CRYOSURGERY OF NORMAL AND LNCAP PRO 5 HUMAN PROSTATE TUMOR TISSUE IN THE DORSAL SKIN FLAP CHAMBER

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ABSTRACT

Vascular injury is an important mechanism of cryosurgical destruction in addition to direct cellular injury mechanisms. Previous work by our laboratory showed that vascular injury was more important than direct cellular injury in the AT-1 Dunning rat prostate cancer line grown in a Copenhagen rat. In this study, we report a similar comparison of cellular to vascular injury after cryosurgery in an *in vivo* microvascular preparation (Dorsal Skin Flap Chamber) with seeded human prostate cancer (LNCaP Pro 5) in the nude mouse. Cryosurgery was performed in the chamber on either normal skin or tumor tissue. The vasculature was then viewed at 3 days after cryoinjury under brightfield and FITC-labeled dextran contrast enhancement to assess the vascular injury. The results showed that there was complete destruction of the vasculature in the center of the lesion followed by an abrupt change to normal patency moving radially outward. The area of vascular injury observed with FITC-labeled dextran quantitatively corresponded to the area of necrosis observed in histologic section after 3 days. A comparison of experimental injury data to the thermal model suggested that the minimum temperature required for causing necrosis was -14.3±4.4 ºC in LNCaP Pro 5 human prostate tumor tissue (n = 8) and -18.5±2.6 ºC in normal skin of the nude mouse (n = 9), and the results are comparable to those in the Copenhagen rat. The other thermal parameters manifested at the edge of the lesion included a cooling rate of ~28ºC/min, 5 minutes freezing time, and a ~7ºC/min thawing rate. Work with the same cell line in suspension suggests that these lethal thermal conditions *in vivo* are well tolerated by individual LNCaP Pro 5 cells *in vitro*. In addition, the size of lesion from cryosurgery is larger in tumor tissue than that in normal skin after the same thermal history ($p < 0.05$). These results are consistent with the hypothesis that vascular-mediated injury is responsible for defining the edge of the cryolesion in microvascular perfused tissue. Additionally, preliminary results using inflammation induction by TNF-α prior to cryosurgery show an increased thermal threshold of injury (1.7±1.5 ºC), which further confirms the importance of vascular injury on cryosurgery and possible means to accentuate it.

MATERIALS AND METHODS

The dorsal skin of the athymic nude mouse was sandwiched between two identical anodized aluminum frames. The skin on the side of the viewing region was removed, exposing the dermis containing the microvasculature on the other side of the skin. Windows milled from glass microslides were used to cover the vascular area. For chambers in which tumors were desired, approximately $5*10^6$ LNCaP Pro 5 human prostate tumor cells were mixed with Matrigel™ matrix (BD Biosciences, Bedford, MA) as described by Lim et al. [1]. 20 µl of the 150-µl cell suspension was applied to the surface of the microvascular bed for tumor cells to grow (approximately 10^6 tumor cells per mouse). The tumor was deemed ready for cryosurgery after 10 days, propagating to a thickness of 450 µm and extending ~10 mm in diameter, being constrained by the presence of the windows. As a control, normal tissue was also used for cryosurgery ten days after implantation of the DSFC. This time period was chosen to allow comparison between normal and tumor tissues while allowing the tissue time to recover from surgery.

Cryosurgery was performed on normal and tumor tissues within the DSFC. Just prior to cryosurgery, the window was removed from the chamber and replaced with a clear Lexan window, which had machined holes in the center and at r = 2, 3, and 4 mm from the center of the window. A ~1 mm diameter brass fin welded to an expanding argon-cooled 5 mm-diameter cryoprobe (EndoCare, Irvine, CA) was inserted into the center. A thermocouple was welded to the base of the fin, and thermocouples were placed in the remaining holes. The thermocouples were type "T" (Omega Tech. Corp., Stamford, CT) and had a 0.5 mm bead diameter. The cryofreezer (EndoCare, Irvine, CA) was then activated for a cooling time of 5 minutes to reach a probe end temperature of -120°C. The ice ball was approximately 5 mm in radius at its greatest extent. The probe was then turned off, and the tissue was allowed to thaw passively at room temperature. The temperature from each thermocouple was recorded using a Hydra Data Logger Series 2 (Fluke, Everett, WA). For sham cryosurgery, the procedure described above was repeated except the probe was not activated. There were a total of 12 nude mice with a normal (non-tumor bearing) DSFC

implant that were used. Of these mice, 9 were cryo-treated and 3 were sham-treated. Similarly, 11 mice were used after tumor propagation in the DSFC described above, with 8 cryo-treated and 3 sham-treated.

The vasculature was imaged by use of fluorescein isothiocyanate (FITC)-labeled dextran (70 kD polyanionic. Molecular Probes, Eugene, OR). At day 3 post-treatment, 0.05 ml of a 10 mg dextran/ml PBS solution was injected into the tail vein of the mouse. The dorsal skin flap chamber was then illuminated with a mercury lamp, and a Silicon Intensified Transmission camera (Hamamatsu, North Central Instruments, Twin Cities, MN) was used to detect the fluorescent signal, which was recorded with a JVC S-VHS video recorder (JVC Company of America, Aurora, IL). The images were then digitized using Adobe Photoshop (Adobe, San Jose, CA) calibrated to a micrometer standard to measure precisely the areas of stasis.

RESULTS

The area of vascular injury observed with FITC-labeled dextran quantitatively corresponded to the area of necrosis observed in histological sections. Absence of fluorescence indicated stasis within the tissue, as confirmed by comparison with the videotape of the vasculature, which allowed viewing of the blood flow in the window. The results showed as before a complete destruction of the vasculature in the center of the lesion and an abrupt change to normal patency moving radially outward. Figure 1 (A) and (B) show the vasculature of normal hypodermis in nude mice under FITC-labeled dextran at day 3 after sham cryosurgery and at day 3 post cryosurgery, respectively. Figure 2 (A) and (B) show the vasculature of LNCaP Pro 5 tumor tissue in nude mice at the same time point after the same treatments.

Only one radial slice of vasculature is shown for each condition and only in the area of 1.5 to 4.9 mm distance from the center of the probe, which shows the zone of transition between vascular stasis to uninjured vasculature. No fluorescence is visible to the right of the image toward the probe, showing vascular stasis, and normal vasculature continues to the left of the image toward the edge of the chamber. There was no peripheral loss of vasculature in the chamber due to sham treatment. A comparison of experimental injury data to the thermal model suggested that the minimum temperature required for causing necrosis was -18.5±2.6 °C in normal skin of the nude mouse (n = 9) and - 14.3±4.4 °C in LNCaP Pro 5 human prostate tumor tissue (n = 8). The results are similar to what we obtained using AT-1 rat prostate tumor in Copenhagen rats, as depicted in Figure 3.

DISCUSSION

To define the thermal history that causes microvascular injury, our laboratory quantified the thermal history of tissue within a dorsal skin flap chamber and modeled the thermal response of the tissue to cryosurgery [2]. The cryosurgery model system allowed for a gradient of thermal parameters, and it was concluded that ~26°C/min cooling rate to an end temperature of approximately -15°C with no hold time and a ~9°C/min thawing rate would lead to destruction the of AT-1 tumor tissue grown in the dorsal skin flap. However, the viability of freezing AT-1 tissue *in vitro* under essentially the same thermal parameters that destroy AT-1 tissue *in vivo* remained 37% viable. We believe that vascular injury mechanisms are implicated in the increased *in vivo* destruction.

In addition, we have now repeated the study in human prostate cancer using LNCaP Pro 5 cells grown in a nude mouse and found very similar results. The data suggests that whereas the tumor and normal skin have similar thermal thresholds in different biological systems (rats vs. mice), the LNCaP tumor has a slightly higher thermal threshold (i.e. easier to destroy) than normal skin with statistical significance of $p < 0.05$.

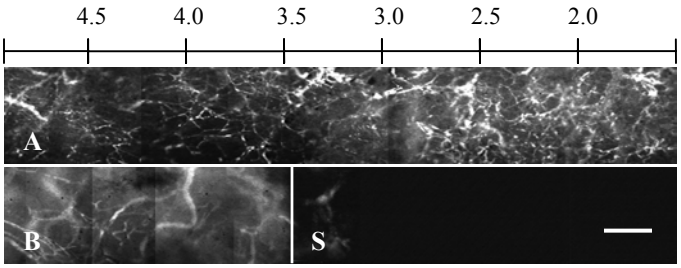

Figure 1. Depiction of the vasculature of normal hypodermis under FITC-labeled dextran at day 3 after sham (A) and at day 3 post cryosurgery (B). Scale bar = 200 µm.

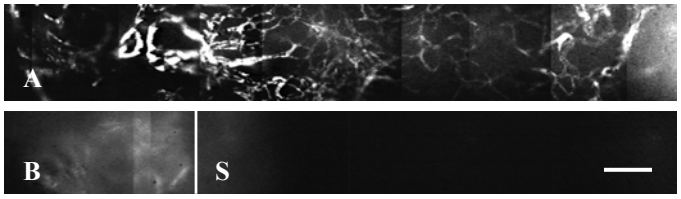

Figure 2. Vasculature of LNCaP Pro 5 tumor tissue in nude mice at day 3 after sham (A) and at day 3 post cryosurgery (B) under FITC imaging. S denotes the edge of the static zone. Radial distances (with 0 being the center of the chamber) are marked above in mm. Scale bar = 200 µm.

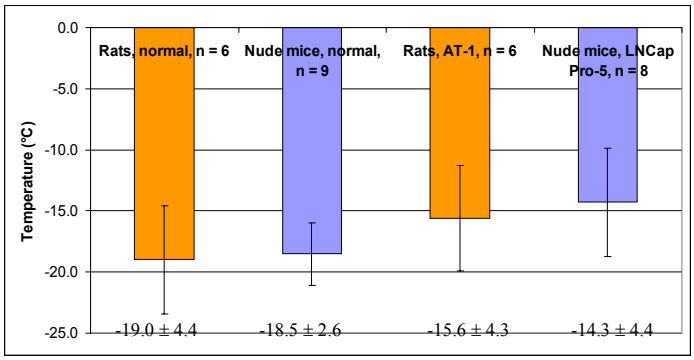

Figure 3. The minimal temperatures required to cause necrosis. Error bars are standard deviations.

The other thermal parameters manifested at the edge of the lesion included a cooling rate of ~28 °C/min, 5 minutes of freezing time and a ~ 7 °C/min thawing rate. The conditions at the edge of the lesion are much less severe than those for destruction of LNCaP Pro 5 cells *in vitro*. These results are consistent with the hypothesis that vascular-mediated injury is responsible for the majority of injury at the edge of the frozen region in microvascular perfused tissue.

ACKNOWLEDGMENTS

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