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A simple method for measuring interstitial fluid pressure in cancer tissues

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Abstract

A novel procedure using a polyurethane transducer-tipped catheter (Millar) is described that allows reliable measurement of interstitial fluid pressure (IFP) in cancer tissues. Before and after each use, the transducer is calibrated at 37°C by a water column. After calibration, the transducer is passed through the lumen of a surgical needle. The sensor is kept in the lumen of the needle during penetration into the tumor. The sensor tip is then introduced into the center core of the tumor as the needle sleeve is withdrawn from the tumor surface. Our new technique is simple and provides IFPs equal to those provided by the well-established, wick-in-needle technique. Using our new technique, we compared IFP in skin melanoma grafts in NG2 knockout and wild-type mice. Knocking out NG2 proteoglycan on vasculogenic and angiogenic pericytes reduced interstitial fluid pressure in melanoma from +4.9 cm H₂O to −0.4 cm H₂O ($P = 0.0054$ Mann–Whitney U test).

Keywords

Cancer; Pericyte; NG2; Interstitial; Fluid; Pressure; Targeting; Wick-in-needle

Introduction

When a piece of bark is peeled off a transpiring tree and a cut is made in the xylem, no sap runs out; in fact, a drop of water placed on the cut is drawn in for the sap is under negative pressure. Similarly, when the subcutaneous tissue of an animal is exposed, fluid does not seep out since the interstitial fluid is under negative pressure. In normal subcutaneous tissue, the interstitial fluid pressure (IFP) is negative (Scholander et al., 1968). By contrast, IFP is often increased in tumor tissue (Young et al., 1950) and forms a barrier against efficient drug delivery into the tumor (Jain, 1987a,b). There are several techniques described for IFP measurements, all of which require experience to use ramified instrumentation and surgical procedures. They include: wick catheter (Scholander et al., 1968; Hargens, 1981; Mubarak and Hargens, 1981), modified wick technique (wick-in-needle technique) (Fadnes et al., 1977; Wiig et al., 1987), servo-micropipette (Wiederhielm et al., 1964) for acute studies of IFP, and subcutaneous capsule implantation for 4–6 weeks (Guyton, 1963) allowing chronic tests of IFP.

Here, we describe a simplified procedure using a transducer-tipped catheter and a precision glide needle that allows reliable measurement of IFP in tumor tissues. We believe this technique

will allow the researchers in the vascular biology field and clinicians in the oncology field to apply IFP measurement easily as a useful tool in research.

Materials and methods

In order to compare and validate the pressures obtained with the miniature pressure transducer with a well-established, conventional method (0.6 mm wick-in-needle) (Fadnes et al., 1977; Wiig et al., 1987), we simultaneously compared a needle-guided Millar SPC 320, 2F Mikro-Tip sensor (<http://www.millarinstruments.com>) and wick-in-needle probes side-by-side in a pilot study in two mice (C57BL/6) bearing B16F1 skin tumor grafts. For wick-in-needle technique, a 0.6 mm needle was provided with a 2-mm-long side hole 2 mm from the tip. The edges of the side hole were polished with a sharpening stone. Strands of nylon fibers were pulled into the needle. The needle was connected to a P23XL pressure transducer (serial number 10153923, Spectramed, Oxnard, CA) by means of a polyethylene tube. The P23XL transducer was connected to a Windograf Model 40-8474 amplifier (serial number 1463, Gould Inc., Valley View, OH). The polyethylene tube and needle were filled with 0.9% saline through another port on the side of the Plexiglas dome of the transducer. Care was taken to prevent air bubbles within the entire system. Zero reference pressure was obtained after placing the needle at the level of needle insertion. Following anesthesia, the needle-guided miniature transducer and wick-in-needle were introduced parallel into the tumor 3 mm apart from one another. The averages of two simultaneous readings for miniature transducer and wick-in-needle were comparable and 9.2 and 9.0 cm H₂O, respectively.

We used an ultraminiature transducer-tipped catheter in which the sensor is side-mounted at the tip: SPC-320 transducer (2 French size, 0.66 mm in diameter) and an 18-gauge, 1.5-in. precision glide needle (Fig. 1). Ultraminiature transducer tipped catheter, TC-510 Control unit, adapter cables, and TAM-D amplifier modules were purchased from Harvard Apparatus (Holliston, MA). All animal studies were performed in accordance with National Institutes of Health Office of Laboratory Animal Welfare (OLAW) guidelines and were approved by the La Jolla Institute For Molecular Medicine animal research committee. NG2 null mice (Grako et al., 1999) were generated via a conventional homologous recombination approach (Mansour et al., 1988; Capecchi, 1989). The mice were back-crossed onto a C57BL/6 genetic background and NG2+/- heterozygotes were mated to establish separate male NG2 knockout (NG2-/-) and wild type (NG2+/+) colonies (Ozerdem and Stallcup, 2004). B16F1 mouse melanoma cells (American Type Culture Collection, Manassas, VA) (5×10^6 cells) were inoculated subcutaneously in the dorsum. The mice were followed for 2 weeks. Following anesthesia with intraperitoneal avertin injection (0.017 ml/g body weight), the size of the tumor in mouse is measured with a caliper. Then, the distance between the skin surface and the center core of the tumor is estimated. Before each use, the transducer is calibrated for accuracy by using a distilled water column at 37°C (Fig. 2A). Calibration in the water column (Fig. 2A) (before and after IFP measurements in tumors) revealed a positive and linear correlation between the transducer output (mV) and pressure (cm H₂O) of the miniature transducer (Fig. 2B). After calibration, the transducer is passed through the lumen of a Becton-Dickinson 18-gauge, 1.5-in. precision glide needle (Franklin Lakes, NJ). The sensor is kept in the lumen of the needle during penetration into the surface of the tumor. The sensor tip is introduced into the core of the tumor as the needle guide is withdrawn from the tumor surface. Tumor pressure (IFP) is read by means of TAM-D module after 15 s.

Results

Polyurethane ultraminiature pressure transducers, recognized for impeccable accuracy in the pressure range between -50 mm Hg to +300 mm Hg, provide a simple, accurate, and thromboresistant method of measuring the pressure at the source (Zimmer and Millar, 1998).

Entire procedure of calibration of the sensor, introduction of the sensor into the tumor, and IFP reading does not take more than 10 min and can be performed with ease by anyone who can perform a subcutaneous injection. Our preliminary studies using this technique to measure IFP in subcutaneous B16F1 melanoma in NG2 knockout and wild type mice 2 weeks following grafting revealed an average core IFP of -0.4 cm H₂O and $+4.9$ cm H₂O in NG2 proteoglycan knockout and wild type mice, respectively. The difference was statistically significant ($n = 24$, $P = 0.0054$ Mann–Whitney U test) (Fig. 3).

Discussion

The interstitial fluid pressure within a tumor is actively regulated through interactions between cells and extracellular matrix molecules. Many anticancer drugs and antibodies used for treating patients with cancer are transported from the circulatory system through the interstitial space by convection (i.e. by streaming of a flowing fluid) rather than by diffusion. Increased tumor IFP causes inefficient uptake of therapeutic agents by decreasing convection. Cancer cells are therefore exposed to a lower effective concentration of therapeutic agents than normal cells, reducing treatment efficiency. Decreasing tumor IFP can thus improve convection of cancer chemotherapeutics into the tumor (Jain, 1987a,b; Heldin et al., 2004).

In this respect, it is also noteworthy that pericytes are known to control interstitial pressure (IFP) by regulating their attachment to a collagen/microfibrillar network, which in turn restrains a proteoglycan/hyaluronan gel from retaining water (Pietras et al., 2001; Heldin et al., 2004; Rodt et al., 1996). In addition, signaling through PDGF β -receptors on pericytes increases IFP, whereas its inhibition reduces IFP (Pietras et al., 2001). Hence, IFP is a dynamic parameter that can potentially be controlled by regulating pericyte activity and their interaction with the extracellular matrix (Pietras et al., 2001). Our results reveal decreased IFP levels in tumors grown in NG2 knockout mice and suggest that NG2 chondroitin sulfate proteoglycan on pericytes has a role on IFP through its interaction with extracellular matrix components. Pericyte–NG2 chondroitin sulfate proteoglycan binds to extracellular matrix components such as type V, type VI, and type II collagen, tenascin, and laminin (Tillet et al., 1997; Burg et al., 1996). Biochemical data also demonstrate the involvement of both galectin-3 and $\alpha 3\beta 1$ integrin in the EC response to pericyte–NG2 and show that NG2, galectin-3, and $\alpha 3\beta 1$ form a complex on the cell surface promoting cell motility (Fukushi et al., 2004). Our recent findings revealed decreased neovascularization following intrinsic (NG2 knockout mice) or extrinsic (hydron polymer pellets containing NG2 neutralizing antibody) targeting of NG2 proteoglycan (Ozerdem, 2004; Ozerdem and Stallcup, 2004). Lowering tumor IFP by targeting NG2 proteoglycan on pericytes may be a useful approach in improving anticancer drug (conventional chemotherapy) efficacy.

This study also demonstrates that IFP is elevated in skin melanoma, corroborating previous studies showing elevated IFP in human melanoma (Boucher et al., 1991), human melanoma xenografts in mice (Kristensen et al., 1996; Tufto et al., 1996), and hamster melanomas (Leunig et al., 1992, 1994).

Critique of four IFP measurement techniques

The micropuncture (0.1 μ m glass micropipette) technique does not allow for measurements in deep tissues; usually recordings can only be made down to about 1 mm from the surface (Wiederhielm, 1981; Adair et al., 1983). Further-more, the glass micropipette breaks very easily during tissue penetration and from the slightest motion of the mouse. The wick catheter technique (Scholander et al., 1968) is vulnerable to clotting if there is extravasated blood within the tissue (Wiederhielm, 1981; Adair et al., 1983). The wick-in-needle technique requires custom-made needles with optimum-size side ports (Wiederhielm, 1981; Adair et al., 1983).

Chronic implantation of the 15-mm capsule is not practical and involves considerable tissue distortion, trauma, and wound healing response (Wiederhielm, 1981; Adair et al., 1983). Capsule implantation is not applicable for human use. In a comparison of two acute (micropipettes and wick-in-needle) and two chronic methods (perforated and porous capsules) in dog skin/subcutis, Wiig et al. (1987) found that transient pressure differences recorded by acute versus chronic methods during changes in hydration result from different physical properties of the capsule lining compared with that of the surrounding skin, in addition to a possible osmometer effect of the capsule lining. Therefore, we chose the wick-in-needle as the best standard by which to compare with Millar SPC-320, 2F Mikro-Tip with side port sensor. One possible disadvantage of this transducer-tipped catheter for IFP measurement is that the transducer may be susceptible to solid tissue pressure artifacts. However, in our pilot studies in tumors with positive pressure, this was found not to be the case, and the values obtained by the Millar probe were essentially equivalent to those obtained by the wick-in-needle technique.

The simplified IFP measurement procedure described in this report will allow the basic researchers and clinicians to apply IFP measurement easily and widely as a useful tool in their research.

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Fig. 1.

Interstitial fluid pressure measurement with ultraminiature pressure transducer. (A) The ultraminiature transducer (arrow) is introduced percutaneously 1 mm through the tumor capsule into the superficial tumor in a protective metal guide (18-gauge needle) (arrowhead). (B) The metal guide is withdrawn slowly, while the sensor is introduced into the center of the tumor. The location of the center of the tumor is estimated by dividing the caliper-measured diameter of tumor in half. The transducer can be marked for millimeter gradation using standard fine point pens (not shown in this picture). (C) Handling of the needle guide and transducer is very easy during microsurgical procedures on mice. Scale bar = 600 μm .

Fig. 2.

Calibration of the miniature transducer. The miniature transducer is calibrated in distilled water column at 37°C (A). Calibration curves both before (pretest) and after (posttest) in vivo measurements show a linear correlation between the depth of water column (pressure in cm H₂O) and transducer output (mV) (B).

Fig. 3.

Targeting NG2 proteoglycan on neovascular pericytes reduces interstitial hypertension in skin melanoma in mice. IFP measurements were performed 2 weeks following subcutaneous tumor grafting. Intrinsic (genetic) inhibition of NG2 proteoglycan decreases IFP in B16F1 melanoma grafts in NG2 knockout mice (average core IFP of -0.4 cm H₂O) compared to B16F1 melanoma grafts in wild type controls[C0](average core IFP of $+4.9$ cm H₂O) ($n = 24$, $P = 0.0054$ Mann-Whitney U test).