The Effect of Various Therapeutic Solutions including Colloidal Chromic $^{32}$P via an Intratumoral Injection on the Tumor Physiological Parameters of AsPC-1 Human Pancreatic Tumor Xenografts in Nude Mice

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Abstract
To overcome the physiological barrier in solid tumors (i.e., tumor hypertension), a large volume of material is required via an intratumoral injection. Alternatively, a method of reduction in tumor hypertension is also feasible. In this study, we focused on the physiological response after an intratumoral injection of various therapeutic agents. Tumor interstitial fluid pressure (TIFP) was intermittently monitored for up to 7 days after treatment using AsPC-1 human pancreatic tumors in nude mice. Macroaggregated albumin (MAA), colloidal chromic $^{32}$P ($^{32}$P-cP), albumin, dexamethasone, 5-fluoro-2'-deoxyuridine, dextrose, saline, and trypan blue increased TIFP within $-5$ min, and TIFP returned to the original level within 1 h, except in the case of MAA and $^{32}$P-cP. We also found that the maximal uptake for AsPC-1 tumors in both the exponential and plateau growth phases occurred at $-100$ min postincubation; the maximum value in the exponential growth phase was $-2$ times less than that of plateau growth phase ($P < 0.01$). Therefore, this study supports intrallesional $^{32}$P-cP brachytherapy for nonresectional pancreatic cancer patients. This may offer a promising treatment modality for delivering high doses of tumor-selective radiation, mainly due to two physiological mechanisms: (a) the high adherence of $^{32}$P-cP to the infused regions; and (b) reduction in either tumor blood flow or TIFP by this therapeutic colloid.

Introduction
Rapid advances in the molecular biology of cancer have led to the development of various genetically engineered molecules, including monoclonal antibodies as well as other useful macromolecules. Due to the elevated TIFP, $^{3}$ radiolabeled monoclonal antibodies and gene therapy in cancer treatment have not been as effective as anticipated. To overcome this physiological barrier, administration of a large volume of material via an i.t. injection is required. When a therapeutic agent is mixed with a large quantity of fluid directly infused into the center of a tumor, it increases the pressure at the core of the tumor relative to its surroundings. Consequently, the drug spreads along an artificially induced pressure gradient by convection from the core through the surrounding region and into the periphery (1).

To elucidate physiological response after an i.t. infusion (volume, 100 $\mu$l) of various therapeutic agents, TIFPs were intermittently monitored up to 7 days posttreatment. Several relevant materials such as saline (0.9% or 30% NaCl), $^{32}$P-cP, MAA (10$^5$ or 10$^7$ particles), human albumin ($\sim$2.5 mg/ml), 30% dextrose, dexamethasone (1 mg/ml), 5-fluoro-2'-deoxyuridine (500 mg/kg), RNase-like ONC, and trypan blue (0.4%) were used on AsPC-1 human pancreatic carcinoma xenografts in nude mice. This study supports the hypothesis that if $^{32}$P-cP was introduced intratumorally, it would maintain highly efficient tumor targeting by $^{32}$P-cP-induced physiological mechanisms.

Materials and Methods
Animals and Tumors. Female 8–10-week-old nude mice (Cox Animal Facility, Massachusetts General Hospital, Boston, MA) were used, and they were kept under pathogen-free conditions in the vivarium maintained at 25°C ± 3°C. AsPC-1 tumor cells were cultured, and they were grown in vitro. Single cell suspensions were prepared using 0.25% trypsin solution. About 1 X 10$^6$ viable cells suspended in 50 $\mu$l of HBSS were injected s.c. into the right thighs of mice. Experiments were carried out when the tumor volume was $\sim$500 mm$^3$ (2).

In Vitro $^{32}$P-cP Cellular Uptake Measurements. AsPC-1 cells were plated in 24-multwell plates and then incubated to grow in either the exponential or plateau growth phase. After exposure to 10 $\mu$Ci of $^{32}$P-cP (Mallinckrodt Medical, Inc.,

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3 The abbreviations used are: TIFP, tumor interstitial fluid pressure; i.t., intratumoral; MAA, macroaggregated albumin; $^{32}$P-cP, colloidal chromic $^{32}$P; TBF, tumor blood flow; ONC, onconase; IFP, interstitial fluid pressure; WIN, wick-in-needle.
Fig. 1  Cellular uptake of colloidal $^{32}$P-CP at 10 μCi in the exponential or plateau growth phase of AsPC-1 tumor cells using 24-multiwell plates. $^{32}$P-CP uptake was assessed in six independent samples per time ($n = 6$; bars, SE). Maximum uptake in both the exponential growth phase and the plateau growth phase occurred at 100 min postincubation.

St. Louis, MO), cells were trypsinized at various time intervals ranging from 1–180 min. The radioactivity was then counted using a gamma counter (3).

Anesthesia. Nude mice were anesthetized with ketamine (90 mg/kg) and xylazine (9 mg/kg) via an i.m. route. Mice were placed on a heating pad to keep the body temperature at $\sim 37.5^\circ$C, which was maintained by monitoring their rectal temperature using a Type-T thermocouple (4).

Measurements of TIFP. TIFP was measured with the WIN technique using 23-gauge needles with a side hole 2 mm from the tip. Measurements were made by introducing WIN needles into the central regions of the tumors using a Mac Lab/4 analogue digital system (AD Instruments, Milford, MA) linked to a Macintosh computer (4). TIFPs were measured after an i.t. injection of various substances and monitored multiple times in the same animals bearing tumors (four times per animal during the 7 days of the treatments; i.e., the first measurement of TIFP was monitored continuously for 1 h, and then the TIFP measurements were repeated at 24 h, 48 h, and 7 days posttreatment).

Measurements of TBF. TBF was measured using the Laserflow Blood Perfusion Monitor 403A (Vasamedics, St. Paul, MN) with a 0.8-mm-diameter laser Doppler needle probe. After an insertion of the WIN needle into the tumor center, a small hole was made in the tumor using a 23-gauge needle, and a laser needle probe was inserted into the tumor center (2 mm away from a WIN needle) and then slightly withdrawn to ensure that there was no compression of the tumor under the probe tip. The electrical signals of flow, volume, and velocity from the laser Doppler system were processed using a Mac Lab/4 analogue digital system linked to a Macintosh computer (4). Laser Doppler flow and TBF were monitored simultaneously for a period of 3 h after hyperglycemia through the i.p. injection of dextrose at 5 mg/g.

Statistical Evaluation. All measured values (except the median values in Table 2) were shown as the mean ± SD or SE of each group. Percentage changes were determined individually for each mouse, based on pretreatment values, and then averaged. Significant differences within a group before and after treatment were evaluated using a paired t test, whereas differences between treatment groups were evaluated with an unpaired t test. $P < 0.05$ were considered significant.

Results

Fig. 1 showed that the mean passive adsorption by 24-multiwell plates was negligible during the 3-h incubation with 10 μCi of $^{32}$P-CP. Maximal uptake for AsPC-1 tumors in both the exponential and plateau growth phases occurred at $\sim 100$ min postincubation. The maximum value in the exponential growth phase was $\sim 2$ times less than the maximum value in the plateau growth phase ($P < 0.01$).

Table 1 shows that human tumor xenografts displayed elevated TIFP compared with ITP in the skeletal muscle of the same nude mice. The mean TIFP in AsPC-1 tumors was 22.5 mmHg (range, 9.5–36.5 mmHg). The mean ITP in skeletal muscle was $-2.0$ mmHg (range, $-4.5$ to 0.0 mmHg). The ITP in kidney was 2.1 mmHg, and the ITP in tail at 3 cm from the anus was 6.8 mmHg. In the case of ITP in the kidney, we observed a continuous rise in ITP values (3 of 16 measurements).

After an i.t. infusion of various therapeutic solutions including $^{32}$P-CP, the trend of changes in TIFP is shown in Table 2. We observed that $^{32}$P-CP, albumin, MAA, dexamethasone, 30% dextrose, saline, and trypan blue increased TIFP within $\sim 5$ min, and TIFP returned to the original level within 1 h, except in the case of MAA (at $10^7$ particles) and $^{32}$P-CP. Interestingly, ONC (5 mg/kg) dropped TIFP within $\sim 2$ min and remained at that level for $\sim 20–30$ min before returning to the original level. Then TIFP decreased significantly to 45% ($P < 0.01$) of the control value at 7 days after treatment with ONC. About 1 h after either MAA (at $10^7$ particles) or $^{32}$P-CP, TIFPs were $\sim 70$% of the original value. TIFP in $^{32}$P-CP then remained at the same level, but the TIFP in MAA decreased further from day 1 to day 7. In contrast, TIFPs at 7 days after treatment with other substances (i.e., MAA at $10^7$, albumin, 30% dextrose, saline, and trypan blue) increased to 30% above the original level. We believe that this trend was due to an increase in TIFP during tumor growth in this tumor model.
Table 2  Relative changes in TIFP of AsPC-1 human tumor xenografts in nude mice after an i.t. infusion (volume, 100 μl) of various substances

<table>
<thead>
<tr>
<th>Substances</th>
<th>0 min</th>
<th>5 min</th>
<th>10 min</th>
<th>1 h</th>
<th>24 h</th>
<th>48 h</th>
<th>7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9% NaCl solution</td>
<td>1.00</td>
<td>1.30</td>
<td>1.10</td>
<td>1.02</td>
<td>1.02</td>
<td>1.05</td>
<td>1.35</td>
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<tr>
<td>32P-CP</td>
<td>1.00*</td>
<td>1.58*</td>
<td>0.70</td>
<td>0.73</td>
<td>0.61</td>
<td>0.65</td>
<td>0.78</td>
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<tr>
<td>MAA-10*</td>
<td>1.00*</td>
<td>1.80*</td>
<td>1.75</td>
<td>1.15</td>
<td>1.05</td>
<td>1.15</td>
<td>1.30</td>
</tr>
<tr>
<td>MAA-10</td>
<td>1.00*</td>
<td>0.70*</td>
<td>0.73</td>
<td>0.73</td>
<td>0.68</td>
<td>0.61</td>
<td>0.34</td>
</tr>
<tr>
<td>Albumin</td>
<td>1.00*</td>
<td>1.55*</td>
<td>0.95</td>
<td>0.95</td>
<td>0.99</td>
<td>1.05</td>
<td>1.25</td>
</tr>
<tr>
<td>30% Dextrose</td>
<td>1.00*</td>
<td>1.13*</td>
<td>1.10</td>
<td>0.94</td>
<td>0.92</td>
<td>1.18</td>
<td>1.30</td>
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<tr>
<td>Dexamethasone</td>
<td>1.00*</td>
<td>1.35*</td>
<td>1.25</td>
<td>1.05</td>
<td>1.02</td>
<td>0.93</td>
<td>0.85</td>
</tr>
<tr>
<td>30% NaCl solution</td>
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<td>1.25*</td>
<td>0.95</td>
<td>1.05</td>
<td>1.05</td>
<td>1.10</td>
<td>1.28</td>
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<tr>
<td>Trypan blue</td>
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<td>1.25*</td>
<td>1.10</td>
<td>1.02</td>
<td>1.02</td>
<td>1.03</td>
<td>1.25</td>
</tr>
<tr>
<td>ONC</td>
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<td>0.35*</td>
<td>0.90</td>
<td>1.05</td>
<td>0.95</td>
<td>0.53</td>
<td>0.55</td>
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<tr>
<td>FdUrd</td>
<td>1.00*</td>
<td>1.30*</td>
<td>1.15</td>
<td>1.05</td>
<td>1.05</td>
<td>0.85</td>
<td>0.80</td>
</tr>
<tr>
<td>No substance</td>
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<td>1.00*</td>
<td>1.00</td>
<td>1.00</td>
<td>1.05</td>
<td>1.05</td>
<td>1.30</td>
</tr>
</tbody>
</table>

* Median value, n = 7 mice/group.

** Treated tumors were compared to the control (0.9% NaCl solution) at each time point, and the TIFPs obtained at various time points were compared to their initial values (0 min) in the same tumors. For each comparison, Wilcoxon’s two-tailed test was applied.

a Value versus 0 min, P < 0.05.
b Value versus 0 min, not significant.
c Value versus 0.9% NaCl solution, not significant.
d Value versus 0.9% NaCl solution, P < 0.05.
e FdUrd, 5-fluoro-2'-deoxyuridine.
f MAA-107, 5-fluoro-2'-deoxyuridine.
g TIFP measurements without an i.t. infusion of substances.

One of the main components of 32P-CP is dextrose (i.e., an aqueous suspension in a 30% dextrose solution). As a result, the high concentration of dextrose present in the colloid may cause an alteration in either TIFP or TBF. Therefore, we measured the changes in both TIFP and TBF after systemic administration of dextrose at the high concentration. We did not observe any changes in both TIFP and TBF after an i.p. injection of saline. However, TBF decreased within 10 min after an i.p. injection of dextrose at 5 mg/g (i.e., 200 μl of 50% dextrose solution), reaching 60% (P < 0.01) of the control value by 1 h (Fig. 2). In contrast, TIFP increased to 60% (P < 0.01) above the control value. Both values returned to the original levels at 3 h post-treatment.

Discussion

In a previous study, we found that 32P-CP had a high adherence to the injected areas (3). The first goal of this study was to evaluate the cellular uptake of 32P-CP, ranging in size from 0.6 μm to 4 μm, on the exponential and plateau growth phases of AsPC-1 tumor cells. As we expected, the uptake of this radionuclide-labeled colloid was dependent on the stage of tumor cell growth. The uptake of 32P-CP in the plateau growth phase was ~2 times more than that in the exponential growth phase (Fig. 1). It was also reported that 32P-CP would fix on the surface by possible adsorption or phagocytosis (5). We have preliminary results using electron micrographs that show that 32P-CP, in part, passed through the cell membranes of AsPC-1 tumors by active phagocytosis. Also, the presence of MAA did not alter the degree of 32P-CP-induced phagocytosis.

When Levine et al. (6) infused 32P-CP via the portal or hepatic artery, ~90% of radioactive colloids remained in the liver. This was mainly due to the high adherence of 32P-CP, although they did not overcome the physiological barrier induced by tumor hypertension in solid tumors. Consequently, i.t. introduction of 32P-CP is one way to overcome tumor hypertension, because it should maintain highly efficient tumor targeting.

The second goal of this investigation was to evaluate the physiological response after an i.t. infusion of various materials including 32P-CP. It is well-documented that when the outflow of the tumor vasculature was obstructed by either an i.t. infusion of substances or mechanical occlusion, TIFP increased significantly.
and remained at those levels (3, 7). However, we did not observe any increased TIFP due to the transient blockade of the tumor vasculature, as shown in Table 2. In particular, the significant increases in TIFP were not caused by an i.t infusion of MAA. The improvement of homogeneous distribution of trypan blue was shown in all of the tumor regions by an i.t injection of trypan blue. The tumor became blue-colored; however, at ~24 h postinjection, nude mice became blue-colored, due to the leakage of trypan blue into the circulation. When MAA was intratumorally infused before trypan blue, there were many blue spots, which indicated that MAA could not travel through the entire tumor region or into the vasculature of tumors. We believe that this was due to the larger sizes of the MAA particles (~5 times larger than a RBC). However, nude mice became blue-colored at ~24 h postinjection. Therefore, it is evident that MAA could not stop the leakage of trypan blue into the circulation (3).

As shown in Fig. 2, an i.p. injection of 5 mg/g dextrose caused a temporary hypovolemic hemoconcentration and significantly reduced TBF in AsPC-1 tumors, reaching 60% of the control value by 1 h. Because $^{32}$P-CP is an aqueous suspension in a 30% dextrose solution, the high concentration of dextrose present in the colloid significantly increased the viscosity, therefore reducing the TBF.

In conclusion, this investigation indicates that the intrale- sional $^{32}$P-CP brachytherapy in outpatient settings may offer a promising treatment modality for nonresectional pancreatic cancer patients due to two physiological mechanisms: (a) the high adherence of $^{32}$P-CP to the infused regions; and (b) the reduction in either TBF or TIFP by this therapeutic colloid. Furthermore, this trial may extend to either intracavity or intralesion al application to brain, to achieve the ability to deliver high doses of tumor-selective radiation in a relatively short period of time. Additional studies of brain tumor models are warranted before clinical trials of $^{32}$P-CP brachytherapy in brain cancer patients.

References


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