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^{13}C-Leucine-tracer-technique for in-vivo measurement of amino acids' metabolism by human colon carcinomas

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Summary. Own investigations on in-vivo tumor metabolism of the malignant human colon tumor showed a significant uptake of branched chain amino acids by the tumor itself. To study the quantitative tumor protein metabolism ("compartment "tumor") the ^{13}C-leucine-tracer-technique was modified.

Beside the common ^{13}C-leucine-breath-test we measured also the AV-differences of ^{13}C-leucine, ^{13}C-ketoisocaproate and $^{13}CO_2$. The "Tumor-blood flow" was measured by "venous-outflow-technique" as well as the tumor mass.

In this way it is possible to get quantitative results in substrate exchange of branched chain amino acids in malignant human colon tumors.

Keywords: Amino acids – Stable isotopes – ^{13}C-Leucine – Tumor metabolism – Malignant colon tumor

Introduction

Up to now the metabolism in patients with a malignant tumor has been investigated much more intensively than the metabolism of the tumor itself. In the literature there are many reports about the metabolic pathways of glucose, fatty acids and amino acids in patients with cancer; but concerning uptake and release of substrates across tumors we had only little information. In addition the available knowledge is based on studies after grafting the tumors on host animals.

Typical of metabolism in malignant tumors is a marked degree of aerobic and anaerobic glycolysis [17] – this is the effect of an activity change for glycolytic key-enzymes [2, 13]. Concerning amino acids' metabolism, there are many reports about an eminent role of glutamine as an energetic source [1, 2, 7, 12, 13]. Pyruvate and lactate are not only derivates of glucose, they can also be

Table 1. Exchange of glucose, lactate, free fatty acids and ketone bodies (μmol/100 g × min). Mean ± standard error, probability level (*/** $p \leq 0.01/0.001$), Wilcoxon Test

	peripheral tissues	tumors
Glucose	+1.05 ± 0.18	+31.65 ± 5.09*
Lactate	−0.51 ± 0.13	−21.73 ± 4.18**
Free fatty acids	−0.43 ± 0.09	+0.09 ± 1.65
Ketone bodies	+0.13 ± 0.04	−0.80 ± 0.96

Table 2. Exchange of amino acids (single or in groups) (nmol/100 g × min). Mean ± standard error, probability level (* $p \leq 0.05$), Wilcoxon Test

	peripheral tissues	tumors
Total amino acids	−357 ± 149	+633 ± 2442
Essential amino acids	−89 ± 40	+620 ± 664
Branched chain amino acids	−34 ± 20	+473 ± 347*
Glutamate	+73 ± 15	+104 ± 216
Glutamine	+111 ± 40	+182 ± 1177
Alanine	−168 ± 50	−988 ± 518*

derivates of glutamine (malate-enzyme). Apparently there is a reversed relationship between glutaminolysis and the sum of energy production from glycolysis and pyruvate oxidation [2, 6, 14, 15].

Our own studies during curative resections of human colon carcinomas showed for the first time "in-vivo" quantitative results of substrate exchange in human tumors [5]. We could demonstrate a marked degree of aerobic glycolysis as we had expected. With regard to metabolism of amino acids we found a significantly high uptake of branched chain amino acids by the tumor in spite of the release of these substrates by peripheral tissues. Contrary to expectations, appreciable use of glutamine in the energy supply of the tumor cells could not be detected in the investigated colon carcinomas (Table 1 and 2).

As a consequence of these results we have developed a new method to study the tumor-metabolism of the branched chain amino acids with the ^{13}C-leucine-tracer-technique [4, 11]. To study the compartment "tumor" we had to modify the described tracer method in several areas.

Materials and methods

Leucine metabolism

First step in breakdown of leucine is the reversible desamination to ketoisocaproate. The next (irreversible) step is decarboxylation to iso-valeryl-coenzyme A (which leads into the citric-acid circle after decomposition to acetyl-coenzyme A) and CO_2, which will be expired through the lungs. If leucine is labelled on its carboxyl-group with ^{13}C and then infused, it

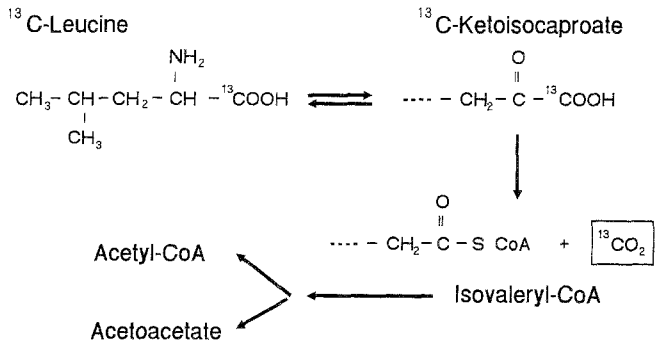

Fig. 1. L-(1-^{13}C)-leucine metabolism

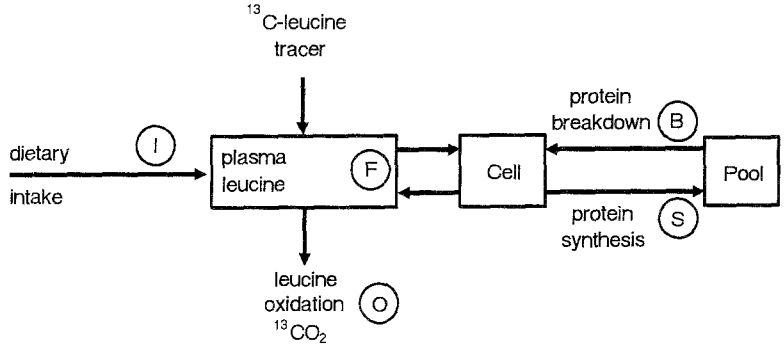

Fig. 2. Model of whole-body leucine metabolism

is possible to determine during the steady state the decarboxylation rate (and so the oxidation rate) by analysing the expired CO_2 (Fig. 1).

By analysing the dynamic aspects of protein metabolism Sprinson and Rittenberg developed their pool model (Fig. 2), which was modified by Matthews and Bier [11]. Their concept was the appearance of a steady state between the single compartments under a continuous ^{13}C-Leucine infusion. By doing that, these parameters, which describe the leucine metabolism, could be determined:

$$\text{Flux} = \text{Oxidation} + \text{Synthesis} = \text{Intake} + \text{Breakdown}$$

The quantity of the infused leucine is known. The oxidation rate is measured by analysing the expired CO_2. Flux is determined by gaschromatic/mass-spectrometry of the ^{13}C-leucine-concentration in plasma. With the above mentioned equation both synthesis rate and breakdown can be calculated [4, 11].

Intraoperative investigations of the tumor

The study is done during curative resection of human colon carcinomas without metastases. We do not investigate very small colon tumors (tumor stage T_1).

At the beginning of the operation a ^{13}C-leucine bolus is given and subsequently the ^{13}C-leucine tracer-infusion is started. Only a "total intravenous anaesthesia" (using Propofol) can be accepted to avoid the side-effects of an inhalation-anaesthesia; eg. reduction of peripheral blood flow and reduction of the blood flow in the gut. When using N_2O – with the same molecular weight [44] as $^{12}CO_2$ – for inhalation anaesthesia another problem arises when analysing the expired $^{13}CO_2/^{12}CO_2$ ratio by GCMS (Gas-Chromatography/Mass-Spectrometry).

The crucial point in the tumor dissection is the perfect preparation of the "tumor-artery" and "tumor-draining-vein". All other vessels which are branching to normal colon tissues have to be ligated. Afterwards the tumor is clamped on both sides.

For the measurement of blood flow in the "tumor-draining-vein" we use the direct method called "venous outflow-technique" [16]. After inserting a cannula of reasonable size in the "tumor-draining-vein" we sample the flowing blood in a syringe (5 ml) and measure the time until this syringe is filled. In addition we take blood samples from the radial artery, the "tumor-draining-vein" and a deep brachial vein. The intraoperative peripheral blood flow is measured by occlusion plethysmography. After the resection the tumor mass is determined.

Analysis of blood samples and calculations

We analyse the A/V-differences between artery and "tumor-draining-vein" and between artery and brachial vein of the following metabolites: ^{13}C-leucine, ^{13}C-KIC, $^{13}CO_2$, plasma amino acids, glucose, pyruvate, lactate, free fatty acids and ketone bodies.

By taking the results of the blood flow-measurements, tumor mass and the A/V-differences, we can calculate the substrate exchange of the analysed metabolites (Fig. 3). The leucine-retention rate can be calculated from the exchange of ^{13}C-leucine, ^{13}C-KIC and $^{13}CO_2$. (Fig. 4).

These results of leucine tumor metabolism can be compared with the results of the whole-body leucine metabolism by using the ^{13}C-leucine-breath-test which is also performed intraoperatively.

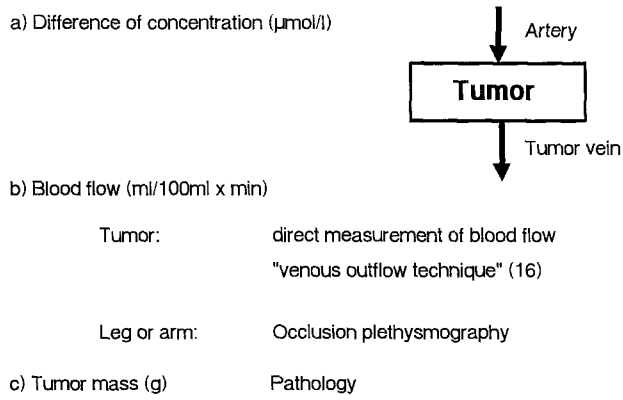

Fig. 3. Method
Measurement of substrate exchange in malignant colon tumors

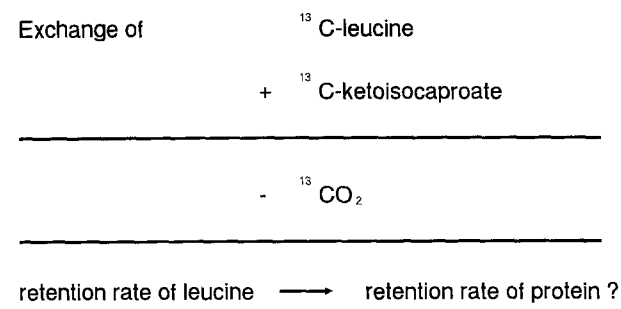

Fig. 4. Calculation of retention rate (leucine), tumor or peripheral tissues

Perspectives

These investigations have been done until now in 19 patients with a malignant colon tumor. For the analyses are not complete yet, the final results will be published soon. The reasonable initial results confirm that the described new method is practicable and can be easily reproduced.

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