumors. Morton *et al.* (6) treated 8 patients who had metastatic malignant melanoma, incurable by surgery, with intratumor injections of BCG. Complete regression occurred in 90% of 184 melanoma nodules in 5 patients who were tuberculin-positive.

Nathanson (7) confirmed Morton’s work by treating 9 patients suffering from metastatic melanoma with injections of BCG into the base of the tumor nodules. An inflammatory response occurred at the injection site, and 7 of the 9 patients experienced a local slough reaction with subsequent healing and disappearance of the tumors. Zbar *et al.* (10) injected living BCG into carcinogen-induced hepatoma in guinea pigs. An intense inflammatory reaction characterized by erythema, induration, and necrosis occurred. Complete tumor regression occurred in 26/35 (74%) guinea pigs. Cheema (1) treated 14 patients who had various s.c. metastatic solid tumors, including melanoma, with direct injections of autochthonous lymphocytes activated by nonspecific mitogens. The regression rate was significantly higher than in untreated patients and those treated with 8.5% NaCl solution.

The above-mentioned findings support our view that the intratumor therapy could be a useful technique for certain tumors.

The mechanism causing tumor regression is not clear. A number of possibilities exist: (a) the local reaction may be caused by some toxic or proteolytic activities in the clam extracts and is therefore different from that produced by BCG or other immunological factors; (b) the same biological reaction may be caused by different biochemical substances, or the clam extracts and BCG may contain some similar components. Prendergast and Suzuki (8, 9) reported the isolation of a heat-labile protein from the coelomocytes (macrophages) of the sea star (*Asterias forbesi*). This protein exhibited delayed skin hypersensitivity in a large number of mammalian species tested. Our clam extracts may contain similar factors; (c) the tumor regression may have resulted from the reaction of the clam extract in combination with the host mechanisms.

Histopathological studies were not included in the present experiments.

Clinically, the management of locally recurrent tumors continues to be a problem. While the use of radiation or cryotherapy for control is successful in some situations, in many areas considerable damage to surrounding normal tissue occurs concomitantly. The development of a substance that would be effective against only the tumor tissue when injected locally would have wide application. With further purification...
Fig. 2. Treatment of a cheek pouch tumor. The hamster was inoculated s.c. at birth with adenovirus 12 on the upper back. A cheek pouch tumor appeared 84 days after virus inoculation. The tumor grew slowly but steadily, hanging from the side of the mouth like a pinkish-red balloon. The 1st photograph (upper left) was taken 10 days before treatment (Day -10), or 132 days after virus inoculation. Intratumor treatment with clam extract (200 mg) was started on Day 0 and was repeated on Days 1, 2, and 4. The size of the tumor was 3.2 x 2.8 cm on Day 3. By Day 7, the tumor became an almost empty black sac. Two pictures were taken on that day, showing both a side and a front view. By Day 11, the tumor became contracted, with discharge of greasy material. No local wound was found afterwards. The hamster was observed for 1 year after the 1st treatment without showing recurrence of tumor or any sign of illness.

Fig. 3. Transplanted S-91 melanoma in 2 CDF, mice; A, treated (above) and B, untreated (below). Treatment consisted of 4 intratumor injections of the clam extract, 50 mg on Days 0 and 2, and 25 mg on Days 1 and 3. The 2 mice were photographed at the same time on the indicated days after the 1st treatment on Day 0. The tumor size of Mouse A was then 0.7 sq cm and that of Mouse B was 0.8 sq cm. The treated mouse tumor went through the same type of reaction as that in the treated hamster. The 1st photograph, taken on Day 7, showed ulceration of the treated tumor. (The size of the untreated tumor on that day was 1.0 x 1.4 cm.) By Day 23, most of the ulcer was healed, and the animal was apparently normal afterwards. No recurrence of the tumor was noted in the following 5 months. The untreated tumor of Mouse B grew progressively larger (4.8 x 3.7 x 3.5 cm on Day 37) and finally killed the host on Day 41.

of the clam extract, active component(s) that may selectively destroy cancer tissue may be found.

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REFERENCES

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