Advances in Brief

Human Tumor Blood Flow Is Enhanced by Nicotinamide and Carbogen Breathing[1]

Melanie E. B. Powell,[2] Sally A. Hill, Michele I. Saunders, Peter J. Hoskin, and David J. Chaplin

Cancer Research Campaign Tumour Biology and Radiation Research Group, Marie Curie Research Wing, Mount Vernon Hospital, Northwood, Middlesex, HA6 2RN [M. E. B. P., M. I. S., P. J. H.], and Tumour Microcirculation Group, Gray Laboratory Cancer Research Trust, Northwood, Middlesex, HA6 2RJ [S. A. H., D. J. C.], United Kingdom

Abstract

Perfusion insufficiency and the resultant hypoxia are recognized as important mechanisms of resistance to anticancer therapy. Modification of the tumor microenvironment to increase perfusion and oxygenation of tumors may improve on the efficacy of these treatments. Using laser Doppler probes to measure microregional RBC flux, this study examines the influence of nicotinamide and carbogen on human tumor perfusion. Ten patients with advanced cancers were studied. Nicotinamide (80 mg/kg) was given p.o., and 60 min later, up to six probes were inserted into the tumor. Readings were taken for 1 h, followed by 10 min of carbogen breathing and 10 additional min of breathing room air. Results were compared with those from a similar group of eight control patients who were not given nicotinamide, but who breathed carbogen. In 44 microregions analyzed, 33 (73%) showed perfusion fluctuations of 50% or more, and 20 (44%) by 100% or more. This compared with the control group in whom 62% and 27% of microregions varied by 50% or more and 100% or more, respectively. Perfusion increases outweighed decreases by 30% with nicotinamide and 20% in the controls. On breathing carbogen, patients pretreated with nicotinamide showed an increase in tumor perfusion of 17% at 5 min and 22% at 10 min, compared with only 0% and 1% in the control group. Pretreatment with nicotinamide made little difference to the random blood flow fluctuations seen in controls. However, when carbogen was introduced, tumor perfusion increased compared with the control group. This may have important therapeutic implications by improving response to treatment and allowing better delivery of systemically administered agents.

Introduction

Modification of the tumor microenvironment may be a method of improving on the therapeutic success not only of conventional anticancer treatment, such as chemotherapy and radiotherapy, but also novel anticancer therapies, such as photodynamic therapy, gene therapy, and biological therapies. This, however, requires a greater understanding of tumor physiology and factors that may influence it. Tumor blood flow is clearly an important factor in the delivery and, hence, the cytotoxic effect of systemically administered agents. It also determines the degree of hypoxia within a tumor, which, in turn, is recognized to be a major cause of resistance to radiation, photodynamic therapy, certain chemotherapeutic drugs, and biological agents (1–4). Hypoxic areas, as defined by a pO_2 of less than 10 mm Hg, are recognized to be a common feature of both experimental and human cancers (2, 5, 6). It was originally believed that such regions of low oxygen tension were due solely to the limited diffusing capacity of oxygen, with cells distant from blood vessels being relatively starved of oxygen and nutrients for long periods (7). More recently, however,

it has been shown that regions of hypoxia can also result from transient fluctuations in tumor blood flow (8).

Improving tumor oxygenation and perfusion is now under renewed scrutiny, with both animal and clinical studies under way evaluating the hypoxic cell sensitizers carbogen and nicotinamide (9–11). The principal mode of action of carbogen (95% oxygen, 5% carbon dioxide) is to increase the amount of dissolved oxygen within the blood, thereby enhancing oxygenation of diffusion-limited or chronically hypoxic cells. Nicotinamide, the amide derivative of vitamin B_3, is believed to overcome perfusion-limited or acute hypoxia by minimizing the frequency and magnitude of changes in microregional tumor erythrocyte flux (12). Animal studies have shown that, combined, carbogen and nicotinamide can achieve a 2-fold enhancement ratio for local tumor control (13).

We have recently developed a clinical technique to monitor real-time fluctuations in microregional erythrocyte flux in experimental and human tumors (14, 15) using commercially available multichannel laser Doppler microprobes.

The aim of this study was to use laser Doppler probes to evaluate the effect of carbogen with and without nicotinamide on microregional RBC flux.

Materials and Methods

Patients. Patients participating in this study all had histologically proven malignancy and gave informed consent to participate in this study. Approval for the study was obtained from the local ethics committee.

Ten patients given nicotinamide were compared to a control group of eight patients previously studied, who had not received nicotinamide. The control group included six patients, two of whom had multiple tumors and were studied on two separate occasions. Patient and tumor details are shown in Table 1.

Laser Doppler Flowmetry. RBC flux was measured using the Oxford Array multichannel laser Doppler system (Oxford Optronix, Oxford, UK), which allows simultaneous measurement of blood flow in up to 12 discrete microregions. The system used in this study comprised six custom-made, cylindrical probes, each measuring 25 mm in length and 300 μm in diameter and with an estimated sampling volume of 0.01 mm³.

Nicotinamide Administration. Nicotinamide was given p.o. in a dose of 80 mg/kg. This dose, which is used in clinical practice, is well tolerated by patients and achieves effective plasma levels in most (10, 16). Salivary or plasma levels of nicotinamide were measured by high-performance liquid chromatography (17, 18) at 15–20-min intervals during the study period.

Carbogen Breathing. Carbogen (95% O_2, 5% CO_2) breathing was carried out using a technique pioneered for clinical use at our center (19). A close-fitting face mask (Intersurgical, Wokingham, UK) covers both the nose and mouth, and the gas is delivered at a flow rate of 15 liters/min through a closed system using a one-way valve and a 3-liter breathing bag.

Experimental Setup. Each patient lay on a bed, and up to six microprobes were inserted into the tumor. The skin adjacent to the lesion was anesthetized using 1% lignocaine, and each microprobe was introduced into the tumor through a 20-gauge cannula. Because LDF[3] measurements were taken some distance from the skin it was felt lignocaine would be unlikely to influence the readings. Laser Doppler readings began, and after 60 min of "baseline"

[3] The abbreviation used is: LDF, laser Doppler flux.

Table 1 *Details of patients and tumors studied, including nicotinamide levels and change in LDF with carbogen breathing[a]*

Case	Site and size of tumor	Histology and site of primary tumor	Plasma nicotinamide levels (nmol/ml)		Relative change in LDF with carbogen after	
			60'	start of carbogen	5 min	10 min
1	Node (SCF), 3 cm	SCC lung	1112	1022	1.16 ± 0.11	1.18 ± 0.05
2	Skin (abdomen), 3 cm	AC colon	2024	1452	1.02 ± 0.04	1.10 ± 0.04
3	Node (neck), 6 cm	SCC pharynx	1595	1054	1.05 ± 0.07	0.92 ± 0.05
4	Skin (thigh), 5 cm	Melanoma	1832	834	1.09 ± 0.07	1.16 ± 0.09
5	Breast, 7 cm	AC breast	3217	1768	1.17 ± 0.12	1.26 ± 0.14
6[b]	Skin (abdomen), 3 cm	AC colon	378	680	1.18 ± 0.06	125 ± 0.07
7[c]	Skin (arm), 3 cm	SCC lung	1364	1246		
8	Skin (flank), 3 cm	TCC kidney	1849	1546	1.11 ± 0.03	1.07 ± 0.03
9	Node (axilla), 5 cm	NHL	721	1676	0.97 ± 0.19	0.96 ± 0.19
10	Node (groin), 4 cm	NHL	1078	913	1.77 ± 0.39	1.77 ± 0.39
1[d]	Breast, 10 cm	AC			1.01 ± 0.11	0.94 ± 0.11
2[d]	Node (neck), 3.5 cm	NHL			0.85 ± 0.07	0.84 ± 0.11
3[d]	Node (neck), 6 cm	SCC larynx			1.10 ± 0.19	1.08 ± 0.12
4[d]	Skin (abdomen), 3 cm	AC colon			1.14 ± 0.18	1.06 ± 0.05
	Skin (abdomen), 3.5 cm				1.21 ± 0.23	1.24 ± 0.11
5[d]	Node (right groin), 4 cm	NHL			0.81 ± 0.06	0.96 ± 0.06
	Node (left groin), 3 cm				0.79 ± 0.20	1.00 ± 0.06
6[d]	Node (axilla), 3 cm	Melanoma			1.25 ± 0.28	1.00 ± 0.05

[a] SCC, squamous cell carcinoma; TCC, transitional cell carcinoma; NHL, non-Hodgkin's lymphoma; AC, adenocarcinoma; SCF, supraclavicular fossa.
[b] Excluded from 60-min observation.
[c] Unable to breathe carbogen.
[d] Control patients (no nicotinamide, breathed carbogen only).

measurements, a 10-min carbogen breathing period commenced. Finally, an additional 10 min of LDF readings were acquired, with the patient breathing room air.

Patients given nicotinamide waited 60 min postadministration before LDF measurements began. This timing was chosen to conform with current clinical practice, in which patients receive radiotherapy and concomitant carbogen 2 h afterward (10, 11). This is based on experimental evidence that suggests that radiosensitization is maximal when radiation is given at peak plasma nicotinamide levels occurring between 30 min and 3 h after administration (16).

Data Analysis. Individual probes generate 20 readings per second. From this, an average flow reading is calculated for each 2-min interval in all channels and plotted against time. The final plots of RBC flux, together with the original recorded data, were examined, and traces showing evidence of patient or probe movement were excluded from analysis.

Flow during carbogen breathing was related to a baseline value that was a single mean calculated from the measurements recorded over the 10-min period prior to breathing carbogen and was designated as the flow at time 0. Blood flow averages at 5 and 10 min after starting carbogen breathing were compared with the time 0 value using a paired two-tailed t test, where $P < 0.05$ was considered to be significant.

Results

Therapeutic levels of nicotinamide (>700 μmol) were achieved in all patients except patient 6, in whom levels were slow to rise and were just suboptimal at the time of carbogen breathing (Table 1). Her readings were excluded from the 60-min observation period but were included for analysis of carbogen breathing. Patient 7 was unable to tolerate the face mask and did not breathe carbogen.

Assessment of the 1-h observation period was made on 52 traces in nine patients. Eight traces were excluded due to probe or patient movement, allowing the RBC flux in 44 separate microregions to be analyzed. As noted in our previous studies, striking heterogeneity of perfusion changes was seen between individual tumors and the separate microregions studied in a tumor (14, 15). Temporal fluctuations in perfusion by a factor of 50% or more occurred in 33 traces (73%),

and 20 traces (44%) showed changes of 100% or more (Table 2). In 12 (27%) of these traces, the initial changes in RBC flux were subsequently reversed; *i.e.,* an increase in blood flow was followed by a period of decreased perfusion or *vice versa*. Table 2 compares fluctuations in microregional RBC flux seen over 60 min in the nicotinamide group with changes observed in the control group. Seven patients whose tumors were included in the latter group did not breathe carbogen and formed part of a previous study (14). Although a slightly higher percentage of the nicotinamide-treated tumors showed fluctuations in RBC flux by a factor of 1.5 or more, the ratio of increases:decreases was lower than in the control group (1.2 in the nicotinamide tumors and 1.3 in the controls). However, taking the hour as a whole, blood flow remained constant (Fig. 1).

Fig. 2 shows the relative changes in RBC flux from nine nicotinamide and eight control patients as a result of carbogen breathing. Values are expressed as means ± SE. The data indicate that in patients pretreated with nicotinamide, erythrocyte flux increased with carbogen, appearing to plateau at 8 minutes. This compares with the control group in whom carbogen effected no overall change in blood flow. Analysis after 5 and 10 min of carbogen breathing showed blood flow in the nicotinamide group to have increased by 17% and 22% relative to the precarbogen value, which reaches statistical significance at $P < 0.004$ and $P < 0.001$, respectively. This compares with the control group, which showed fluctuations of 0 and 1% at 5 and 10 min ($P = 0.94$ and $P = 0.9$, respectively). Table 1 summarizes the effect of carbogen on RBC flux in individual patients.

Discussion

There are reports that in certain experimental tumors, carbogen can improve tumor blood flow and oxygenation (9, 20). If such increases in perfusion were a common feature of human tumors, the finding would be of importance not only to radiotherapy but also to systemically delivered treatments, such as chemotherapy and biological

Table 2 *Changes in microregional erythrocyte flux over a 60-min observation period in 15 control tumors and 10 treated with nicotinamide[a]*

Factor of change	Controls		Nicotinamide	
	No. of traces changing	No. and direction of changes	No. of traces changing	No. and direction of changes
≥1.5	45 (62%)	46 (+) 35 (−)	33 (73%)	28(+) 23 (−)
≥2	20 (27%)	17 (+) 13 (−)	20 (44%)	14(+) 10 (−)

[a] +, increase; −, decrease.

therapies. Our clinical data indicate that blood flow response to carbogen can be variable, with both increases and decreases occurring (15). This agrees with more recent detailed data from animal systems, which show that decreases as well as increases in tumor blood flow are observed in response to carbogen (21, 22). The current study shows that in a heterogeneous group of tumors, pretreatment with nicotinamide more reliably enhances tumor perfusion compared with carbogen alone, with only one tumor not showing increased perfusion.

Nicotinamide is believed to improve oxygenation by improving the homogeneity of microregional tumor blood flow. It might therefore be expected that nicotinamide would lead to a rise in blood flow, or at least an alteration in the ratio of blood flow fluctuations with fewer decreases occurring. These data, however, show a similar result to random blood flow fluctuations seen in a group of untreated patients in whom 62% of traces changed by 50% or more (73% in this series) and 27% changed by 100% (44% in this series), with increases in blood flow outnumbering decreases by 1:1.3 (Ref. 14; 1:1.2 in this study). Thus, nicotinamide, at the doses administered in the clinic, does not prevent transient fluctuations in human tumor microregional blood flow. This finding contrasts with studies in experimental tumor systems in which nicotinamide has been shown to reduce or eliminate such blood flow instability. It should be emphasized, however, that animal studies have used much higher doses of nicotinamide that cannot be achieved in humans without unacceptable toxicity (16).

Our study demonstrates that, together, carbogen and nicotinamide give rise to an increase in blood flow. The overall increase of 22% may seem modest, but such a global view ignores the spatial hetero-geneity of both blood flow and oxygenation known to exist in tumors. If increases were occurring where capillary flow has either ceased or is severely reduced, i.e., where microregional perfusion is most com-promised, they could bring important benefits for drug delivery and the eradication of hypoxia.

The mechanism responsible for the combination of carbogen and nicotinamide increasing tumor blood flow is unclear but may involve a complex interaction between tumor cells and tumor vasculature. For example, it is known that tumor and incorporated host cells produce vasoactive compounds, such as the vasodilator nitric oxide and the vasoconstrictor peptide endothelin. Their production, which may vary quite markedly between different tumor types, is independently al-tered by both carbon dioxide levels and nicotinamide (23, 24). Thus, a selective alteration in local production of such vasoactive com-pounds that might, e.g., prevent vasoconstriction of vessels within a tumor, may be a possible explanation for the effects on tumor
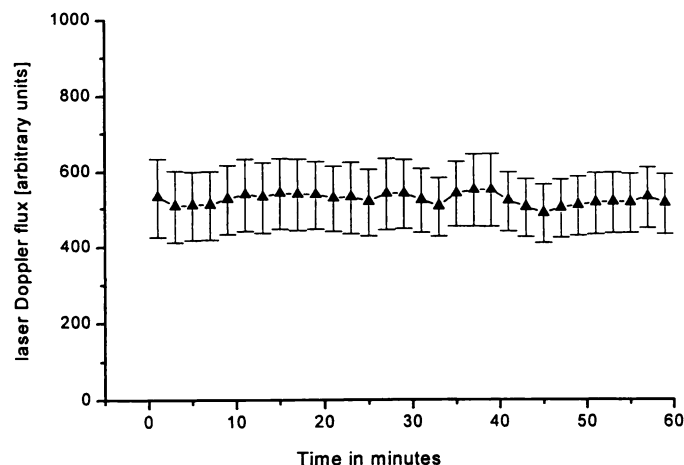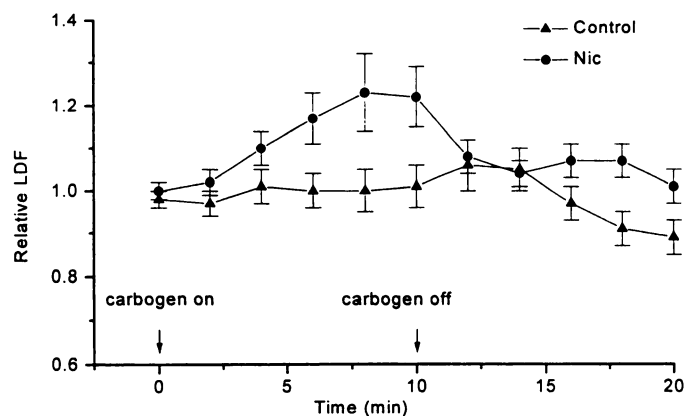

Fig. 2. The effect of carbogen breathing on relative LDF in control patients (34 traces) and patients pretreated with nicotinamide (45 traces). Data are means; bars, SE.

perfusion seen in our study with the combination of carbogen and nicotinamide.

Our study confirms that tumor perfusion can be increased with the addition of carbogen and nicotinamide. This may, in turn, lead to higher tumor cure rates by enhanced radioresponsiveness and improved delivery of systemic treatments. Clearly, consistent increases are necessary if the use of nicotinamide and carbogen is to become routine. Our work, however, provides a basis for further investigation of a wider range of tumors and the evaluation of other agents that might offer larger and more dependable improvements in tumor perfusion.

Acknowledgments

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Fig. 1. Change in LDF over 1 h in patients pretreated with nicotinamide (44 traces). Data are means; bars, SE.

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