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Influence of hydralazine on interstitial fluid pressure in experimental tumors - a preliminary study

Blaž Podobnik and Damijan Miklavčič

Faculty of Electrical Engineering, University of Ljubljana, Ljubljana, Slovenia

Background. *Interstitial fluid pressure (IFP) has been recognised as the most important obstacle in macromolecular drug delivery to solid tumors. Our interest was to reduce differentialy tumor IFP with respect to IFP in surrounding and normal tissues in order to increase drug delivery to tumors as well to increase tumor blood flow and potentialy tumor tissue oxygenation. In this preliminary study we used hydralazine, a long-acting arterial vasodilator.*

Materials and methods. *Measurements of interstitial fluid pressure were performed in vivo on CBA mice bearing SAF tumors using wick-in-needle technique. Altogether eleven measurements were obtained on different animals with tumors of different size.*

Results. *IFP in tumors after hydralazine administration was significantly lower than initial values in corresponding tumors. On average tumor IFP decreased for 33% from initial value. On the contrary, no change in IFP in normal tissue was observed after hydralazine administration. Also, after injection of physiological saline instead of hydralazine there was no change in IFP neither in tumors nor in muscle. The results of our preliminary study on the effect of hydralazine on IFP in SAF tumor model is in accordance to previously reported studies. The decrease in tumor IFP was only observed in tumors, but not in muscle and surrounding subcutis.*

Conclusion. *Hydralazine is a vasodilator which is capable of decreasing tumor IFP, reproducibly and with favorably long lasting dynamics.*

Key words: sarcoma; experimental-drug therapy; hydralazine; extracellular space; interstitial fluid pressure; manometry

Introduction

Interstitial fluid pressure (IFP) has been recognised as the most important obstacle in macromolecular drug delivery to solid tumors.[1,2,3] IFP was also correlated with tumor blod flow.[4,5] Recent clinical study involving patients with cervical carcinoma[6] reported that tumors with high IFP were more likely to be hypoxic and less likely to regress completely after radiotherapy. Elevated IFP in solid tumors hinders fluid filtration from tumor vasculature which is the prime driving force for macromolecular transvascular flow. However, it is not clear at the moment how elevated IFP would affect tumor blood flow

Corespondence to: Damijan Miklavčič, DSc, Faculty of Electrical Engineering, Tržaška 25, 1000 Ljubljana. Tel: +386 61 176 84 56; Fax: +386 61 126 46 58; E-mail: damijan@svarun.fe.uni-lj.si

and oxygenation. Nevertheless, there is an obvious and increasing interest in modulating IFP in solid tumors thus by decresing it, facilitating macromolecular drug delivery like monoclonal antibodies into tumor tissue and possibly modyfing tumor blood flow and tissue oxygenation. Various vasoactive drugs have been used with variable success to modulate solid tumor IFP. Most of the drugs used (e.g. nikotinamide, angiotensin II, epinephrine, norepinephrin, nitroglycerin and hydralazine) have been reported to modulate tumor IFP.[7] In general, vasoconstricting agents resulted in increase of tumor IFP whereas vasodilating agents produced decrease in tumor IFP. Our interest was to reduce differentialy tumor IFP with respect to IFP in surrounding and normal tissues in order to increase drug delivery to tumors as well to increase tumor blood flow and potentialy tumor tissue oxygenation. In this preliminary study we used hydralazine, a long-acting arterial vasodilator. After i.v. administration we measured IFP in solid subcutaneous tumors (SAF anaplastic sarcoma) and subcutis close to tumor and/or muscle tissue in CBA mice.

Materials and methods

Animals and tumor model

All experiments were performed on 8 to 10 week old female CBA mice which were maintained under standard laboratory conditions with food and water *ad libitum*. The SAF (anaplastic sarcoma; 0.1 ml of crude tumor cell suspension) was transplanted subcutaneously under sterile conditions dorsolaterally on a right flank of the mice. Experiments were performed on tumors of different size ranging from 95 mm^3 to 800 mm^3. All experiments were performed at the Department of Tumor Biology, Institute of Oncology, Ljubljana in accordance with ethical provisions for research on animals.

Anesthesia

The treatment and measurements of animals which could cause discomfort or pain to animals were performed under general anesthesia. The mice were anesthetized with Isoflurane (Flurane-Isoflurane, Abbot Labs Ltd., UK; waporizer-Isotec 337C, Ohmeda, USA) gas anesthesia (1.5-2% of Isoflurane was mixed with NO_2, O_2 mixture; flow of NO_2 and O_2 was 0.6 l/min). Animals were anesthesied and placed on a heating pad (TCU 035,27S, Cheshire, UK) which maintained stable body temperature. Throughout the experiment rectal temperature and heating pad surface temperature were monitored. The rectal temperature was between 37 and 38 °C and the maximal surface temperature of heating pad was lower than 40 °C.

Drugs

Hydralazine (Hydrazinophthalazine, Sigma Chemical Co., St. Louis, MO) was dissolved in sterile saline (0.9% NaCl) prior to each experiment. A dose of 2.5 mg/kg was injected intravenously (i.v.) into retroorbital sinus.

Measuring technique and experimental protocol

IFP was measured by the wick-in-needle technique[8,3,9] using a 0.5 mm (25G) needle probe (Terumo Belgium) with a 2 mm sidehole about 3 mm from the tip. The needles were filled with two surgical thread fibers (5-0, Seide Silk). Prior to each experiment the measurement system was calibrated. All recordings of IFP were performed as two channel measurements, measuring IFP in tumor and in subcutis close to tumor or muscle. Needles were connected to pressure transducers (TSD104 and TSD104A, Biopac Systems Inc., CA-Goleta, USA) by a polyethylene tube and the entire system was filed with physiological saline (0.9% NaCl) which contained heparin (Krka, Slovenia) 72 u/ml for preventing blood clots to be formed. Special care was taken to

avoid trapping of air bubbles in a system during the filling. Saline in system was used as a conductor of pressure. Pressure transducers were connected via amplifier (DA100A, Biopac System Inc.) and data acquisition unit (MP100, Biopac Systems Inc.) on personal computer. The sampling frequency was 10 Hz.

During calibration of a measurement system zero reference pressure was obtained by placing the needles in a heparinesed physiological saline-filled beaker and calibration of pressure was done by elevating or lowering the beaker. At different levels (1cm and 30 cm) of liquid column (level 0 cm was equal to level of needle insertion into a tumor or muscle), output voltages of pressure sensors were measured and used for calibration. After the calibration one needle was inserted into the center of a tumor and the other one into subcutis or a muscle of a right hind limb. After that needles were slightly withdrawn to avoid compression of the tumor and muscle under the probe tip and left in place without external fixation. All measurements lasted for 2 hours or longer.

One of complete IFP measurements is given in Figure 1. After the initial equilibration period, compression/decompression (C/D) test was performed. This test allowed us to verify the continuum between the fluid phase in interstitium and needle lumen. By tightening the clamp on a polyethylene tube so as to inject a volume of approximately 0.2 µl into the tissue caused a sudden pressure rise (Figure 1). The pressure then declined, first rapidly and then more slowly, restabilized to the initial level within 30 seconds to 2 min. Withdrawal of the same amount of fluid by loosing the clamp gave a reverse response, a sudden fall in pressure with gradual return to the original level (Figure 1). This test was performed at the beginning and at the end of

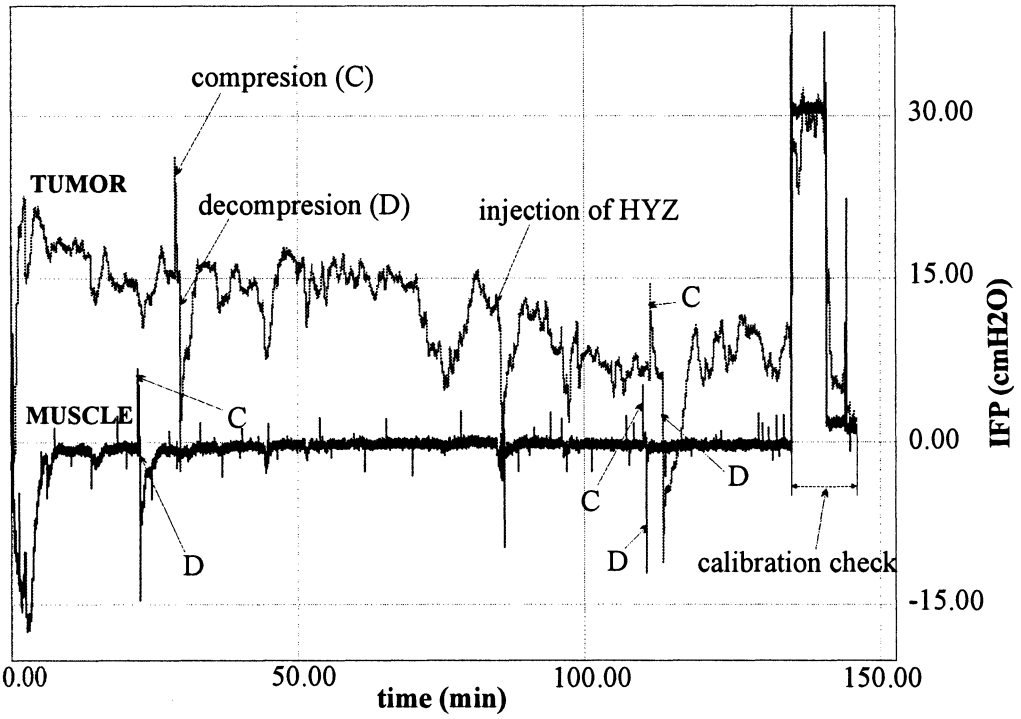

Figure 1. An example of IFP recording during the entire experiment. IFP in tumor and muscle tissue including compression (C) /decompression (D) test, injection of hydralazine (HYZ) and calibration chack are given.

each experiment and gave us the information about the quality and reliability of IFP measurements. Only results obtained in experiments where both tests were correct, were accepted and considered as reliable. Hydralazine was injected i.v. after a stable recording of IFP was obtained. The response of IFP to hydralazine was monitored for approximately one hour. After that period C/D test was performed again and needles removed from tissue. Measurement was finished with the calibration test in order to verify the calibration procedure performed prior to the beginning of the experiment.[10]

Data processing and statistical analysis

Initial IFP values in tumors and muscle or subcutis was determined as the mean value of IFP recording in the interval of app. 20 minutes duration after the first C/D test and prior to hydralazine injection. The values of IFP after hydralazine injection were determined as the mean value of IFP recording in the interval of app. 30 minutes duration starting at 10-15 minutes after hydralazine (or physiological saline) injection. All values are reported in tables and figures as mean±standard deviation. Statistical analysis of the data was performed with a paired t-test comparing measured values of IFP values in tumors before and after hydralazine injection, IFP in muscle tissue or subcutis before and after hydralazine injection after normality test was performed and fulfilled. Exact p-values are reported.

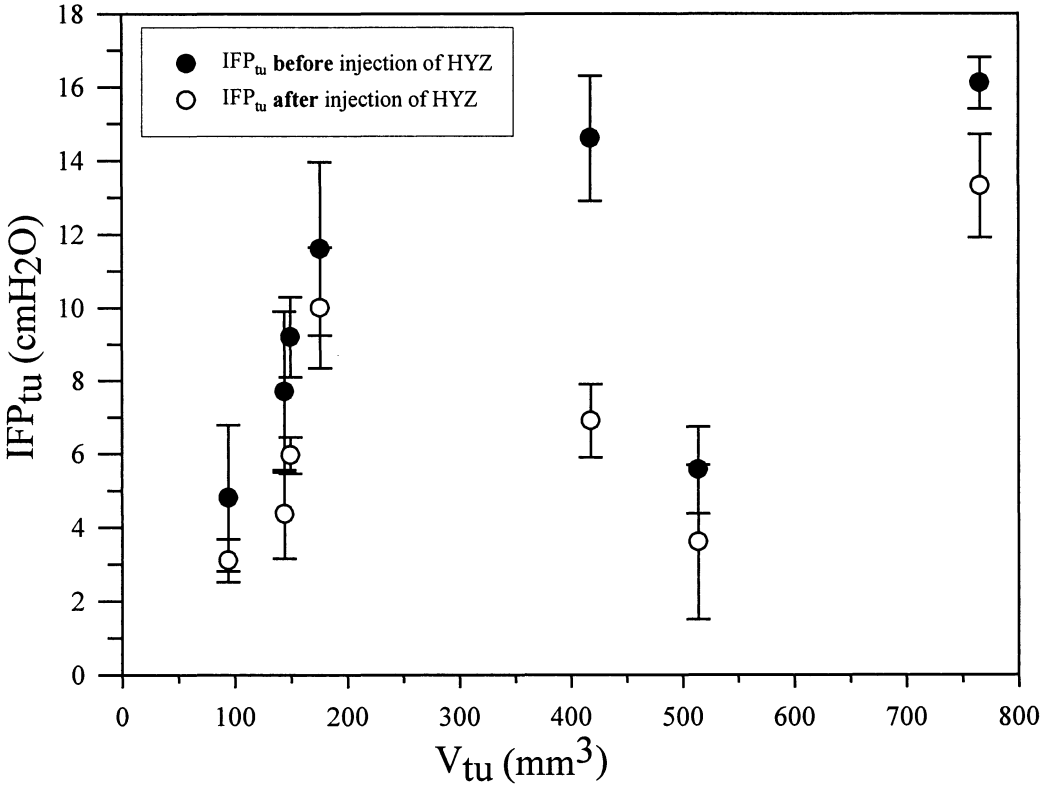

Figure 2. Picture shows interstitial fluid pressure (IFP) with standard deviation in SAF tumors respective on tumor volume. Black dots present IFP before injection of hydralazine (HYZ) and white dots present IFP after HYZ injection.

Table 1. Mean values of interstitial fluid pressure (IFP) with standard deviations in tumor (IFP_{tu}) and in muscle (IFP_{mu}) before and after injection of 2.5 mg/kg hydralazine (HYZ) with respect to tumor volume (V_{tu}). Decrease induced by HYZ in tumor and muscle IFP are given (ΔIFP_{tu} and (ΔIFP_{mu}, respectively)

V_{tu} (mm^3)	IFP_{tu} *BEFORE HYZ* (cmH_2O)	IFP_{tu} *AFTER HYZ* (cmH_2O)	Δ IFP_{tu} (cmH_2O)	IFP_{mu} *BEFORE HYZ* (cmH_2O)	IFP_{mu} *AFTER HYZ* (cmH_2O)	Δ IFP_{mu} (cmH_2O)
94.20	4.80 ± 1.9	3.10 ± 0.6	*1.70*	1.00 ± 0.3	0.90 ± 0.4	*0.10*
144.10	7.70 ± 2.2	4.36 ± 1.2	*3.34*	1.20 ± 1.5	0.77 ± 0.6	*0.43*
149.30	9.20 ± 1.1	5.96 ± 0.5	*3.24*	0.72 ± 1.1	0.79 ± 0.3	*-0.07*
175.80	11.60 ± 2.4	10.00 ± 1.7	*1.60*	0.37 ± 0.4	0.33 ± 0.7	*0.04*
417.80	14.60 ± 1.7	6.90 ± 1.0	*7.70*	***-0.20 ± 0.3**	***-0.15 ± 0.5**	***-0.05**
514.10	5.60 ± 1.2	3.60 ± 2.1	*2.00*	0.35 ± 0.2	0.40 ± 1.0	*-0.05*
765.80	16.10 ± 0.7	13.30 ± 1.4	*2.80*	***-0.10 ± 0.3**	***0.05 ± 0.3**	***-0.15**

*IFP values in subcutis

Results

Measurements of interstitial fluid pressure were performed in vivo on CBA mice bearing SAF tumors using wick-in-needle technique. Altogether seven measurements were obtained on different animals with tumors of different size in the range of 95 mm^3 to 800 mm^3. At the same time IFP in muscle or subcutis before and after hydralazine administration was measured. In addition four control measurements were performed, where instead of hydralazine physiological saline (80 to 100 µl, depending on mouse weight) was injected. We determined initial value of IFP in tumors and normal tissue (*i.e.* muscle or subcutis) during the stable recording of IFP signal (approximately 20-25 minutes before injection) and IFP after hydralazine or physiological saline injection during app. 30 minutes interval starting 10-15 minutes after injection as described in materials and methods section.

In general, immediately after needle insertion we recorded high negative IFP in tumor and in muscle or subcutis (Figure 1). Under control conditions when physiological saline was injected, the IFP returned rapidly and stabilized at a level around 0 cmH_2O for muscle and somewhere between 4 and 16 cmH_2O for tumor, depending on tumor size (Figure 2). After 10 to 15 minutes following hydralazine injection the IFP in tumor decreased on average for 33 % from initial level but there was no change of IFP in muscle or in subcutis (Table 1 and 2). The level which was observed lasted at least 30 minutes and then it slowly raised up towards the initial value.

Initial values of IFP in tumors based on measurements prior to any manipulations were between 4.8 and 16.1 cm H_2O with the mean±std value of 9.6±4.0 cm H_2O (n=11). The initial values of IFP in tumors were higher in larger tumors, as previously observed.[10] Initial values of IFP in muscle were between 0.3 and 1.6 cmH_2O and in subcutis between -0.1 and -0.2 cmH_2O with the mean±std value of 0.7±0.4 cm H_2O (n=9) and -0.15±0.07 cmH_2O (n=2), respectively.

The values of IFP in tumors and normal tissue (muscle and subcutis) after hydralazine administration are given in (Table 1) for each tumor and corresponding muscle or subcutis IFP measured at the same time. IFP in tumors after hydralazine administration was significantly lower than initial values in corresponding tumors (paired t-test: p=0.016). On aver-

Table 2. Mean values of interstitial fluid pressure (IFP) with standard deviations in tumor (IFP_{tu}) and in muscle (IFP_{mu}) before and after injection of 80-100 µl physiological saline (PHYS.SAL.) with respect to tumor volume (V_{tu}). Decrease induced by PHIS.SAL. in tumor and muscle IFP are given (ΔIFP_{tu} and ΔIFP_{mu}, respectively)

V_{tu} (mm^3)	IFP_{tu} *BEFORE PHYS. SAL* (cmH_2O)	IFP_{tu} *AFTER PHYS. SAL* (cmH_2O)	ΔIFP_{tu} (cmH_2O)	IFP_{mu} *BEFORE PHYS. SAL* (cmH_2O)	IFP_{mu} *AFTER PHYS. SAL* (cmH_2O)	ΔIFP_{mu} (cmH_2O)
94.50	9.41 ± 1.0	10.60 ± 0.9	*-1.19*	0.32 ± 0.2	0.50 ± 0.3	*-0.18*
136.00	14.00 ± 0.9	14.30 ± 1.2	*-0.30*	0.36 ± 0.4	0.30 ± 0.4	*0.06*
195.60	5.10 ± 1.0	5.70 ± 0.9	*-0.60*	1.60 ± 0.3	1.60 ± 0.3	*-0.00*
228.80	7.85 ± 1.0	7.60 ± 1.3	*0.25*	0.41 ± 0.2	0.45 ± 0.2	*-0.04*

age tumor IFP decreased for 33% from initial value. This decrease was between 14 to 53% in individual tumors (mean±std ΔIFP = 2.37±0.8 cmH_2O). On the contrary, no change in IFP in normal tissue was observed after hydralazine administration resulting in mean±std ΔIFP = 0.09±0.2 cmH_2O (p=0.376) (Table 1). In addition, injection of physiological saline instead of hydralazine produced no change neither in tumors with mean±std ΔIFP = -0.46±0.6 cmH_2O (p=0.223) nor in muscle with mean±std ΔIFP = -0.04±0.1 cmH_2O (p=0.490) (Table 2).

Discussion

The results of our preliminary study on the effect of hydralazine on interstitial fluid pressure in SAF tumor model is well in accordance to previously reported studies[4,7], both in the amplitude and the duration of response. The choice of hydralazine dose was based on previous studies, where comparable doses resulted in 50% deacrease in mean arterial blood pressure which occured within 10-15 minutes after hydralazine injection and lasted for at least 30 minutes.[4] In another study[7] both mean arterial blood pressure as well as tumor IFP were measured after 60µg of hydralazine injection (which corresponds to app. 3 mg/kg dose). The reduction of mean arterial blood pressure of 50% was obtained which is the same as in previously mentioned study[4] and approximately 50% reduction in tumor IFP was reported. In our study we observed an average of 33% (range: 14-53%) decrease in tumor IFP with respect to initial value. This response was noticed within 15 minutes after hydralazin injection and lasted for at least 30 minutes. The exact relation between mean arterial blood flow pressure and interstitial fluid pressure is not well known. In the same study[7] they developed a network model where they explored the role of mean arterial blood flow and tumor IFP. In addition, we did not observe any significant changes in IFP in muscle or subcutis, demonstrating a potentialy interesting differential effect. Other authors stipulated that observed decrease in tumor interstitial fluid pressure after hydralazine injection could be explained by the reverse steal-effect, which remains to be confirmed.

Our results on initial elevated interstitial fluid pressure and its dependence on tumor size are also well in accordance with our previous results[10] and the model we developed.[10,11]

In additional control experiments where we injected physiological saline only, no significant decrease in IFP was noticed after the

injection of phisiological saline apart from a short lived decrease in IFP which was present in both signals (in tumor and in muscle). This decrease was however a transient and IFP returned to the initial level within two to three minutes.

In conclusion, hydralazine is a vasodilator which is capable of decreasing tumor interstitial fluid pressure, reproducibly and with favorably long lasting dynamics. The decrease in tumor interstitial fluid pressure was only observed in tumors, but not in muscle and surrounding subcutis. Whether the response depends on tumor size and is accompanied by changes in tumor blood flow as suggested by other authors[4,7] and how would these affect tumor oxygenation remains to be determined.

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