

Effect of Hydralazine on Interstitial Fluid Pressure in Experimental Tumours and in Normal Tissue

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Abstract. *Interstitial fluid pressure (IFP) has been recognised as the most important obstacle in macromolecular drug delivery to solid tumours. The aim of our study was to measure the IFP simultaneously in tumour and in muscle or in subcutis and to determine whether injection of hydralazine reduces differentially tumour IFP with respect to IFP in surrounding and normal tissues. In addition, it was of interest whether the decrease in IFP due to hydralazine depends on tumour volume and/or on initial IFP. Measurements of IFP were performed by means of the wick-in-needle technique and they were obtained on tumours of different size. In both tumour models, hydralazine significantly reduced the pre-treatment IFP level. On average IFP decreased by 31% and 14% from the initial value in SAF and LPB tumours, respectively. On the contrary, hydralazine did not decrease IFP in normal tissue. Injection of NaCl solution instead of hydralazine had no effect on IFP either in tumours or in subcutis/muscle. The results of our study on the effect of hydralazine on IFP in SAF and LPB tumour model are in accordance to previously reported studies. The initial IFP in tumour is positively-correlated with the tumour size, while the decrease in the tumour IFP is independent of the initial IFP value. In addition, the decrease in tumour IFP is not correlated to tumour volume.*

Elevated interstitial fluid pressure (IFP) has been recognised as the most important obstacle in macromolecular drug delivery to solid tumours (1-3). Elevated IFP in solid tumours hinders fluid filtration from tumour vasculature, which is the prime driving force for macromolecular transvascular flow (4). Elevated tumour

IFP was also suggested to lead to reduction in tumour blood flow, which may in turn contribute to the development of hypoxia (5). The latter is an important cause of radiation treatment failure in many tumours. Indeed, in recent clinical study involving patients with cervical carcinoma (6), it was reported that tumours with high IFP were more likely to be hypoxic and less likely to regress completely after radiotherapy. However, it is not clear at the moment how elevated IFP would affect tumour blood flow and oxygenation.

Due to developments in macromolecular drugs for tumour treatment and to a possible causal relationship between IFP, blood flow and tumour oxygenation, there is an increasing interest in modulating IFP in solid tumours. Decreasing IFP could facilitate macromolecular drug delivery, like monoclonal antibodies, into tumour tissue (7) and could possibly modify tumour blood flow and tissue oxygenation. Various vasoactive drugs have been used with variable success to modulate IFP in solid tumours. Most of the drugs used (e.g. nicotinamide, angiotensin II, epinephrine, norepinephrine nitroglycerine and hydralazine) have been reported to modulate tumour IFP (8). In general, vasoconstricting agents resulted in an increase of tumour IFP whereas vasodilating agents induced a decrease in tumour IFP. Whether this decrease is induced in tumour only or also in nonnal tissue, is not known. In this study we used hydralazine (9), a long-acting arterial vasodilator, which is used for the treatment of hypertension. IFP was measured simultaneously in tumours and in muscle or subcutis before and after hydralazine administration. In control experiments we injected NaCl solution instead of hydralazine. Measurements were obtained on different mice with tumours of different size. After i.v. hydralazine or NaCl solution administration, we measured IFP in solid subcutaneous tumours (SAF-anaplastic sarcoma, LPB - mouse sarcoma) and subcutis close to tumour and/or muscle tissue in CBA and C57B1/6 mice.

Our interest was to simultaneously measure IFP in tumour and in muscle or in subcutis and to reduce differentially tumour IFP with respect to IFP in

Abbreviations: IFP, interstitial fluid pressure; HYZ, hydralazine.

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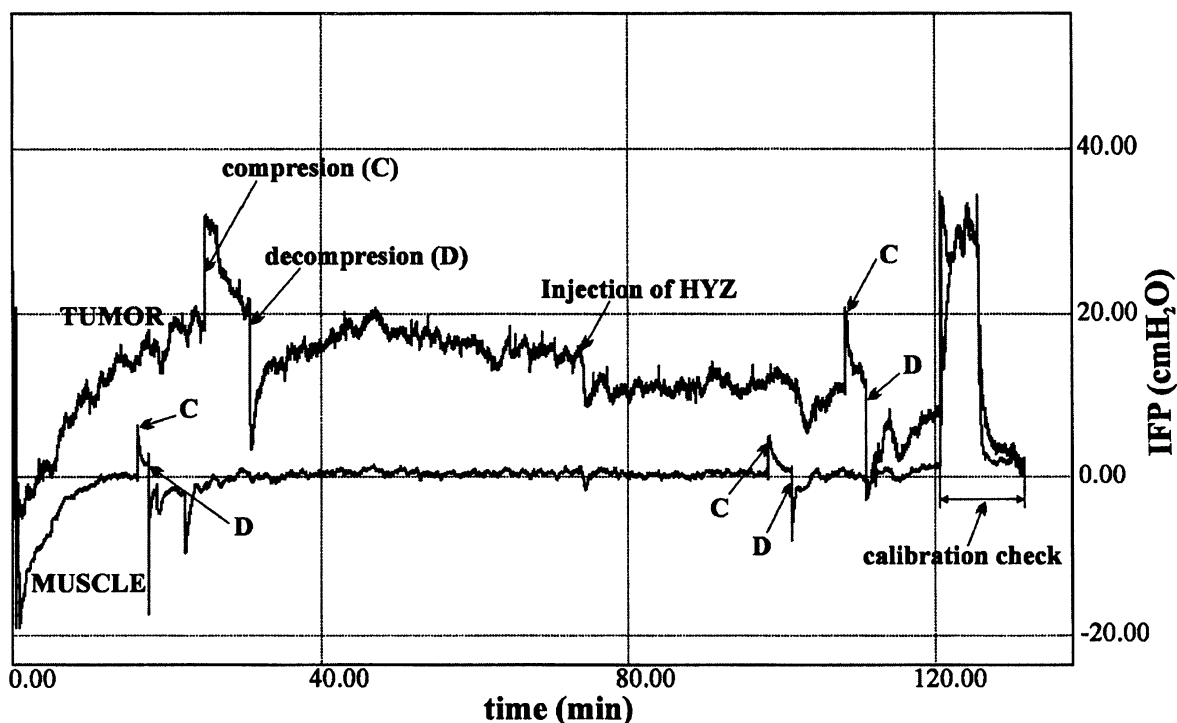


Figure 1. An example of IFP recording during the entire experiment. IFP in tumour and muscle tissue including the compression (C) /decompression (D) test, injection of hydralazine and calibration check are given.

surrounding and normal tissues. In addition we also investigated whether the decrease in IFP induced by hydralazine is dependent on tumour volume and/or initial tumour IFP.

Materials and Methods

Animals and tumour model. All experiments were performed on 8 to 10-week-old female CBA and C57Bl/6 mice, which were maintained under standard laboratory conditions with food and water *ad libitum*. The SAF (anaplastic sarcoma; 0.1 ml of crude tumour cell suspension) which were grown in RPMI 1640 culture media (Sigma, USA), supplemented with 10% foetal calf serum (Sigma, USA) and LPB (mouse sarcoma; $1.3 \cdot 10^6$ cells) which were routinely maintained *in vitro* in Eagle minimal essential medium (EMEM) (Sigma, USA), supplemented with 8% foetal calf serum (Sigma) and antibiotics, were transplanted subcutaneously under sterile conditions dorsolaterally on the right flank of the mice. The experiments were performed on tumours of different size ranging from 95 mm^3 to 800 mm^3 . All experiments were performed at the Department of Tumour Biology, Institute of Oncology, Ljubljana, Slovenia, in accordance with the ethical provisions for research on animals.

Anaesthesia. The experiments were performed under general anaesthesia. The mice were anaesthetised with Isoflurane (Flurane-Isoflurane, Abbot Labs Ltd., UK) gas anaesthesia (1.5-2% of Isoflurane was mixed with a N_2O and O_2 mixture; the flow of N_2O and O_2 was 0.6 l/min). The animals were anaesthetised and placed on a heating pad (TCU 035, 27S, Cheshire, UK), to maintain stable body

temperature. Throughout the experiment rectal temperature and heating pad surface temperature were monitored. The rectal temperature was kept between 37 and 38 °C and the maximum surface temperature of the heating pad was kept below 40 °C.

Drugs. Hydralazine (Hydrazinophthalazine, Sigma Chemical Co., St. Louis, MO, USA) was dissolved in sterile saline (0.9% NaCl) prior to each experiment. A dose of 2.5 mg/kg (125 to 250 μl , depending on mouse weight) was injected intravenously (i.v.). The dose was chosen on the basis of different doses tested, according to the literature (10). In control experiments, we injected 125 to 250 μl of 0.9% NaCl solution instead of hydralazine.

Measuring technique and experimental protocol. IFP was measured by the wick-in-needle technique (11, 3, 12) 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 fibres (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 the tumour and in the subcutis close to the tumour or in the thigh muscle. The needles were connected to pressure transducers (TSD104 and TSD104A, Biopac Systems Inc., CA-Goleta, USA) by a polyethylene tube and the entire system was filled with NaCl solution (0.9% NaCl) which contained heparin (Krka, Slovenia) 72 U/ml to prevent blood clotting. Special care was taken to avoid trapping of air bubbles in the system during the filling. Saline in the system was used as a conductor of pressure. The pressure transducers were connected via an amplifier (DA100A, Biopac System Inc.) and data acquisition unit (MP100, Biopac Systems Inc.) to a personal computer. The sampling frequency was 10 Hz.

During calibration of the measurement system, zero reference pressure was obtained by placing the needles in a heparinised NaCl

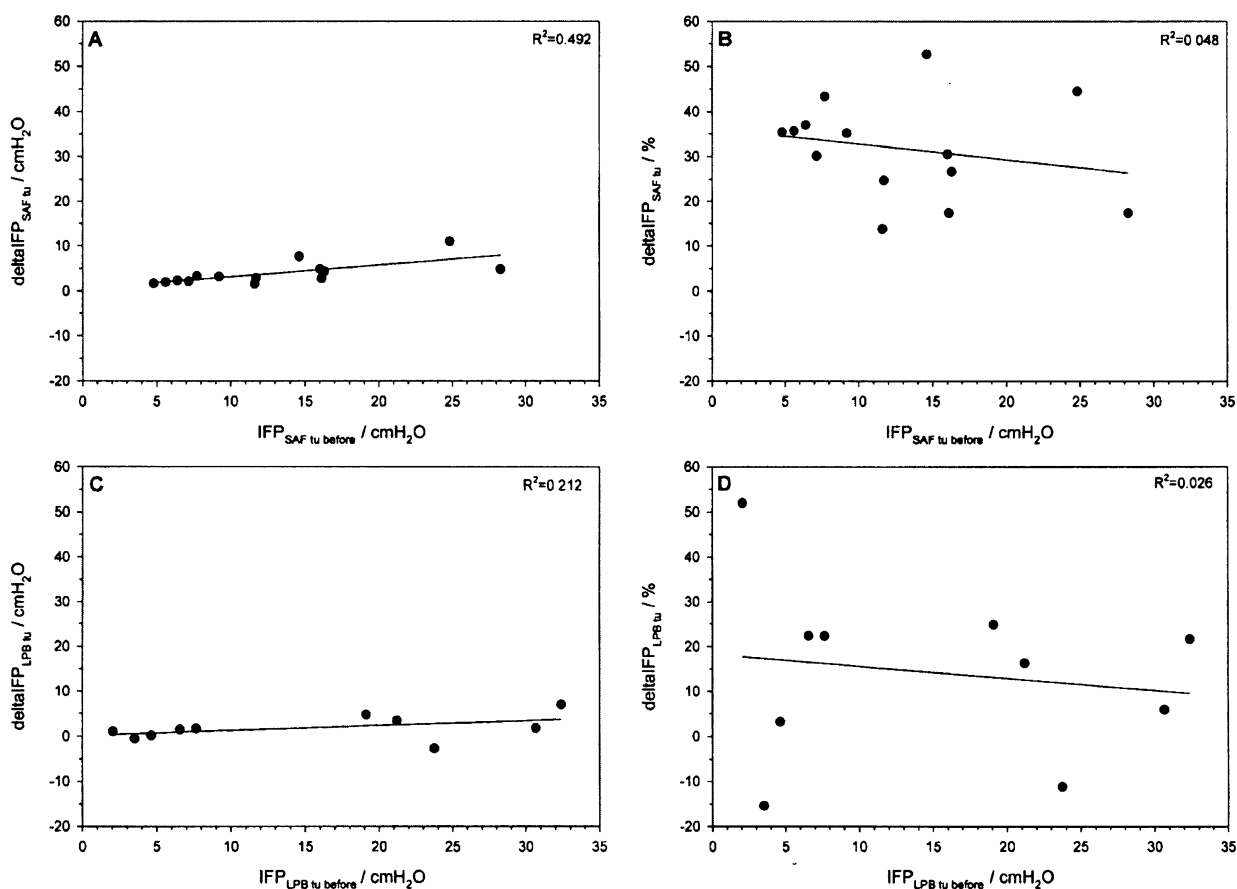


Figure 2. Relation between absolute change of IFP in tumour after injection of 2.5 mg/kg hydralazine and initial value of IFP in tumours before injection of hydralazine with linear regression and correlation factor are given in Figures A and C. Figures B and D show a relation between percentage change of IFP in tumour after HYZ injection and initial value of IFP in tumour before injection of HYZ. There is a linear regression and correlation factor too.

solution-filled beaker and calibration of pressure was done by elevating or lowering the beaker. At different levels (1 cm and 30 cm) of water column (level 0 cm was equal to the level of needle insertion into tumour or muscle), the output voltages of the pressure sensors were measured and used for calibration. After the calibration one needle was inserted into the centre of a tumour and the other needle was inserted into subcutis or into the muscle of a right hind limb. After insertion, the needles were slightly withdrawn to avoid compression of the tumour and muscle under the probe tip and left in place without external fixation. All measurements lasted for 2 hours or longer.

A typical complete IFP measurement is given in Figure 1. After the initial equilibration period, a compression/decompression (C/D) test was performed. This test allowed us to verify the continuum between the fluid phase in the interstitium and in the needle lumen. Compressing the clamp on a polyethylene tube so as to inject a volume of approximately 0.2 μ l into the tissue caused a sudden pressure rise (Figure 1). The pressure then declined, first rapidly and then more slowly, re-establishing the initial level within 30 seconds to 2 minutes. Withdrawal of the same amount of fluid by decompressing 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 each experiment and gave us the information about the quality and reliability of the IFP measurements. Only results were

accepted (21 measurements for CBA and 13 for C57Bl/6 mice) and considered as reliable. Other measurements where compression, decompression tests were not good (10 for CBA and 11 for C57Bl/6 mice), were rejected. Depending on the mouse weight, hydralazine or NaCl solution (125 to 250 μ l) dose was injected i.v. after a stable recording of IFP was obtained. The response of IFP to hydralazine was monitored for approximately 30 minutes to one hour. After that period the compression/decompression test was performed again and the needles removed from the tissue. Measurement was finished with the calibration test in order to verify the calibration procedure performed prior to the beginning of the experiment (3).

Data processing and statistical analysis. Initial IFP values in tumours and muscle or subcutis were determined as the mean value and standard deviation of IFP recording in the period of approximately 25 minutes duration after the first compression/decompression (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 period of approximately 30 minutes duration starting at 10-15 minutes after hydralazine (or NaCl solution) injection. All the values are reported in the tables and figures as mean \pm standard deviation. Statistical analysis of the data was performed using a paired t-test comparing IFP values in tumours

Table 1. Interstitial fluid pressure (IFP) in SAF tumour (IFP_{SAF tu}) and in muscle (IFP_{mu}) before and after injection of 2.5 mg/kg (125-250 µl) hydralazine (HYZ) or 125-250 µl NaCl solution (NaCl sol.) with respect to tumour volume (V_{SAF tu}). The decrease induced by HYZ or NaCl solution in tumour and muscle IFP are given (ΔIFP_{SAF tu} and ΔIFP_{mu}, respectively). A paired t-test performed on data signed with (a), shows a significant change (p_{SAF}≤0.001) between IFP values in tumour (before/after) HYZ administration, while there was no statistically significant change observed between values in muscle (p_{CBA}=0-473). Also there was no statistically significant change between values in tumour (p_{SAF}=0.850) on in muscle (p_{CBA}=0.950) before/after NaCl solution administration.

	V _{SAF,tu} (mm ³)	IFP _{SAF tu} ^a BEFORE (cmH ₂ O)	IFP _{SAF B tu} ^a AFTER (cmH ₂ O)	Δ IFP _{SAF tu} ^c (cmH ₂ O)	IFP _{mu} ^a BEFORE (cmH ₂ O)	IFP _{mu} ^a AFTER (cmH ₂ O)	Δ IFP _{mu} ^c (cmH ₂ O)
Hydralazine	94.20	4.80 ± 1.9	3.10 ± 0.6	1.70	1.00 ± 0.3	0.90 ± 0.4	0.10
	123.40	24.82 ± 1.2	13.77 ± 0.9	11.05	1.83 ± 0.3	1.87 ± 0.3	-0.04
	141.60	16.01 ± 1.1	11.12 ± 0.8	4.89	0.42 ± 0.27	0.30 ± 0.3	0.12
	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
	185.10	7.15 ± 1.4	4.99 ± 0.9	2.16	1.06 ± 0.57	0.78 ± 0.5	0.28
	272.40	6.40 ± 1.1	4.03 ± 1.1	2.37	1.79 ± 0.4	2.10 ± 0.4	-0.31
	294.70	16.30 ± 1.2	11.94 ± 1.0	4.36	0.38 ± 0.2	0.41 ± 0.2	-0.03
	310.60	28.27 ± 2.7	23.36 ± 2.0	4.91	1.85 ± 0.4	1.90 ± 0.3	-0.05
	368.40	11.70 ± 1.7	8.80 ± 1.1	2.90	0.85 ± 0.3	0.79 ± 0.3	0.06
	417.80	14.60 ± 1.7	6.90 ± 1.0	7.70	-0.20 ± 0.3^b	-0.15 ± 0.5^b	-0.05^b
	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^b	0.05 ± 0.3^b	-0.15^b
NaCl sol.	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
	154.40	6.57 ± 1.3	6.20 ± 0.2	0.37	0.78 ± 0.3	0.58 ± 0.2	0.20
	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
	238.50	10.30 ± 1.3	9.30 ± 0.9	1.00	1.10 ± 0.7	1.10 ± 0.6	0.00
	303.97	14.00 ± 2.3	13.30 ± 1.3	0.70	1.34 ± 0.3	1.40 ± 0.3	-0.06

^a Values are mean ± std

^b IFP values in subcutis

^c Values are obtained with equation [1] (addenda)

before and after hydralazine injection, IFP in muscle tissue or subcutis before and after hydralazine injection, after the normality test was performed and fulfilled. Exact p-values are reported.

Results

Measurements of IFP were performed by means of the wick-in-needle technique in two different solid murine tumour

models. Altogether 21 SAF tumours of CBA and 13 LPB tumours of C57B1/6 mice were measured. IFP was measured simultaneously in tumours and in the muscle or subcutis before and after hydralazine administration. In addition, 10 control measurements were performed (7 on SAF and 3 on LPB tumours) where instead of hydralazine, NaCl solution was injected. We determined the initial value of IFP in

Table II. Interstitial fluid pressure (IFP) in LPB tumour ($IFP_{LPB\ tu}$) and in muscle (IFP_{mu}) before and after injection of 2.5 mg/kg (125-250 μ l) hydralazine (HYZ) or 125-250 μ l NaCl solution with respect to tumour volume ($V_{LPB\ tu}$). The decrease induced by HYZ or NaCl solution in tumour and muscle IFP are given ($\Delta IFP_{LPB\ tu}$ and ΔIFP_{mu} respectively). A paired *t*-test done on data signed with (a), shows a significant change ($P_{LPB} \leq 0.064$) between IFP values in tumour (before/after) HYZ administration, while there was no statistically significant change observed between values in muscle ($p_{C57B1/6} = 0.019$). Also there was no statistically significant change between values in tumour ($P_{LPB} = 0.850$) or in muscle ($p_{C57B1/6} = 0.595$) before/after NaCl solution administration.

	$V_{LPB\ tu}$ (mm^3)	$IFP_{LPB\ tu}^a$ BEFORE (cmH_2O)	$IFP_{LPB\ tu}^a$ AFTER (cmH_2O)	$\Delta IFP_{LPB\ tu}^c$ (cmH_2O)	IFP_{mu}^a BEFORE (cmH_2O)	IFP_{mu}^a AFTER (cmH_2O)	ΔIFP_{mu}^c (cmH_2O)
Hydralazine	135.70	2.06 \pm 1.2	0.99 \pm 0.8	1.07	1.69 \pm 0.6	1.68 \pm 0.6	0.01
	138.40	6.56 \pm 1.6	5.09 \pm 1.2	1.47	0.89 \pm 0.5	1.09 \pm 0.3	-0.20
	139.45	4.64 \pm 1.0	4.49 \pm 1.0	0.15	3.26 \pm 0.3	3.49 \pm 0.4	-0.20
	156.60	19.10 \pm 1.2	14.35 \pm 1.8	4.75	3.03 \pm 0.7	3.86 \pm 1.1	-0.83
	165.70	7.65 \pm 0.9	5.94 \pm 0.7	1.71	2.68 \pm 0.4	2.90 \pm 0.3	-0.22
	179.20	3.52 \pm 0.7	4.06 \pm 0.6	-0.54	1.49 \pm 0.4	1.39 \pm 0.6	0.10
	187.90	21.19 \pm 1.7	17.7 \pm 1.1	3.47	2.67 \pm 0.5	3.00 \pm 0.6	-0.39
	241.90	23.73 \pm 1.3	26.38 \pm 1.5	-2.65	1.99 \pm 0.4	2.30 \pm 0.3	-0.31
	318.90	32.40 \pm 1.7	25.37 \pm 1.2	7.03	0.51 \pm 0.3	0.62 \pm 0.2	-0.11
	321.49	30.66 \pm 1.4	28.84 \pm 1.8	1.82	1.38 \pm 0.3	1.51 \pm 0.4	-0.13
NaCl	136.60	1.42 \pm 0.7	2.38 \pm 0.5	-0.96	1.04 \pm 0.3	1.16 \pm 0.3	-0.12
	290.28	5.19 \pm 1.2	6.50 \pm 1.1	-1.31	2.94 \pm 0.5	4.20 \pm 0.5	-1.26
	329.57	24.31 \pm 0.3	22.10 \pm 1.1	2.21	0.38 \pm 0.3	-0.06 \pm 0.4	0.44

^a Values are mean \pm std

^c Values are obtained with equation [1] (addenda)

tumours and normal tissue (*i.e.* muscle or subcutis) during the stable recording of IFP signal and IFP after injection, as described in the Materials and Methods section.

In general, immediately after needle insertion we recorded negative IFP in the tumour and in muscle or subcutis (Figure 1). Under control conditions when NaCl solution was injected, the IFP returned rapidly and stabilised at a level around 0 cmH_2O for muscle and somewhere between and 33 cmH_2O for tumour, depending on the tumour size (Tables I, II, III and IV). After 10 to 15 minutes following hydralazine injection, IFP in the tumours decreased from the initial level on average by 31% (3.71 ± 2.59 cmH_2O) in SAF and by 14% (1.83 ± 2.74 cmH_2O) in LPB tumour, but there was no decrease of IFP in muscle or in subcutis (Tables I, II, III and IV). This reduced IFP level lasted at least 30 minutes and then slowly returned to the pre-treatment level.

Initial values of IFP in tumours, based on measurements prior to any manipulations, were between 4.8 and 28.27 cmH_2O for SAF and between 2.06 and 32.40 cmH_2O for LPB tumours with the mean \pm std value of 11.78 ± 6.21 cmH_2O ($n_{SAF} = 21$) and 14.03 ± 11.42 ($n_{LPB} = 13$), respectively. The initial values of IFP in tumours were higher in larger tumours, as we previously observed (13, 14). The initial values of IFP in

muscle were between 0.3 and 1.8 cmH_2O in CBA and between 0.3 and 3.3 in C57Bl/6 mice with the mean \pm std value of 0.93 ± 0.55 cmH_2O ($n_{CBA} = 19$) and 1.84 ± 0.99 cmH_2O ($n_{C57B1/6} = 13$), respectively.

The values of IFP in tumours and normal tissue (muscle and subcutis) after hydralazine administration are given in (Tables I and III) for each tumour and corresponding muscle or subcutis IFP measured at the same time. The IFP in tumours after hydralazine administration was significantly lower than the initial values in corresponding tumours (paired *t*-test: $P_{SAF} < 0.001$, $P_{LPB} = 0.064$). On average tumour IFP in SAF and LPB tumours decreased by 31% and by 14% from the initial value, respectively. This decrease was in individual tumours, between 14 to 53% in SAF and between 6 and 52% in LPB tumour (mean \pm std $\Delta IFP_{SAF} = 3.93 \pm 2.6$ cmH_2O , $\Delta IFP_{LPB} = 1.83 \pm 2.7$ cmH_2O). On the contrary, no decrease in IFP in normal tissue was observed after hydralazine administration resulting in mean \pm std $\Delta IFP_{CBA} = 0.04 \pm 0.2$ cmH_2O and $\Delta IFP_{C57B1/6} = -0.23 \pm 0.3$ cmH_2O ($p_{CBA} = 0.473$, $p_{C57B1/6} = 0.019$) (Tables I and III). In the control experiment, where NaCl solution was injected instead of hydralazine, the injection produced no decrease either in tumours with mean \pm std $\Delta IFP_{SAF} = 0.03 \pm 0.8$ cmH_2O , $\Delta IFP_{LPB} = -$

Table III. Change in interstitial fluid pressure (IFP) in SAF tumour and in muscle ($\Delta IFP_{SAF\ tu, mu\ before}$, $\Delta IFP_{SAF\ tu, mu\ after}$) before and after injection of 2.5 mg/kg (125-250 μ l hydalazine (HYZ) and after injection of 125-250 μ l NaCl solution, with respect to tumour volume ($V_{SAF\ tu}$) and percentage decrease induced by HYZ in tumour IFP are given ($\Delta IFP_{SAF\ tu}$ respectively).

	$V_{SAF\ tu}$ (mm ³)	$\Delta IFP_{SAF\ tu, mu\ before}$ (cmH ₂ O) ^d	$\Delta IFP_{SAF\ tu, mu\ after}$ (cmH ₂ O) ^d	$\Delta IFP_{SAF\ tu}$ (%) ^e	ΔIFP_{mu} (%) ^e
Hydalazine	94.20	3.80	2.20	35.42	10.00
	123.40	22.99	11.90	44.52	-2.19
	141.60	15.68	10.82	30.54	28.57
	144.10	6.50	3.59	43.38	35.83
	149.30	8.48	5.17	35.22	-9.72
	175.80	11.23	9.67	13.79	10.81
	185.10	6.09	4.21	30.21	26.42
	272.40	4.61	1.93	37.03	-17.32
	294.70	15.92	11.53	26.75	-7.89
	310.60	26.42	21.46	17.37	-2.70
	368.40	10.85	8.01	24.79	7.06
	514.10	5.25	3.20	35.71	-14.29
NaCl	94.50	9.09	10.10	-12.65	-56.25
	136.00	13.64	14.00	-2.14	16.67
	154.40	5.79	5.62	5.63	25.64
	95.60	3.50	4.10	-11.76	0
	228.80	7.44	7.15	3.19	-9.75
	238.50	9.20	8.20	9.71	0
303.97	12.66	11.90	5.00	-4.48	

^dValues are obtained with equations [3, 4] (addenda).

^eValues are obtained with equation [2] (addenda).

0.02±1.9 ($p_{SAF} = 0.850$, $p_{LPB} = 0.987$) or in muscle with mean±std $\Delta IFP_{CBA} = -0.003±0.2$ cmH₂O, $\Delta IFP_{C57B1/6} = -0.31±0.9$ cmH₂O ($p_{CBA} = 0.950$, $p_{C57B1/6} = 0.595$) (Tables II and IV).

Discussion

The aim of our study was to measure the IFP simultaneously in tumour and in muscle or in subcutis and to determine if injection of hydalazine reduces differentially tumour IFP

Table IV. Change in interstitial fluid pressure (IFP) in LPB tumour and in muscle ($\Delta IFP_{LPB\ tu, mu\ before}$, $\Delta IFP_{LPB\ tu, mu\ after}$) before and after injection of 2.5 mg/kg (125-250 μ l hydalazine (HYZ) and after injection of 125-250 μ l NaCl solution, with respect to tumour volume ($V_{LPB\ tu}$) and percentage decrease induced by HYZ in tumour IFP are given ($\Delta IFP_{LPB\ tu}$ respectively).

	$V_{LPB\ tu}$ (mm ³)	$\Delta IFP_{LPB\ tu, mu\ before}$ (cmH ₂ O) ^d	$\Delta IFP_{LPB\ tu, mu\ after}$ (cmH ₂ O) ^d	$\Delta IFP_{LPB\ tu}$ (%) ^e	ΔIFP_{mu} (%) ^e	
Hydalazine	135.70	0.37	-0.69	51.94	0.59	
	138.40	5.67	4.00	22.41	-22.47	
	139.45	1.38	1.00	3.23	-7.06	
	156.60	16.07	10.49	24.87	-27.39	
	165.70	4.97	3.04	22.35	-8.21	
	179.20	2.03	2.67	-15.34	6.71	
	187.90	18.52	14.66	16.38	-14.61	
	241.90	21.74	24.08	-11.17	-15.58	
	318.90	31.89	24.75	21.70	-21.57	
	321.49	29.28	27.33	5.94	-9.42	
	NaCl	136.60	0.38	1.22	-67.61	-11.54
		290.28	2.25	2.30	-25.24	-42.86
329.57		23.93	22.16	9.09	115.79	

^dValues are obtained with equations [3, 4] (addenda).

^eValues are obtained with equation [2] (addenda).

with respect to IFP in the surrounding and normal tissues. Measurements of IFP were performed in two different solid murine tumour models. IFP in tumours after hydalazine administration was significantly reduced from the initial values in both tumour models. On average the tumour IFP in SAF and LPB tumours decreased by 31% and 14% from the initial, values, respectively. On the contrary, hydalazine did not decrease IFP in normal tissue. Injection of NaCl solution instead of hydalazine had no effect on IFP either in tumours or in the subcutis/muscle.

The decrease in IFP was only observed in tumours, while in the surrounding tissue and muscle no decrease was observed. We found that the initial IFP in tumours positively correlated with tumour size, while the decrease obtained by hydalazine injection on tumour IFP was independent of the initial IFP. In addition, the decrease in IFP was not correlated with tumour volume nor with initial value of IFP (IFP_{tu})

The results of our study on the effects of hydalazine on IFP in SAF and LPB tumour models are in accordance to previously reported studies (10, 8), both in the amplitude and the duration of response. The choice of hydalazine dose (2.5

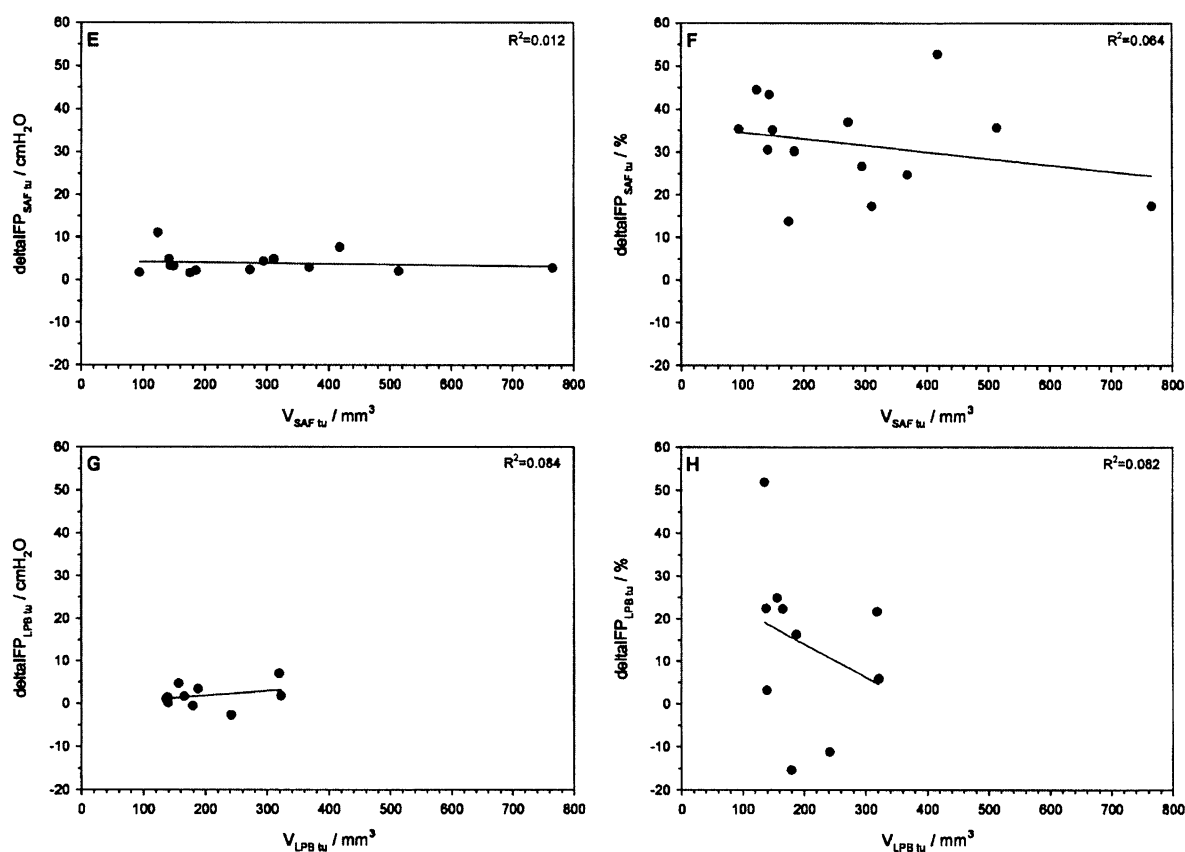


Figure 3. Relation between absolute change of IFP in tumour after injection of 2.5 mg/kg. HYZ and tumour volume with linear regression and correlation factor are given in Figures E and G. Figures F and H show a relation between percentage change of IFP in tumour after HYZ injection and tumour volume before injection of HYZ. There is a linear regression and correlation factor too.

mg/kg) was based on previous studies, where comparable doses resulted in a 50% decrease in mean arterial blood pressure that occurred within 10-15 minutes after hydralazine injection and lasted for at least 30 minutes (10). In another study (8), both mean arterial blood pressure as well as tumour IFP were measured after 60 μ g of hydralazine injection (which corresponds to approximately 3 mg/kg dose). The reduction of mean arterial blood pressure (50%) was obtained, being the same as in the previously mentioned study (10). In our study we observed a decrease in tumour IFP with respect to the initial value with an average of 31% (range: 14-53%) in SAF and 14% (range: 6-52%) in LPB tumour model. This response was observed within 15 minutes after hydralazine injection and lasted for at least 30 minutes thereafter. The exact relationship between mean arterial blood flow and IFP is not well-established. In addition, we did not observe any significant changes in IFP in muscle or subcutis, demonstrating a potentially interesting differential effect. Other authors have stipulated that an observed decrease in tumour IFP after hydralazine injection could be

explained by the reverse steal-effect, which remains to be confirmed. Our results on initially elevated IFP and its dependence on tumour size are well in accordance with our previous results and the model we developed (15).

Comparison between Figures A, B, C, D and E, F, G, H from Figures 2 and 3 shows that there is no correlation (correlation factors R^2 are low) between tumour volume (V_{tu}) and absolute or percentage value of decrease in IFP (ΔIFP_{tu}) in tumour or decrease in IFP after hydralazine injection. Except in Figure A, moderate correlation between the absolute value of change in IFP and the initial value of tumour IFP can be seen. In general, the results show that neither the absolute nor percentage value of decrease in IFP is correlated with tumour volume or initial value of IFP (IFP_{tu}).

In additional control experiments where we injected NaCl solution only, a non-significant short-liver decrease in IFP, present in both signals (in tumour and in muscle), was noticed. This decrease was, however transient and IFP returned to the initial level within two to three minutes.

In conclusion, hydralazine is a vasodilator, which is capable of decreasing differentially tumour IFP reproducibly and with favourably long-lasting dynamics. The decrease in tumour interstitial fluid pressure was only observed in tumours, not in muscle and surrounding subcutis. The decrease in tumour IFP was neither correlated with tumour volume nor with initial value of IFP.

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Addenda

All data in Tables I, II and III, were calculated based on the following equations:

$$\Delta IFP_{tu/mu} = \Delta(IFP_{tu/mu \text{ before}} - IFP_{tu/mu \text{ after}}) \quad [1]$$

$$\Delta IFP_{tu/mu} = \frac{\Delta(IFP_{tu/mu \text{ before}} - IFP_{tu/mu \text{ after}})}{IFP_{tu/mu \text{ before}}} \cdot 100\% \quad [2]$$

$$\Delta IFP_{tu, mu \text{ before}} = IFP_{tu \text{ before}} - IFP_{mu \text{ before}} \quad (\text{cmH}_2\text{O}) \quad [3]$$

$$\Delta IFP_{tu, mu \text{ after}} = IFP_{tu \text{ after}} - IFP_{mu \text{ after}} \quad (\text{cmH}_2\text{O}) \quad [4]$$

IFP_{tu} - interstitial fluid pressure in SAF or in LPB tumours
 IFP_{tu before} - interstitial fluid pressure in tumour before injection of HYZ,
 IFP_{tu after} - interstitial fluid pressure in tumour after injection of HYZ,
 IFP_{mu before} - interstitial fluid pressure in tumour before injection of HYZ,
 IFP_{mu after} - interstitial fluid pressure in tumour after injection of HYZ,
 and all tumour volumes were calculated with equations below.

$$V_{tu} = (L \cdot W \cdot T / 6) \cdot \pi \quad [5]$$

L - length of tumour
 W - width of tumour
 T - thickness of tumour

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