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Influence of malignancy and cyctostatic treatment on microcalorimetric behaviour of urological tissue samples and cell cultures ☆

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Abstract

Microcalorimetry allows direct, continuous measurement of metabolic rate in tissue samples and cell cultures. In the oncological field, it may complement morphological tumour grading by information on the "biological activity" of tumour material. However, calorimetry, although widely accepted in haematological research, has up to now rarely been used in the study of solid tumours. In this investigation 44 tumorous and non-tumorous tissue samples of various urological organs and 10 cell lines, cultured in RPMI 1640 medium, from patients with renal cell carcinomas were measured in a microcalorimeter to study the metabolic activity and the responsiveness to cytostatic treatment. The determination of the maxima (P_{max}) and the mean values (P), and the contour integrals (W) of the measured heat evolution shows a distinctly higher metabolic activity of tumorous than of non-tumorous material. We conclude that through microcalorimetric analysis, it is possible to differentiate between healthy and tumorous tissue samples on the basis of a varyingly higher metabolic activity of the malignant samples. The renal cell carcinoma lines were incubated without and with the cytostatic drug (5-fluorouracil) and with two "biological response modifiers"

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(alpha-interferon-2a and interleukin-2). We found that untreated cell lines, which showed a high increase in heat production, react more sensitively to the antimetabolic agent and that in all cases the combination of 5-fluorouracil with alpha-interferon-2a creates an improved cytostatic effect.

Keywords: Benign; Cytostatic treatment; Malignant; Microcalorimetry; Tumour; Urogenital tract

1. Introduction

Histological examination and flow cytophotometry (FCM) are commonly used for determining the malignancy of a tumour [1,2]. These procedures allow no precise prediction of the development of the disease. Microcalorimetry may give additional information in diagnosis and prognosis by detection of differences in biological behaviour and the response of cells to cytostatic treatment. Unsatisfactory results in the treatment of urogenital cancer, especially renal cell cancer, underline the necessity of an individual treatment [3–6]. Microcalorimetric investigations of tissue samples and tumour cell lines are practical for optimizing the treatment. With the help of microcalorimetry we try to select patients who will profit from cytostatic treatment and to find the most efficient agent. This is an important fact in a clinical situation with a response rate of less than 20% for chemotherapeutic treatment.

2. Method I

A total of 44 human tissue samples from urogenital tract organs as well as from the mamma and the colon were available for microcalorimetric measurements using a thermal activity monitor (TAM, ThermoMetric, Sweden). The samples originating from patients in the urological, gynaecological and surgical clinics were divided into three parts to allow microcalorimetric measurement in addition to routine histological and impulse-cytophotometrical diagnosis. The use of the established reference methods determined that the tissue samples were non-tumorous or tumorous, at all stages, respectively. For the examination, glass ampoules filled with 2.5 ml of Ringer solution were used. The measurements were carried out at the physiological body temperature of 37°C for a 90 min time period. Finally, after the samples were dried to weight constancy, the calorimetric values were converted into microwatts per gram dry weight (dw). Under the static ampoule conditions used in this study, typically decaying calorimetric curves are obtained indicating the successive loss of metabolic activity of the non-perfused tissue samples. For the evaluation of the power–time curves the maximal thermal power (p_{max} in $\mu W\ g^{-1}$ dw), the integral of the curve serving as the measure for the total energy released (W in mJ g^{-1} dw) and the mean thermal power (P in $\mu W\ g^{-1}$ dw) of the tissue sample during the period of measurement were used (Fig. 1).

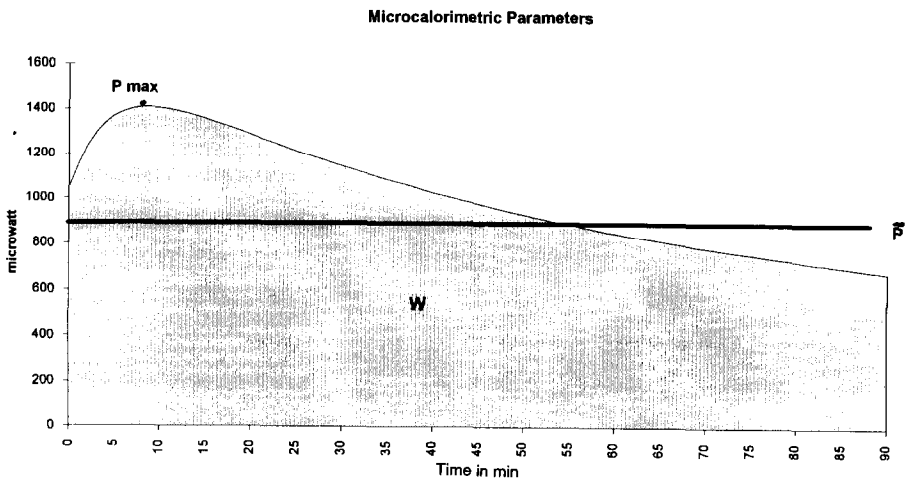

Fig. 1. Parameters used to analyse microcalorimetric records obtained from tissue samples: shaded area, the total energy released W; horizontal line, mean thermal power P; P_{max}, maximum of the calorimetric curve.

Table 1
Histological grading and staging, DNA index, and cell cycle division (PDT, population doubling time)

Cell lines	Staging	Grading	DNA index	G0-G1/S/G2-M in %	PDT in h	Plating efficiency
BN4	pT2,Nx,Mx	G-2	2.3	80/11/9	22.4	74
BN7	pT3b,Nx,Mx	G-3	1.8	79/12/9	24	88
BN8	pT3a,pN0,Mx	G-1	1.9	81/10/9	23.7	89
BN9	pT3b,pN0,Mx	G-1	2.1	88/7/5	23	91
BN12	pT2,pN0,Mx	G-2	1.9	84/9/7	24	76
BN13	pT3,pN0,Mx	G-2	2.0	80/11/9	21	81
BN14	pT3b,pN0,Mx	G-2	2.1	81/10/9	19	75
BN15	pT2,pN0,Mx	G-2	2.1	78/12/10	18	89
BN16	pT3a,pN0,Mx	G-3	2.0	75/13/12	24	79
BN17	pT3,pN0,Mx	G-3	1.8	72/15/13	23	78

3. Method II

Tumour samples from renal cell carcinomas (RCC) were cultured from 10 patients who underwent nephrectomy at our department. This tissue was prepared by mechanical disaggregation and single cells were gained by sedimentation. The human cell lines were cultured in RPMI 1640 medium which were supplemented with 10% FCS (fetal calf serum). For flow-cytometric analyses the tissue samples

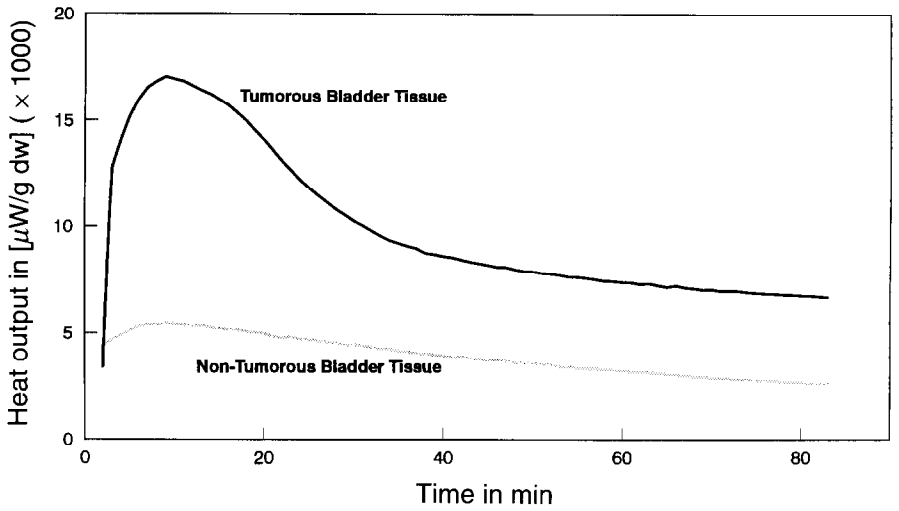

Fig. 2. Microcalorimetric response of bladder tissues.

were stored in ethanol (96%). First the samples were washed in physiological solution (0.9% NaCl), then monocellular suspensions were obtained by mechanical treatment and enzymatic treatment with 0.5% pepsin for 10 min at 37°C. The pepsin was neutralized and washed with 0.1 M Tris buffer solution. The DNA was stained with ethidium bromide and mithramycin C. The disturbing fluorescences of RNA were eliminated by ribonuclease. For an internal standard we used isolated autolog peripheral blood lymphocytes which were processed by the same procedure. The DNA index was calculated by the differences in DNA-fluorescences in an ICP 11/22 (PHYWE) of the lymphocytes and the tumour cells (Table 1). For microcalorimetric assay, we used the cell lines after 3 passages. The cells were incubated for 72 h in 4 separate ampoules with and without drugs at an initial cell density of 150 000 cells per tube. The cytostatic potency of 5-fluorouracil (5-FU)was calculated as a monosubstance and in combination with alpha-interferon-2a and interleukin-2. For evaluation of the cell proliferation, the increase in heat-output in the first 35 h was investigated. The reduction in heat output through 5-FU was analysed for classification of the cells into drug-resistant and sensitive groups. The modified cytostatic effect of 5-FU in combination with alpha-interferon-2a and interleukin-2 was calculated in the course of heat decline in the death phase (45–72 h). As reference we used the course of the 5-FU-treated cells.

4. Results I

Taking the parameters P_{max}, P and W into consideration, the tumorous tissue samples generally showed a higher metabolic activity than the non-tumorous specimens. For instance in bladder tissues, tumorous samples clearly exhibited,

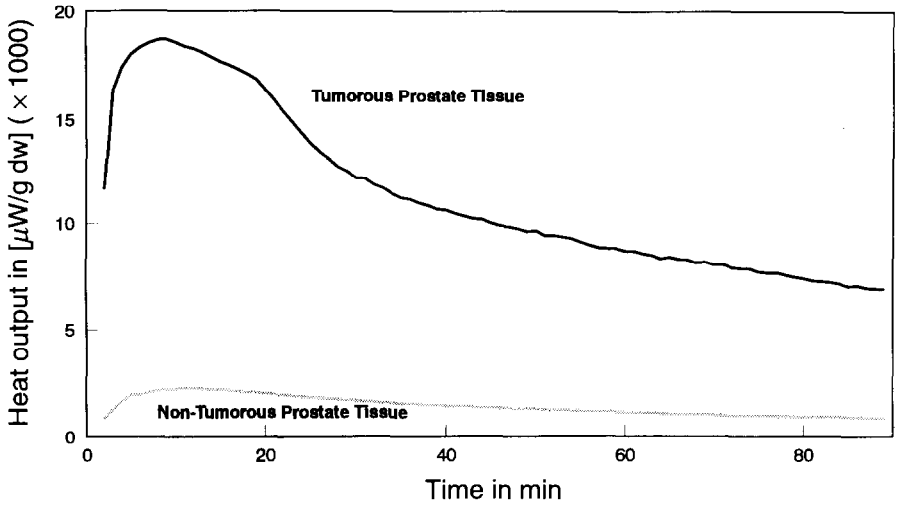

Fig. 3. Microcalorimetric response of prostate tissues.

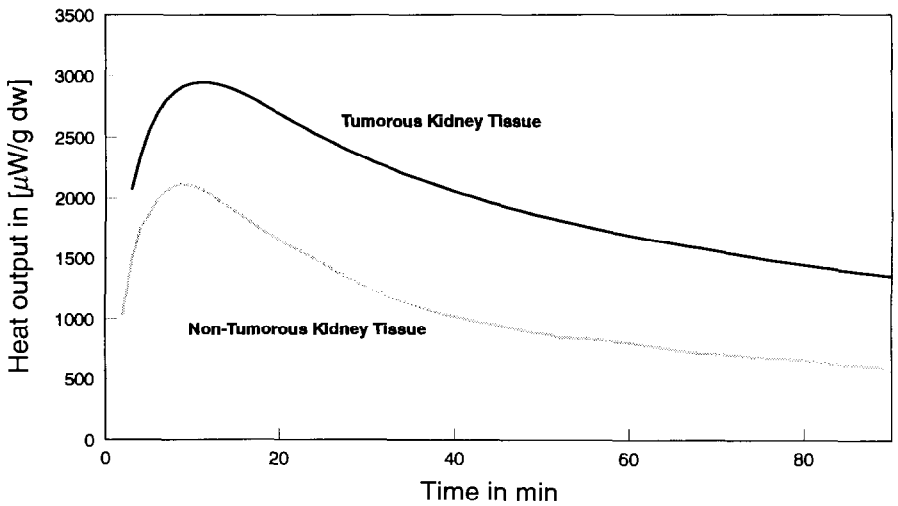

Fig. 4. Microcalorimetric response of kidney tissues.

from the beginning and for the whole course of measurement, a distinctly higher metabolic activity than the non-tumorous tissue samples of this organ. The metabolic activity of the tumorous material was higher by a factor of 2.5–3.1 than the non-tumorous samples (Fig. 2). Among the 13 measurements of prostate tissues, the tumorous samples likewise showed a distinctly higher activity than the non-tumorous tissues. The total released energy (W) during the measurement

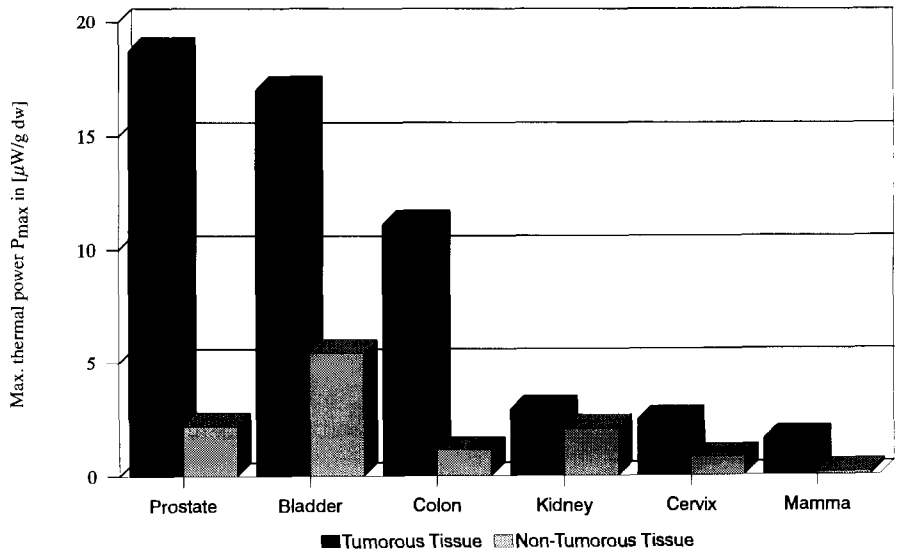

Fig. 5. Comparison of the maximal thermal power (P_{max}) values of all tissue samples.

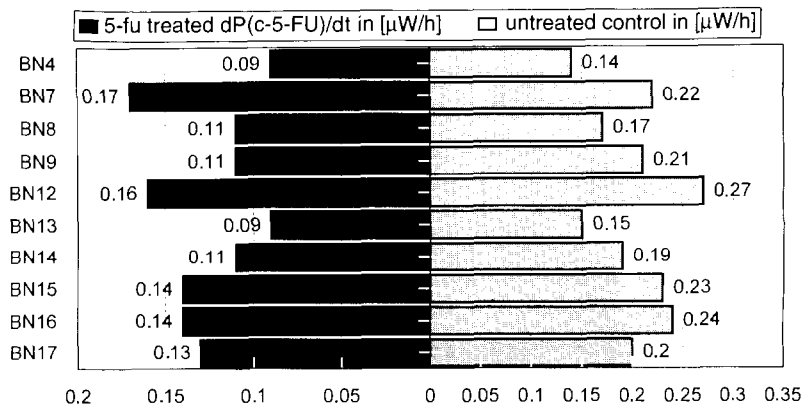

Fig. 6. Comparison of the heat output of untreated versus 5-FU-treated cells during cell proliferation (2 to 5).

period and the mean thermal (P) were increased for tumorous tissue samples by a factor of 8, and likewise, the maximum thermal power (P_{max}) was increased by a factor of 8.4 (Fig. 3). The lowest differences in metabolic activity was recorded in tumorous and non-tumorous kidney tissues. A total of 11 measurements of tissue samples from six patients were available. As the calorimetric curves show, the tumorous kidney tissue manifests a higher metabolic activity by a factor of 1.4–1.8

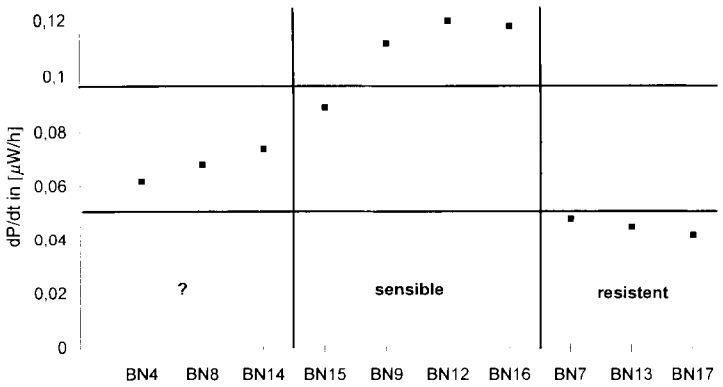

Fig. 7. Classification of the tumours concerning their drug sensitivity. Increase of the heat production (2 to 35 h) as a parameter of the cell response on 5-fluorouracil-treated cell lines from renal cell carcinoma; 5-FU concentration 4 μg ml^{-1}, incubation time 72 h.

than non-tumorous tissue (Fig. 4). In a direct comparison, e.g. for the parameter P_{max}, between tumorous and non-tumorous samples of the tissues it can be seen that, without any exception, the metabolic activity in the tumorous samples is higher than in the non-tumorous samples of the same tissue (Fig. 5). Therefore, a differentiation between malignant and benign tissues is possible by the differing metabolic activities.

5. Results II

Of the 10 RCC tumours, all exhibited sufficient growth in vitro. Table 1 shows the histological grading and staging, the DNA index and the cell cycle division. The increase in the heat production in these tumours is determined by their cell proliferation. The results reflect great individual differences and thus heat production seems to be a usable parameter for the biological proliferation potential under optimal growth conditions. Comparing the increase in the heat production rate dP/dt of untreated cells with that of the 5-FU-treated cells yields a strong correlation (Fig. 6). The differences in the 5-FU cytotoxic effects allow a classification of the cells, as is practised in antibacterial treatment (Fig. 7). The improved cytotoxic effect of 5-FU in combination with alpha-interferon-2a was observed in all cell lines. The modified effect of alpha-interferon-2a was different for each cell line. We calculated a RF (reduction factor) of the additional cytostatic effect due to the different calorimetric responses of all samples treated with 5-fluorouracil and with the combination of 5-FU, alpha-interferon-2a and interleukin-2 (Table 2). With interleukin-2, only 5 of the 10 cell lines showed an additional cytostatic effect which was significant for BN9 and BN13. For BN 12, no effect was determined.

Table 2
RF reduction factor of 5-FU-treated cells versus 5-FU + α-IFN-2a-versus 5-FU + α-IFN-2a + IL-2-treated cells; negative values reflect lower toxicty than 5-FU alone

Cell lines	RF 5-FU/5-FU + α-IFN-2a	RF 5-FU/5-FU + α-IFN-2a + IL-2
BN4	18.74	2.46
BN7	7.81	−1.34
BN8	1.48	1.76
BN9	7.23	11.89
BN12	2.81	2.85
BN13	1.71	5.65
BN14	13.79	12.64
BN15	7.25	8.84
BN16	2.48	2.88
BN17	1.92	2.16

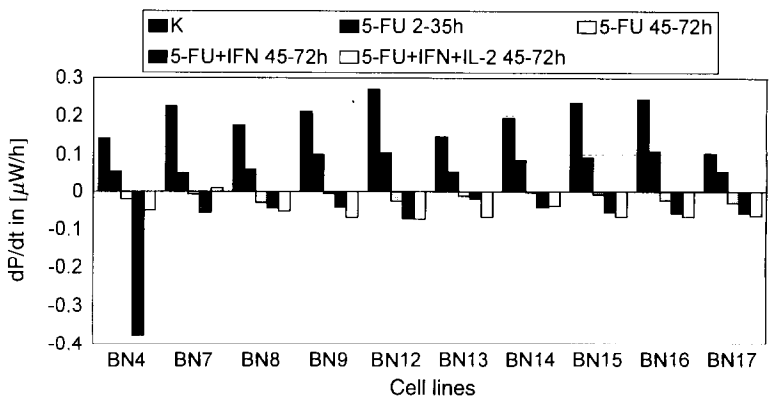

Fig. 8. Course of heat production in the different phases of incubation and the toxic-modified effect of alpha-interferon-2a (IFN) and interleukin-2 (IL-2) in combination with 5-fluorouracil (5-FU) in the death phase. dP/dt positive values: cell proliferation ($t = 2$ to 35 h); negative values: death phase ($t = 45$ to 72 h).

Three cell lines showed a better cytotoxic effect in the combination of 5-FU alpha-interferon-2a and interleukin-2. The course of the heat output of the cells in the different incubation phases demonstrates the toxic-modified effect of the biological response modifiers and the efficiency of the 5-fluorouracil exposure (Fig. 8).

6. Discussion

RCC represents about 2% of all human malignancies and is the most common renal tumour (90%) [7]. Metastatic RCC is still a therapeutic challenge in urological

oncology. Of RCCs, about 20–25% have metastasized at the time of diagnosis [8]. Discussion concerning the different therapeutic strategies is still controversial. Immunotherapy, especially using interferon and interleukin-2 [9], or a combination of chemotherapy and immunotherapy [10], are the most popular regimes. It is a well-known fact that renal cell carcinomas are very heterogeneous in their DNA status and biological behaviour [11]. The classification of the tumours is based on the clinical course, the histological features of anaplasia as manifested by hypercellularity, cellular pleimorphism, and frequencies of mitoses, but not by biological parameters like growth or metabolic activity. A procedure for disclosing additional data on tumour proliferation has been developed which uses microcalorimetry of certain cell parameters, e.g. the measurement of anaerobic glycolysis capacity of the tumour and the proliferation behaviour [12]. The poor prognoses of renal cell carcinomas are caused by aggressive infiltrating growth and a bad response rate to cytostatic treatment. Multiple attempts at chemosensitivity testing have failed, partially due to the practical problems. We examined 10 RCC using microcalorimetry. Cell cultures, when incubated under favourable conditions, show a typical proliferation behaviour producing an appropriate heat output. The suppression of this metabolic increase may be used as an in vitro assay for the cytostatic effect.

The higher heat output of tumorous tissue samples allows a differentiation between tumorous and non-tumorous tissue. Looking at the increased metabolic activity of tumorous material, one would expect, however, a steeper decay rate under ischemic conditions. Yet, as the measurements have revealed, the opposite is the case. In correlation with these results, examinations carried out in 1957 by Opitz [13] are of interest. Opitz investigated the percental survival rate of various tissues after different ischemic periods. Whereas the survival rate of brain tissue declines to zero within a few minutes, a distinctly higher ischemia tolerance is achievable in kidney tissues. In these examinations, the highest survival rates were shown by carcinoma tissue. This raises the question as to which causes may be responsible for a delayed loss of metabolic activity found in the tumorous tissue compared to that of non-tumorous tissue samples. Examinations performed by Warburg [14] pointed to an increased rate of anaerobic glycolysis in tumorous tissues [15–17], which is partly due to an altered enzyme pattern of neoplastic cells [18–20]. Because the survival of tissues under ischemic conditions is mainly dependent on anaerobic glycolysis, an increased anaerobic capacity will lead to a slower metabolic decay of tumorous samples when compared to that of non-tumorous ones [21] and might be a good explanation for the microcalorimetric results. Glycolysis is the energy-yielding process in tumorous tissue. Thus, microcalorimetry is a suitable method to investigate the energy metabolism of the tumour tissue and gives valuable information on the metabolic activity of solid tumours and their individual responsiveness to chemotherapy.

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