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LOWERING OF TUMORAL INTERSTITIAL FLUID PRESSURE BY PROSTAGLANDIN E_1 IS PARALLELED BY AN INCREASED UPTAKE OF ^{51}Cr-EDTA

Kristofer RUBIN[1]*, Mats SJÖQUIST[2], Ann-Marie GUSTAFSSON[1], Britta ISAKSSON[2], Gerd SALVESSEN[3] and Rolf K. REED[3]

[1]*Department of Medical Biochemistry and Microbiology, University of Uppsala, Uppsala, Sweden*
[2]*Department of Physiology, University of Uppsala, Uppsala, Sweden*
[3]*Department of Physiology, University of Bergen, Bergen, Norway*

High intra-tumoral fluid pressure (TP_{IF}) may impair uptake of anticancer drugs into tumors, contributing to poor efficiency in treatment of carcinomas. Here, we demonstrate that lowering of TP_{IF} parallels increased transport of ^{51}Cr-EDTA (m.w. 341) into tumor interstitium. Introduction of 15 μg prostaglandin E_1 (PGE^1) -methyl ester into the s.c. tissue surrounding transplanted rat colonic (PROb) carcinomas or chemically-induced rat mammary carcinomas, lowered TP_{IF} by 30%. Transcapillary transport into the interstitium of PROb tumors quantified by microdialysis increased by 39.6% after PGE_1 treatment 40 min prior to administration of ^{51}Cr-EDTA (n=6; $p<0.05$) compared to vehicle (n=10). In mammary tumors, PGE_1 increased transport into the tumors by 86.9% over controls (n=16; $p<0.05$). Both tumors had well developed stroma containing collagen and hyaluronan. Our data demonstrate that adjuvant treatment with PGE_1 lowers TP_{IF}, and enhances transport into the tumors. This principle may be of value as adjuvant therapy in treatment of solid malignancies with currently used anticancer drugs. *Int. J. Cancer* 86:636–643, 2000.
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Successful pharmacological treatment of solid tumors requires that the anticancer agents reach the tumor cells. They must cross capillary barriers, and diffuse through the extracellular matrix of the tumor stroma, to reach the malignant cells. The problem of how anticancer agents reach the malignant cells has received relatively little attention. It has been suggested that the high intratumoral interstitial fluid pressure (TP_{IF}), typical of human (Less *et al*., 1992; Nathanson and Nelson, 1994) and experimental (Gullino *et al*., 1964; Wiig *et al*., 1982) solid tumors, impose a hydrodynamic barrier to intratumoral transport of macromolecular antitumor agents (Jain, 1987, 1996; Netti *et al*., 1999). Nicotinamide (Lee *et al*., 1992) and TNF-α (Kristensen *et al*., 1996) decrease TP_{IF} in experimental tumors. Treatment of athymic mice, carrying transplanted human colon adenocarcinoma, with dexamethasone for several days results in lowering of TP_{IF} (Kristjansen *et al*., 1993). It is, however, presently not clear whether a lowering of TP_{IF} increases capillary-to-interstitium transport of low m.w. compounds.

The Starling hypothesis states that the interstitial fluid pressure (P_{IF}) participates in the control of transcapillary fluid fluxes (for a review, Aukland and Reed, 1993) according to the equation $J_V = \Delta P K$, where J_V is filtration rate and K is a constant expressing capillary area and permeability, where $\Delta P = (P_{PL} - P_{IF}) - \sigma (COP_{PL} - COP_{IF})$ in which P_{PL} equals the capillary hydrostatic pressure, P_{IF} the interstitial fluid pressure, COP_{PL} the colloid osmotic pressure in plasma, COP_{IF} the colloid pressure in the interstitial fluid and σ plasma protein reflection coefficient. A lowering of P_{IF} will enhance transcapillary fluid flux and is the main explanation for the rapid development of edema formation associated with inflammation. Thus, attenuating the lowering of P_{IF} will limit or abolish edema formation under these conditions. A large body of data demonstrates that the connective tissue actively can regulate P_{IF} and thereby control fluid content in the tissue (Aukland and Reed, 1993). We have proposed a model for how connective tissue cells control P_{IF} by a mechanism involving a regulating cellular tension. This cellular tension acts on the extracellular matrix, the fiber network restraining the ground substance, thus preventing the swelling of the hyaluronan and proteoglycan ground substance (Reed *et al*., 1997; Rubin *et al*., 1996).

We have demonstrated that infusion of prostaglandin E_1 or carbaprostacyclin in rat dermis induces a lowering of P_{IF} and formation of edema (Berg *et al*., 1998). In the present study, we took advantage of this observation and investigated whether PGE_1 would also decrease P_{IF} in experimental tumors which have a high TP_{IF}. Furthermore, we have investigated, employing the technique of microdialysis, whether a lowering of TP_{IF} is paralleled by an increase in transport of the low molecular mass compound ^{51}Cr-EDTA into the tumors.

MATERIAL AND METHODS

Animals and tumors

BD-IX rats were obtained from Charles River Laboratories (Uppsala, Sweden) and bred at the animal facility at the Biomedical Center in Uppsala. Female Sprague Dawley rats were from Möllergaard (Lille Skensved, Denmark). The animal experiments had been approved by the ethical committees for animal experiments in Uppsala (Sweden) and at University of Bergen (Norway).

The PROb subclone of the cell line DHD-K12 (Martin *et al*., 1996) was employed to generate s.c. growing rat colonic carcinoma in syngeneic BD-IX rats. PROb cells (5×10^6), suspended in PBS, were injected s.c. in the left shoulder on male or female BD-IX rats weighing 200–400 g. Tumor growth was monitored externally on the rats. After approximately 2 months, s.c. tumors with an average volume of around 2 cm^3 appeared. Experiments were conducted with tumors of various sizes.

Female Sprague-Dawley rats, 7 weeks of age, received 16 μg di-methyl-benz- anthracene (DMBA) in olive oil by gavage. This was done at the animal facility at Möllergaard, Denmark. After DMBA gavage the rats were housed in isolation for 1 week and subsequently transferred to the animal facility at University of Bergen, Norway. The rats were housed under normal animal house conditions until mammary tumors had developed along the mammary crest about 6 to 8 weeks later.

Measurement of TP_{IF}

On the day of the experiment, PROb tumor bearing BD-IX rats were anesthetized with Inactin (sodium 5-sec-butyl-5-ethyl-2-thiobarbiturate; RBI, Natic, MA) given i.p. at a dose of 120 μg kg^{-1} body weight and placed on a servo-controlled heating pad maintaining a rectal temperature of 37.5°C. After tracheostomy, the left femoral vein was cannulated for continuous infusion of Ringer's

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*Correspondence to: Department of Medical Biochemistry and Microbiology, University of Uppsala, BMC, Box 575, SE-751 23 Uppsala, Sweden. Fax: +46-18 471 4975. E-mail: rubin@medkem.uu.se

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solution (129 mM NaCl, 2.5 mM KCl, 25 mM $NaHCO_2$, 0.75 mM $CaCl_2$) at a rate 5 mL hr^{-1} kg^{-1} body weight to compensate for fluid losses during the experiment. The right femoral artery was cannulated to continuously measure mean arterial blood pressure (MAP). TP_{IF} was monitored continuously with the "wick-in-the needle" technique using a 23G needle inserted blindly into the tumor. The needle was attached to a PE50 catheter filled with saline supplemented with 50 IE/mL of heparin and connected to a pressure transducer (Hewlett Packard, PaloAlto, CA). After having obtained a stable pressure, the PE50 catheter is slightly compressed using a clamp placed on the catheter. This should result in a transient rise in pressure with return to a stable pressure within a few minutes at the same level as prior to compressing the catheter. Subsequent release of the clamp should result in the opposite response, followed by a return to the same level as prior to release of the clamp within a few minutes. The maneuver of tightening and releasing a clamp on the catheter ensures communication between the fluid inside the needle and interstitial fluid. A measurement was accepted when the pressures obtained prior to clamping, after clamping and finally after release of the clamp did not differ by more than 0.5 mmHg. Control measurements using 2 needles for measurements of TP_{IF} in the same tumor did not reveal significant differences between the TP_{IF} recorded from the 2 needles. In the case of bleeding and subsequent coagulation in the tip of needle, the typical response is a pressure recording which does not become stable but typically fluctuates by 2–4 mmHg. Reference level for interstitial fluid pressure (zero mmHg) was set in a saline cup at the level of implantation of the needle. When MAP and TP_{IF} had stabilized, 15 μg of PGE_1-methyl ester (Sigma P439, St Louis, MO) or 15 μg of carbaprostacyclin (Sigma, C3305) both dissolved in PBS with 2.5% ethanol was administered locally around the tumor. Control animals received 1 mL of vehicle alone (PBS with 2.5% ethanol), which was installed locally around the tumor. All injections were made as 4 or 5 doses and care was taken not to penetrate the tumor tissue with the injection needles.

TP_{IF} measurements in chemically induced mammary tumors were performed as described above. However, here the MAP was not recorded routinely and here the rats were anesthetized with 50 μg kg^{-1} pentobarbital given i.p.

Microdialysis technique

Microdialysis was used to determine the transcapillary transport of ^{51}Cr-EDTA (m.w. 341.3) (NEN, Sollentuna, Sweden). Rats with PROb tumors were anesthetized, tracheotomized, and the left and right femoral veins and arteries, cannulated, all as described above. In animals carrying PROb tumors implantable 10 mm long microcdialysis probes (CMA20; CMA/Microdialysis, Solna, Sweden) with nominal m.w. cut-off at 20 kDa were implanted into the tumors and in the left jugular vein. Between 40 and 80 μCi of ^{51}Cr-EDTA was injected in the right jugular vein 10 or 40 min after the administration of 15 μg PGE_1 into the s.c. tissue surrounding the tumors. The dialysis probes were perfused at a rate of 2.5 mL per min and fractions collected in a Microfraction Collector CMA/140 (CMA/Microdialysis). The dialysate for each 10 min period after i.v. injection of ^{51}Cr-EDTA was pooled in aliquots. These aliquots were collected for 2 hr. Radioactivity in samples were measured by scintillation counting using a LKB Wallac (Bromma, Sweden) γ-counter. The areas under the plasma curve, and the tumor curve (*i.e.*, the product of radioactivity, in cpm and time), were simply taken as the total amount of counts in the respective pools of samples. Experiments in which the tumor collapsed when the microprobes were inserted (n=8) were rejected.

Quantification of the uptake of ^{51}Cr-EDTA into rat mammary tumors was performed according to the same protocol as that for PROb tumors described above but with some modifications. Dialysis probes were perfused at 1 μL per min and 30 μCi ^{51}Cr-EDTA (Amersham, Aylesbury, UK) was injected 0 or 40 min after administration of 15 μg PGE_1 around the tumors. Plasma levels of ^{51}Cr-EDTA were determined in 10 μL of the plasma. This plasma was drawn at the end of the periods when the microdialysate was sampled. The area under the plasma curve was obtained by numerical integration (Simpson's rule). The radioactivity was determined with an LKB-Wallac γ-counter with automatic background subtraction.

Permeability of the probes was checked by a calibration procedure, which involved immersing the probes into PBS with a known amount of ^{51}Cr-EDTA. If the permeability differed by more than 10%, the probes were discarded.

Tumor edema formation

PROb tumors were treated *in situ* with 15 μg PGE_1-methyl ester or vehicle alone, as described above. After 2 hr, the animals were sacrificed and tumors excised and tumor wet weights (Tw) determined. Dry weights (Td) were determined after lyophilization to complete dryness. From these figures, tumor water content (Twc) was calculated according to the formula $Twc=Tw-Td/Tw\times100\%$ and expressed as % tumor water content. To determine fluid uptake of tumors *ex vivo*, tumors were excised, weighed and immersed in sterile PBS supplemented with penicillin/streptomycin to suppress bacterial growth. The tumors were allowed to swell for 28 hr (PROb) or 48 hr (mammary carcinoma). The amount of swelling was calculated as the percentage increase in tumor weight after immersion in PBS, relative to the initial weight.

Histochemical analyses of tumor morphology in sections

Tumors were excised from anesthetized animals and snap frozen in liquid nitrogen or fixed in 4% paraformaldehyde. Paraformaldehyde-fixed tumors were imbedded according to standard protocols, sectioned and routine stained with Hematoxylin and van Gieson stain. Van Gieson stain highlights extracellular matrix rich in collagen. Expression of hyaluronan was investigated by incubating 4 μm paraffin imbedded sections with biotinylated aggrecan hook domains (kindly donated by Dr. P. Heldin, University of Uppsala) and further incubated by Vectastain, ABC Elite kit (Vector, Burlingame, CA), according to the manufacturers recommendations. Immobilized horse reddish peroxidase was detected with ethyl-carbazole.

Statistical methods

One-way analysis of variance was used to test for differences within the experimental groups followed by subsequent *post-hoc* Bonferroni, Student-Newman-Keul tests or *t*-tests. When comparing 2 experimental groups, *t*-tests or Wilcoxon tests were used. These comparisons were paired when possible.

RESULTS

Morphology of PROb and mammary experimental tumors

The transplantable PROb rat colonic carcinoma typically grow from minor tumors (≤2 mm in diameter) to around 10–15 mm over a period of 5–6 weeks. Tumor cell proliferation was evident in a zone in which the tumor cells organized into semi-glandular structures (Fig. 1*a*,*b*), in agreement with what has been previously been reported for this type of tumor (Martin *et al*., 1996). A zone of cells with pycnotic nuclei and deposits of cell debris could be distinguished at the center of the tumors. In most tumors, the central region was largely acellular. Infiltrating leukocytes were not evident in any part of the tumors. The tumors possessed an extracellular matrix that surrounded growing tumor glands. This matrix was also present in the more acellular, central parts of the tumors (Fig. 1*c*,*d*). Collagen depositions (Fig. 1*c*), visualized by van Gieson staining was deteceted throughout the tumors. Hyaluronan was present in all regions of the tumor. In the proliferative region, hyaluronan was present in the stroma between glands and following vessels (Fig. 1*d*).

Microscopically, the chemically induced mammary tumors displayed a cribriform pattern, as well as infiltrating cells of the anaplastic spheroidal-cell type (Fig. 1*e*,*f*). The tumor stroma was

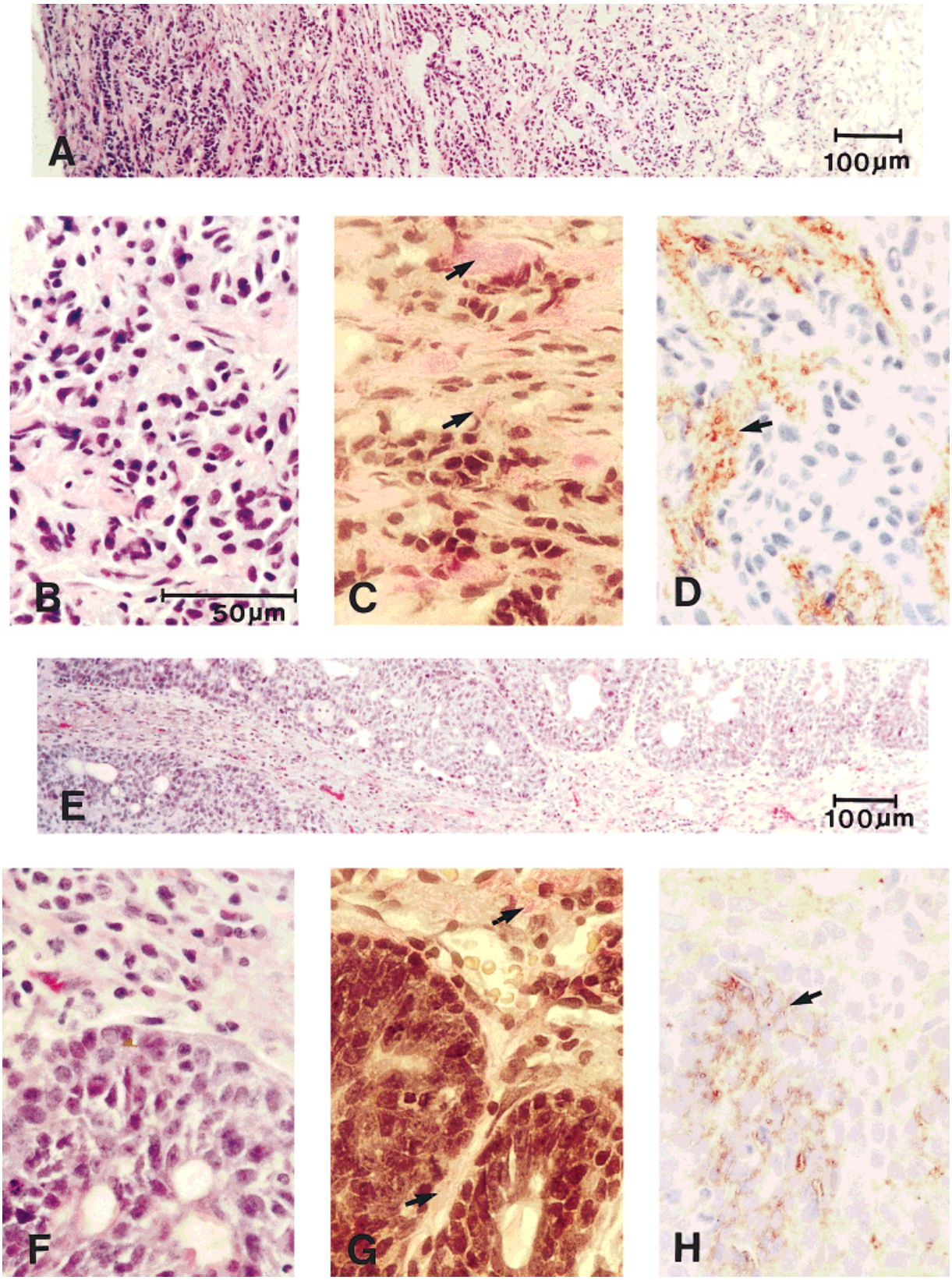

FIGURE 1 – Morphology of experimental PROb colonic carcinoma and chemically induced mammary carcinoma. Sections from PROb tumors (*a–d*) and mammary tumors (*e–h*) were stained with Hematoxylin (*a,b,e,* and *f*); van Gieson stain to highlight collagen fiber bundles seen as pink stain (arrows) in stroma around tumor cells or glands (*c,g*); or by biotinylated link-protein to detect hyaluronan, seen as brown stain (arrow) (*d,h*). Scale bars: 100 μm (*a,e*) and 50 μm (*b–d,* and *f–h*).

rich in collagen, as revealed by van Gieson staining (Fig. 1*g*), and also contained hyaluronan (Fig. 1*h*).

The effect of PGE_1 or prostacyclin on TP_{IF} and MAP

Local administration of 15 μg PGE_1-methyl ester around PROb tumors caused a time dependent lowering in TP_{IF} (Table I, Fig. 2). PGE_1 also induced a decrease in MAP in rats carrying PROb tumors. However, this effect was more transient and less marked (Fig. 2). The decrease in normalized TP_{IF} values (Fig. 2) were significant ($p<0.05$) at all time points after time zero. The decrease in MAP was significant only at the 5 min time point. Vehicle alone had no effect on either TP_{IF} or MAP (Table I, Fig. 2). TP_{IF} in untreated PROb tumors varied largely (Table I). No correlation of TP_{IF} with tumor size was evident in the PROb tumors (data not shown). Administration of PGE_1 around chemically induced mammary tumors also induced a decrease in TP_{IF} (Table II). In both types of tumors TP_{IF} decreased by around 30% as a result of PGE_1 treatment. The effect of PGE_1 on TP_{IF} was already evident 10 min after installment. Local administration of 15 μg prostacyclin around PROb tumors did not significantly change TP_{IF} or MAP (Fig. 3).

Swelling of tumors immersed in PBS and after installment of PGE_1

Excised PROb tumors that had been immersed in PBS for 28 hr increased their weight by between 6.9 and 38.7% relative to their initial values. The average increase was 18.1±11.2% (±SD; n=7). The wet weight of excised mammary tumors increased between 12 and 39.6%, relative to their initial weights, after immersion for 48 hr in PBS, with an average of 20.3±4.4% (±SD) for 5 mammary tumors investigated. PGE_1-methyl ester installment around PROb tumors *in situ* caused an increase in tumor water content (Twc) after 2 hr from 83% (n=6) to 85.2% (n=5, $p=0.090$). The latter data suggest that treatment with PGE_1 for 2 hr, induce edema formation but only in a subpopulation of PROb tumors.

Effect of PGE_1 on transcapillary transport of ^{51}Cr-EDTA in experimental tumors measured by microdialysis

The plasma clearance of ^{51}Cr-EDTA was rapid, whereas uptake in tumors proceeded during 30– 60 min to then decline (Fig. 4). The half-life of plasma clearance of ^{51}Cr-EDTA was not affected by PGE_1 in any of tumor two tumor models studied (data not shown). Area under the curve (AUC) for ^{51}Cr-EDTA levels in the

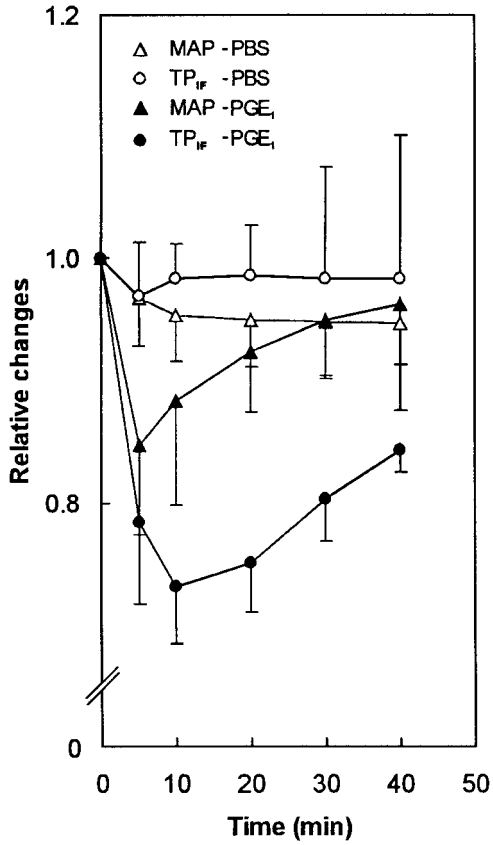

FIGURE 2 – Time course of PGE_1-induced lowering of PROb tumoral interstitial fluid pressure and mean arterial pressure in rats. Animals were first treated with 1 mL vehicle (PBS with 2.5% ethanol) for a period of 40 min during which TP_{IF} and MAP were recorded. Subsequently, 15 μg of PGE_1 in 1 mL of vehicle was installed and TP_{IF} and MAP recorded for an additional 40 min. The values for TP_{IF} and MAP at the start of the experiments are set to 1.0 and the following points are means from the 7 different rats at the indicated times. Vertical bars represent ±SD. For experimental details, see Material and Methods.

TABLE I – TIME COURSE OF PGE_1-INDUCED LOWERING OF TP_{IF} AND MAP IN PROb TUMORS[1]

Time	TP_{IF} (mmHg)[2]	MAP (mmHg)
	Vehicle alone (n = 7)	
0 (start)	19.2 ± 5.4 (10.0–27.0)	127 ± 8.9 (118–135)
5 min	18.7 ± 5.5 (9.25–27.0)	120 ± 8.9 (103–131)
10 min	19.0 ± 5.7 (9.5–27.5)	118 ± 9.1 (102–130)
20 min	19.1 ± 5.7 (9.75–27.75)	118 ± 11.1 (101–132)
30 min	19.0 ± 7.0 (9.5–28.25)	118 ± 12.6 (97.0–133)
40 min	18.9 ± 6.9 (9.5–28.75)[3]	120 ± 13.9 (97.0–135)[2]
	PGE_1 (n = 7)	
0 (start)[3]	18.6 ± 6.5 (9.5–28.8)[2]	118 ± 12.5 (97.0–131)
5 min	14.6 ± 5.2 (7.3–23.0)[2]	99 ± 11.7 (84.0–114)
10 min	13.5 ± 4.6 (7.5–21.3)[2]	100 ± 12.5 (86.0–111)
20 min	13.8 ± 4.5 (7.5–21.3)[2]	106 ± 11.1 (86.0–119)
30 min	14.9 ± 5.0 (7.8–22.8)[2]	111 ± 11.3 (87.0–122)
40 min	15.8 ± 5.7 (7.8–24.5)[2]	113 ± 12.8 (87.0–127)

[1]Tumor interstitial fluid pressure (TP_{IF}) and mean arterial pressure (MAP) were continuously recorded as described in Material and Methods. Animals were first treated with 1 mL vehicle (PBS with 2.5% ethanol) for a period of 40 min during which TP_{IF} and MAP were recorded. Subsequently, 15 μg of PGE_1 in 1 mL of vehicle were administered and TP_{IF} and MAP recorded for an additional 40 min. The normalized data from this Table are shown in Figure 2.–[2]n: number of rats; Mean ± SD, range in parenthesis.–[3]n = 6.

TABLE II – EFFECT OF LOCAL ADMINISTRATION OF PGE_1-METHYL ESTER ON TP_{IF} IN CHEMICALLY INDUCED RAT MAMMARY TUMORS[1]

Experimental group	n	TPIF (Start)[1]	TPIF (PGE_1)	Ratio PGE_1/Start
Control (vehicle alone)	6	4.6 ± 1.3	5.0 ± 1.7	1.091 ± 0.122
PGE_1 (Group I[2])	6	5.8 ± 1.1	4.2 ± 1.4	0.708 ± 0.170
PGE_1 (Group II[3])	4	12.0 ± 3.6	9.1 ± 1.9**	0.775 ± 0.095*
PGE_1 (all animals)	10	8.3 ± 3.9	6.3 ± 3.0*	0.735 ± 0.143*

[1]TP_{IF} was measured before administration of 15 μg PGE_1 around tumors (TP_{IF} Start), and 20 min after administration (TP_{IF} PGE_1).
[2]Group I consisted of tumors with $TP_{IF} < 9$ mmHg at start.
[3]Group II consisted of tumors with $TP_{IF} > 9$ mmHg at start.
Mean ± SD. *$p < 0.05$ compared to control (paired *t*-test), **$p = 0.125$ using Wilcoxon-test.

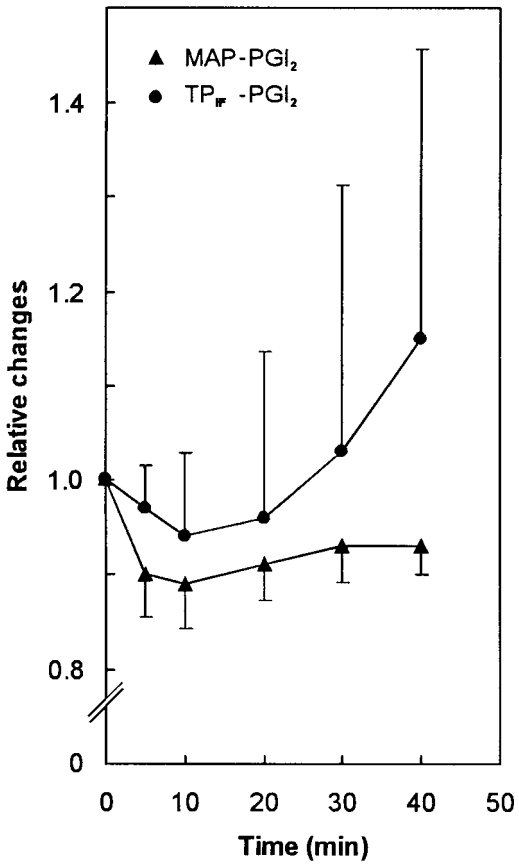

FIGURE 3 – Time course for effects of carbaprostacyclin on PROb tumoral interstitial fluid pressure and mean arterial pressure in rats. Continuous recordings of TP_{IF} and MAP after administration of 15 μg carbaprostacyclin, or vehicle alone, around tumors. Points are means from seven different rats at the indicated times. Vertical bars represent ±SD. For experimental details, see Material and Methods.

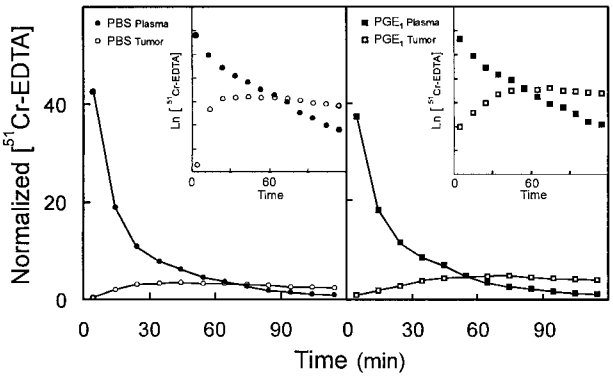

FIGURE 4 – Plasma and tumor levels of i.v. injected ^{51}Cr-EDTA *vs.* time. ^{51}Cr-EDTA levels as recovered by microdialysis by probes inserted in the jugular vein or in 2 separate PROb tumors after intravenous injection of ^{51}Cr-EDTA. Tumors had been treated with vehicle alone (PBS; left panel) or with 15 μg of PGE_1 (PGE_1; right panel), installed around the tumors 40 min before the injection of ^{51}Cr-EDTA. The total radioactivity recovered from each of the probes during the 120 min collection time was set to 100. The radioactivity recovered at each sample time point was normalized against the total radioactivity. Inserts show the natural logarithm of the values. For experimental details, see Material and Methods.

dialysates from plasma and tumor, respectively, were determined. Transcapillary transport was calculated from AUC plasma/AUC tumor. After administration of vehicle alone, AUC plasma/AUC tumor ratios averaged around 0.30 in PROb tumors, and around 0.14 in mammary tumors (Table III, Fig. 5*a,b*). Only a marginal difference in transcapillary transport in PROb tumors was observed after vehicle alone was administered at time intervals of either 10 or 40 min before the i.v. injection ^{51}Cr-EDTA (Table III). Installment of 15 μg PGE_1-methyl ester increased average AUCtumor/AUCplasma ratios in both PROb and mammary tumors (Table III, Fig. 5*a,b*). The ratios (AUCtumor/ AUCplasma) for PGE_1-treated PROb tumors displayed a large scatter (Fig. 5*a*). One set of PROb tumors, within each group treated with PGE_1, responded with an increase in AUCtumor/AUCplasma ratios of around 0.4–0.6, whereas the other set of PROb tumors had ratios similar to tumors treated with vehicle alone (Fig. 5*a*). The average value for transcapillary transport of ^{51}Cr-EDTA into PROb tumors increased with 39.6% (n=6) in animals treated with PGE_1, 40 min before the injection of ^{51}Cr-EDTA, compared to the control group (n=10), which had been treated with vehicle alone ($p<0.05$). In the group treated with PGE_1, 10 min before injection of ^{51}Cr-EDTA (n=7), the uptake increased by 26.1% in comparison with the control group (n=10, $p>0.05$).

Administration of PGE_1 around the mammary tumors induced a significant increase in transcapillary transport of ^{51}Cr-EDTA from plasma into tumor interstitium (Table III, Fig. 5*b*). The values in mammary tumors also displayed a wide scatter (Fig. 5*b*). Compared to the control group which received vehicle alone (n=7), uptake increased by 79.3% when PGE_1 was administered simultaneously with the i.v. injection of ^{51}Cr-EDTA (n=5; $p<0.05$), and with 16.4% when the tracer was injected 40 min after administration of PGE_1 (n=8; $p>0.05$).

DISCUSSION

We report that administration of PGE_1-methyl ester into the s.c. tissue around experimental rat carcinomas induces a transient lowering of TP_{IF}, an increase in tumor water content and an increased transcapillary transport into the tumor interstitium of the low molecular mass compound ^{51}Cr-EDTA. These results suggest that acute lowering of TP_{IF} can be of therapeutic value to increase uptake of low m.w. anticancer drugs into tumors. Poor uptake of

TABLE III – EFFECT OF LOCAL ADMINISTRATION OF PGE_1-METHYL ESTER ON UPTAKE OF ^{51}Cr-EDTA INTO TUMORS

Experimental group	n	Transcapillary transport AUCtumor/AUCplasma (±SD)	Percent increase in transcapillary transport
PROb tumors			
Vehicle alone, ^{51}Cr-EDTA at 10 min	5	0.285 ± 0.048	—
Vehicle alone, ^{51}Cr-EDTA at 40 min	5	0.308 ± 0.062	—
Vehicle alone (10 min + 40 min)	10	0.297 ± 0.054	—
PGE_1, ^{51}Cr-EDTA at 10 min	7	0.374 ± 0.135	26.1
PGE_1, ^{51}Cr-EDTA at 40 min	6	0.414 ± 0.126*	39.6
Mammary tumors			
Vehicle alone, ^{51}Cr-EDTA at 0 min	9	0.131 ± 0.062	—
Vehicle alone, ^{51}Cr-EDTA at 40 min	7	0.140 ± 0.067	—
Vehicle alone (0 min + 40 min)	16	0.135 ± 0.062	—
PGE_1, ^{51}Cr-EDTA at 0 min	5	0.251 ± 0.056**	86.9
PGE_1, ^{51}Cr-EDTA at 40 min	8	0.163 ± 0.087	21.3

*$p < 0.05$ compared to vehicle alone (^{51}Cr-EDTA at 10 min + 40 min, *i.e.*, combined control groups).
**$p < 0.05$ compared to vehicle alone (^{51}Cr-EDTA at 0 min + 40 min, *i.e.*, combined control groups).

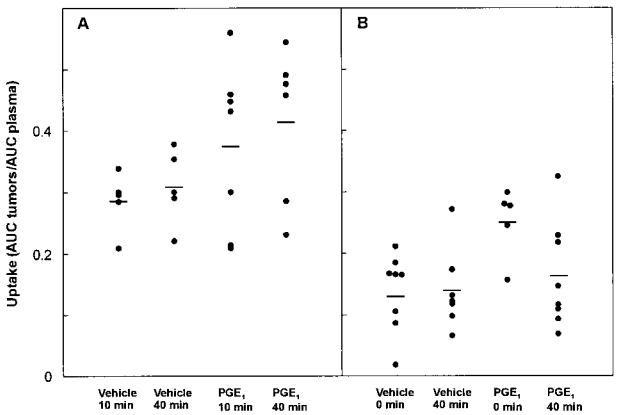

FIGURE 5 – PGE_1-induced increase in uptake of ^{51}Cr-EDTA into tumor interstitium. Uptake in PROb tumors (*a*) or in mammary carcinoma (*b*). The ratio between total radioactivity in plasma (AUC plasma) and in tumors (AUC tumors) was taken as a measure of uptake. Vertical bars show averages of the groups.

drugs into tumor interstitium is thought to, at least in part, be responsible for the low efficiency in pharmacological treatment of many solid malignancies (Jain, 1996; Müller *et al*., 1998, 1997; Philips *et al*., 1990).

The appreciation of the existence of interstitial hypertension in solid tumors goes back to the work of Gullino *et al*. (1964) and has been observed in several human and experimental tumors (Less *et al*., 1992; Nathanson and Nelson, 1994; Wiig *et al*., 1982). The most extensive studies on interstitial hypertension in tumors, and the effects of the hypertension on transcapillary and interstitial transports, have been performed by Jain (1987, 1996). The general problem addressed in these studies is how this interstitial hypertension in tumors influences the delivery of, especially macromolecular, anticancer agents to the tumors. Transcapillary exchange of solutes is by diffusion, which depends on the concentration of the agents across the capillary barrier and by convection, which depends upon the net capillary filtration pressure. In normal tissues capillary-to-interstitium transport of low m.w. components depends mainly on diffusion, whereas transport of macromolecules to a large part seem to depend on convection (Rippe and Haraldsson, 1994). The microvascular exchange barrier in tumors is considered non-selective; hence the transcapillary transport in tumors is normally considered to occur by convection (Jain, 1987, 1996). In a non-selective microvasculature the convective transport can be raised by either raising the capillary pressure, or by lowering the interstitial fluid pressure. Increased mean arterial pressure, achieved by periodic infusion of angiotensin II, creates a more favorable pressure gradient across the tumor vasculature and subsequently higher uptake of tumorspecific IgG into experimental tumors (Netti *et al*., 1999). Although several agents have been found to decrease TP_{IF} (Kristensen *et al*., 1996, 1993; Lee *et al*., 1992), a correlation of a lowered TP_{IF} and increased uptake of low m.w. substances has not previously been established. Starting from this point we wanted to investigate whether a lowering of TP_{IF} would enhance the transmicrovascular transport in the tumors by enhancing the convective transport.

The approach of lowering TP_{IF} taken in this present study is based on our recent series of studies in which we have studied on how the interstitial fluid pressure in skin can be altered by means of endogenous and pharmacological substances (Reed *et al*., 1997; Rubin *et al*., 1996). Here, we used an PGE_1 that lowers interstitial fluid pressure and forms edema in rat dermis (Berg *et al*., 1998). In addition, PGE_1 inhibits fibroblast-mediated collagen gel contraction (Berg *et al*., 1998). Our present findings, namely that administration of PGE_1-methyl ester, into the s.c. tissue around 2 experimental carcinomas, induced a time-dependent decrease in TP_{IF}, and an increase in tumor water content in PROb tumors, are in line with these earlier observations. PGE_1-methyl ester was chosen since it is highly diffusible. We have previously shown that carbaprostacyclin, a PGI_2 agonist, induced a decrease in P_{IF} and induces edema formation in rat dermis (Berg *et al*., 1998). Notably administration of carbaprostacyclin had no effect on TP_{IF} or MAP. The discrepancy between effects of this prostaglandin on P_{IF} in tumors and skin may be due to difference in diffusion of the 2 prostaglandins into tumors, or differences in expression of IP and EP receptors (Coleman *et al*., 1994) in dermis and tumor stroma.

Microdialysis has been introduced to record *in vivo* pharmacokinetics in the interstitial space of human (Müller *et al*., 1998, 1997) and experimental solid tumors (Dukic *et al*., 1998). This technique allows a continuous recording of capillary-to-interstitium transport of low m.w. solutes *in vivo*. Clinical studies with a limited number of human breast cancer patients, employing microdialysis for monitoring the pharmacokinetics of cytostatics in the tumor interstitium, has not provided clear evidence for drug transport into tumors to have predictive value for clinical response (Müller *et al*., 1998, 1997). To our knowledge, it has not been established whether pharmacological intervention, aimed at increasing uptake of anticancer drugs into tumors can favorably change the clinical response to the cytostatic treatment. Our present data show that PGE_1-methyl ester, administered locally around experimental tumors, significantly increased the transcapillary transport into tumors of a low molecular mass tracer, ^{51}Cr-EDTA as measured by microdialysis. In these experiments we used 10 mm long microprobes, thus recording uptake from a major segment of the experimental tumors. The data indicate that, particularly the PROb tumors, encompassed two subpopulations: one that did responded by increased transcapillary transport and one that did not. In spite of the large variations in values, the differences in average uptake values reached significance ($p<0.05$). Significant PGE_1-induced increases in ^{51}Cr-EDTA uptake were evident at different time points. Moreover, for the 2 types of

experimental tumors investigated, these increases were of different magnitudes. Mammary tumors showed the largest increase when the ^{51}Cr-EDTA was injected simultaneously with the installment of PGE_1. In PROb tumors, the largest increase was seen 40 min after the administration of PGE_1. However, 4 out of 7 investigated PROb tumors from the group in which PGE_1 was administered 10 min after the injection of ^{51}Cr-EDTA showed a 43% increase in transcapillary transport, whereas none of the controls reached this value. Despite the observation that the increase in average uptake compared with the controls has not yet reached significance at this time point ($p=0.118$), a majority of the PROb tumors appeared to have responded. Furthermore, only 2 time points were investigated for each of the 2 types of tumors, and it is possible that the increase in transcapillary transport would be larger at other time points. The finding that the PGE_1-induced increase in uptake was larger in mammary tumors, compared to PROb tumors, can be due to differences in stromal compartments between the respective types of tumors. Indeed, microscopical analyses of sections from the 2 tumors showed that the mammary tumors possessed a more developed stroma than the PROb tumors (data not shown).

There could in theory be several mechanisms by which PGE_1 could exert its effects on TP_{IF} and uptake of ^{51}Cr-EDTA. First, PGE_1 increases blood flow and vascular permeability in skin (Bisgaard, 1990; Fürstenberger, 1991). PGE_1 is also a potent vasodilator (Lumley *et al*., 1982). Systemic infusion of PGE_1 in an experimental rabbit tumor model leads, however, to a decrease both in tumor blood flow and MAP, whereas blood flow in tissues surrounding the tumors increased (Morita *et al*., 1991). In the present study we confirmed that PGE_1 administration decreased MAP. TP_{IF} is reportedly influenced by microvascular pressure which in turn is directly proportional to MAP in several experimental tumors (Netti *et al*., 1999). To test whether the decrease in MAP, induced by PGE_1, was responsible for the decrease in TP_{IF} in PROb tumors, we performed experiments with nipride in doses that lowered MAP by around 20%. This treatment did not reduce TP_{IF} within a 40 min study period. Instead TP_{IF} increased, after a varying lag period, in response to the infusion of nipride in most animals studied (data not shown). Taken together, it is not possible to conclude that the PGE_1-induced lowering of TP_{IF} in PROb tumors can be accounted for by changes in MAP or increased blood flow through the tumors. The potential effect of PGE_1 to increase vascular permeability, and its relation to TP_{IF} and uptake of ^{51}Cr-EDTA into the tumor interstitium, in the 2 presently investigated types of experimental tumors is not clear. Tumor vascularity is functionally underdeveloped with highly permeable capillaries (Yaun, 1998), making it reasonable to assume that the PGE_1-induced lowering of TP_{IF} and increase in uptake of ^{51}Cr-EDTA not primarily is due to increased vascular permeability.

Previous reports from our laboratories point to that dermal connective tissue have an active role in control of P_{IF}. This is in part based upon the finding that P_{IF} can be modulated in connective tissue after circulatory arrest, *i.e.*, independently of vascular events (Aukland and Reed, 1993; Reed and Rodt, 1991). The general concept that has emerged from these studies is that the interstitial matrix is a dynamic structure in which the physical properties can be altered. This concept is a further development of the ideas put forward by Meyer (1983), demonstrating that the swelling of a tissue ground substance rich in hyaluronan and glycosaminoglycan is balanced by a restraining collagen and/or microfibril network. We have extended this concept by showing that connective tissue cells contribute to the properties and behavior of the interstitial matrix (Reed *et al*., 1997; Rubin *et al*., 1996). Thus, agents that relax the tension exerted by connective tissue cells, such as PGE_1 (Berg *et al*., 1998), on the extracellular matrix will lower the interstitial pressure. Our previous experimental data suggest that the mechanism by which connective tissue cells generate tension on the extracellular matrix *in vivo* is related to "traction forces" (Harris *et al*., 1981), which are operative also in fibroblast-mediated contraction of collagen gels *in vitro* (Grinnell, 1994). In the present study we demonstrated that both the PROb and mammary tumors encompassed a stromal compartment which contained collagen fibers and hyaluronan. Furthermore, both types of tumors swelled when immersed in PBS, albeit to a lower degree than rat dermis. Tumors swelled by around 20%, compared to the up to 100% increase in volume observed for the rat dermal tissue under the same conditions. It therefore remains an interesting possibility that the cellular targets for PGE_1, effecting the decrease in TP_{IF} and increase in transcapillary transport, are stromal fibroblastoid cells or pericytes. Further studies aimed at elucidating the cellular distribution of prostanoid receptors in tumors, as well as effects on TP_{IF} and capillary-to-interstitium transport of more selective prostanoid receptor agonists, are needed for an assessment of this possibility.

In conclusion, the present data show that acute lowering of TP_{IF} in experimental tumors by local treatment with PGE_1 is paralleled by increased transcapillary transport of a low molecular mass solute into the tumor interstitium. These findings may be important for the establishment of better treatment regimes for currently used low molecular weight anticancer drugs, based on manipulations of the hydrodynamic properties of tumors. Specifically, it is possible that adjuvant treatment with PGE_1, or PGE_1 analogues, would be beneficial in increasing efficiency of regional cytostatic therapy, *e.g.*, for the treatment of liver metastasis using arterial or portal shunts for administration of anticancer drugs and adjuvant.

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