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Energy turnover of human large bowel adenocarcinoma in comparison to adenomatous and normal mucosa

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Abstract

Malignant tumors differ from healthy tissue, among other things, in their cell kinetics and in a changed energy metabolism. Therefore the objective of this study is to find an answer to the question, whether these cell-biological differences result in a changed thermal output. For this purpose biopsies were taken from human colorectal carcinomas (n=10), on the occasion of colonoscopic examinations; the former were placed into a microcalorimeter, and the occurring thermal flow rates were measured. The results were then compared with corresponding data of biopsies taken from normal colorectal mucosa (n=16) and with data of biopsies taken from colorectal adenomatous tissue (n=18), which can be looked upon as a precancerosis. While tissue from adenocarcinomas showed a significantly higher (t=0/1 h: p<0.005/0.025) energy turnover than normal mucosa, adenomatous tissue did not differ from healthy mucosa. Thus an enhanced overall energy turnover with colorectal adenocarcinomas cannot be verified until the stage of malignancy has been arrived at. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

It has recently been demonstrated, that microcalorimetry enables the reliable measurement of very small thermal flow rates, as they occur when human mucosa biopsies are measured [1,2]. Tumor tissue is, on the one hand, distinctive from normal mucosa in its changed cell kinetics, which is influenced by several molecular-biological mechanisms; on the other hand, it differs because of the prioritization of the quantitative importance, which is pertinent to individual pathways of energy metabolism [3–8]. Therefore the objective of this study lay in the examination, in which way the thermal output of biopsy samples taken from colorectal adenocarcinomas differ from those taken from normal mucosa.

2. Methods

The mucosa samples for the microcalorimetric measurements were taken from patients, who had reported for colonoscopy for diagnostic or therapeutic reasons and from whom, for the aforesaid reasons, biopsies had to be taken anyway. From all patients parallel samples were taken for histological analysis,

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insofar as the diagnosis had not been confirmed previously. None of the patients had been treated chemotherapeutically or radiotherapeutically prior to their examination. Before the examination was made, the patients had been informed about the planned additional biopsy taking.

All biopsies were taken from that marginal area of the tumor, which was still definitively pathological. For the control examinations biopsies were taken from patients who did not show a pathological colon finding. After the extraction the biopsies were transported at room temperature in the same incubation solution, in which they were later incubated during the measurement. The composition of the antiseptically produced incubation solution (mM) was the following: NaCl 140, KCl 4, $CaCl_2$ 1.2, $MgCl_2$ 1, phosphate buffer 10 (pH 7.4), butyrate 2, L-glutamic acid 2, D-glucose 5. The solution contained 40 mg/l gentamycin as an antibiotic.

Within 10 min after the extraction the samples reached the lab and were placed into 4 ml steel ampoules, which were half-filled with the nutrient solution described above. Oxygen was filled into the residual 2 ml. Then the ampoules were locked and together with the reference ampoule, filled with NaCl, placed into the microcalorimeter (Thermal Activity Monitor (TAM) 2277-201, Thermometric AB, S-17561 Järfälla, Sweden) for the purpose of equilibration to the measuring temperature of 37°C. After a further 20 min the ampoules were lowered into their final measuring position and the measuring was initiated. After 6 h the measurement was terminated and the samples were dried to the point of weight constancy. The heat output values of the individual biopsies were then normed to dry weight. Altogether, 16 biopsies were taken from 10 individuals without a pathological colon finding, 18 colon adenoma biopsies from 12 patients and 10 colorectal adenocarcinoma biopsies from four patients. As to the grading see Table 1.

For the evaluation of the data the time of extraction was set as zero term. For the graphic presentation of the measuring progression which, for the sake of lucidity, has only been illustrated for the first 4 h, the programme Microsoft Excel 5.0 has been applied. With the help of the values measured, in addition to each measurement, a back-extrapolation was made to the initial value of the thermal production at the time of the sample-taking; in the case of an exponential curve course a two-phased exponential decay curve was calculated, utilizing a GraphPad Prism 2 programme. For details of the curve analysis and for details of the calculation see [1]. The achieved level value was also taken as an initial value in the case of an immediate direction of the measured values into a horizontal curve structure, without an exponential drop preceding. The data are given as mean values and standard deviations from the mean values. The testing of the level of significance was done by means of the Wilcoxon-U-Test [9].

Table 1
Colorectal energy turnover in vivo (μW/mg) calculated by mathematical back-extrapolation, tumor grading in brackets (MB=macrobiopsy)

Normal mucosa	Polyps	Adenocarcinoma
9.0	6.5	14.0 (G2)
7.5	12.0	30.2 (G2)
19.2	8.9	8.8 (G3)
10.5	7.3	17.2 (G3)
14.5	13.3	9.3 (G3)
11.4	11.0	17.3 (G3)
8.5	11.2	9.5 (G2)
8.1	10.0	23.0 (G2)
5.4	14.8	56.4 (G2)
8.2	9.0	24.1 (G2)
2.2	5.3	
5.0	6.3	
10.7	9.0	
8.0	8.1	
8.2	7.1	
7.9	7.1	
	5.8	
	10.5 (MB)	
Mean=9.0	Mean=9.1	Mean=21.0

3. Results

The average dry weight with the inconspicuous mucosa biopsies was 0.63±0.06 mg, in the carcinoma group it was 1.06±0.23 mg and in the adenoma group it was 1.30±0.25 mg; among the latter was one biopsy with a dry weight of 5.3 mg, which was markedly higher than the next smaller biopsy with 1.6 mg. The values of this macrobiopsy, however, were located in

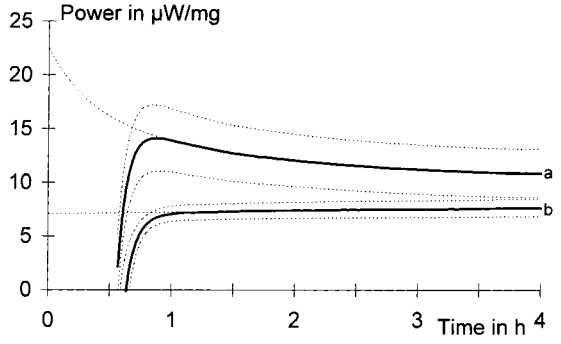

Fig. 1. Specific heat power of biopsies from colorectal tumors (a: n=10) and normal mucosa of the colorectum (b: n=16): mean values (solid lines) and standard error of the mean (dotted lines). Also the back-extrapolated curves are indicated (dotted lines).

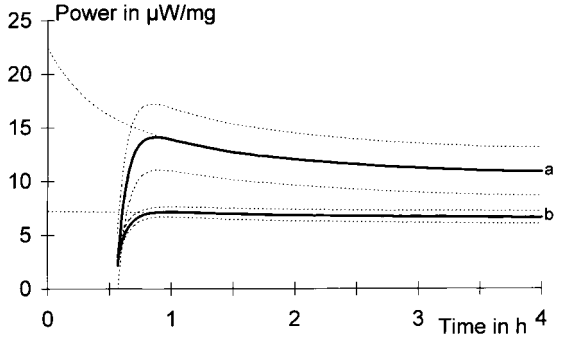

Fig. 2. Specific heat power of biopsies from colorectal tumors (a: n=10) and colon adenomas (b: n=18): mean values (solid lines) and standard error of the mean (dotted lines). Also the back-extrapolated curves are indicated (dotted lines).

the middle of the dispersion area of the other values measured (Table 1).

As soon as measurable thermal flow rates were reached after the conclusion of the initial thermal equilibration, the measured values in the group containing the biopsies from adenocarcinomas of the colon were found to be significantly higher (after 1 h: $p<0.025$), than in the control group containing biopsies with inconspicuous colon mucosa. The curve then showed a falling tendency, so that the mean values of both curves approached each other somewhat in the course. The values which had been back-extrapolated to the time of extraction differed by the factor 2.3 ($p<0.005$) (Table 1). Altogether, the dispersion in the carcinoma group was considerably wider than in the control group Fig. 1.

The dispersion of the values in the group of the adenomas was small, despite the divergence of the dry weights, as described above. While no difference could be verified between the mean values of the control group and the adenoma group, the values of the colon carcinoma group differed again markedly in the first two and a half hours from those measured with the adenomas (t=0/1 h: $p<0.005$, t=2.5 h: $p<0.05$) Fig. 2.

4. Discussion

It has been demonstrated recently that the thermal flow rate of biopsy samples taken from human gastrointestinal mucosa can reliably be measured by microcalorimetry. At that, differences in the dependence from the point of extraction as well as between carcinomatous and healthy tissue of the same region could be verified [1,10]. An enhanced thermal production had already previously been proved microcalorimetrically with malignant cells [11–13].

In 1926 Warburg [14] had described the enhanced glycolysis rate of malignant tumors. This finding had later repeatedly been confirmed with numerous tumors [2,4,5,12]. Today it is well known that proliferation and an enhanced glycolysis rate are closely linked with each other [15,16]. An increased proliferation rate, however, represents only one of the several possible mechanisms of tumor growth. By means of biological markers and using histochemical methods, Kikuchi et al. [6] characterized changes of the proliferation rate and of induced cell death (apoptosis) of the colorectal adenocarcinoma. Starting from normal mucosa, via the stage of hyperplasia and the adenoma up to the carcinoma, an increasing proliferation rate was found, whereas an increase of the apoptosis rate could only be identified up to the stage of adenoma. According to these findings the effects of an enhanced proliferation rate towards tumor growth and an inversely inhibited apoptosis rate cumulate at the stage of carcinoma.

Our findings of the thermal flow rate of adenomas, as it was found unchanged in relationship to normal colon mucosa, contradicts an essential enhancement of energy turnover as a consequence of an apoptosis

induction, as it had been described for leukemia cells [17]. Due to the parallel proliferation enhancement, an enhancement of energy requirement should already have been expected for adenomas. We were, however, not able to verify an enhanced thermal production until the stage of adenocarcinoma was reached. In all likelihood, a measurable enhancement of the overall energy turnover, as it must be assumed for the adenoma stage, fails to occur with a glycolysis that is only moderately enhanced, owing to its energetic limitations.

Whereas there is a lack of corresponding examinations of the colon carcinoma, breast cancer cells, whose proliferation is closely associated with a marked glycolysis enhancement [18], have been found to reveal a 3.5-fold enhancement of microcalorimetrically measurable thermal production in the G2-phase [13]. Thus the enhanced thermal flow rate of colorectal adenocarcinomas, as it has been described by us, could be a manifestation of a – compared with adenomas – markedly enhanced glycolysis intensity, a fact that would match the literature data [19]. Current reports about increased thermal flow rates only for the squamous cell carcinoma of the esophagus, but not for its adenocarcinomas, suggest, however, that alternative metabolic paths to glycolysis with malignant tumors can be enhanced, too, since squamous cell carcinomas might rather reveal a lower glycolysis intensity than adenocarcinomas [10]. With butyrate and glutamine, apart from glucose, other aerobically usable substrates were, at any rate, present in the nutrient solution [20]. Prior to extended conjectures about therapeutic consequences to be drawn from our findings, the underlying mechanisms of the thermal production enhancement should be clarified further.

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