In Vivo Nutrient Uptake by Head and Neck Cancers

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ABSTRACT

The arteriovenous uptake differences for glucose, lactate, pyruvate, β-hydroxybutyrate, and acetacetate were measured in vessels across squamous cell carcinomas in ten selected patients with tumors of the head and neck. All tumors measured took up glucose, pyruvate, β-hydroxybutyrate, and acetacetate. They could either produce, take up, or maintain a constant level of lactate. These tumors demonstrate a heterogeneous affinity for various metabolites, including ketone bodies which are elevated in the nutritionally depleted, tumor-burdened patient.

INTRODUCTION

Sixty years ago Warburg (1) studied glucose consumption using tumor slices incubated aerobically and noted high rates of lactic acid production. From these experiments and others, an attitude regarding the high rate of aerobic glycolysis and lactate production became a biochemical hallmark of tumors. Recent in vivo work with Morris hepatomas and Walker carcinomas (2–5) grown in rats has changed our perspective by adding further insights into this aspect of tumor energy metabolism. A model developed by Sauer et al. (2) has allowed the growth of rat tumors on a vascular pedicle composed of the superficial epigastric artery and vein. As the tumor grows, the vascular connection to the host is through this single artery and vein. The ability to sample nutrients which flow into and out of these tumors has led to detailed data on the nutrient uptake in vivo. While glucose was utilized at fast rates, the tumors were observed to either produce or take up lactic acid (4, 5). Also, these rodent tumors have been shown to utilize β-hydroxybutyrate and acetoacetate.

We designed this study to measure AV3 differences across the tumor bed of patients undergoing composite resections for head and neck malignancies. The human system is much more complex than the rat model because there are various anesthetics used, different tumor sites, and multiple draining veins. We thought, however, that sampling AV differences would yield significant insight into the nutrient uptake of these tumors and would possibly extend observations made in the rat model to the human system.

We chose the head and neck tumor model because it provided a circumstance where surgical treatment of tumors was commonly the first mode of therapy for lesions with a known vascular supply. Previous attempts to estimate nutrient uptake for human tumors measured AV differences across entire tumor-bearing extremities (6). They have been relatively nonselective in their venous cannulation or may have had significant metabolic activity in adjacent normal tissues.

MATERIALS AND METHODS

Patient Population. Patients for the study were recruited at the Seattle Veterans' Administration Medical Center from the Otolaryngology/Head and Neck Surgery Tumor Service. Patients had previously untreated squamous cell carcinomas with a variety of primary tumor site presentations. They presented different nutritional states and different histories with regard to pretreatment weight loss. In general, the patients had advanced (Stage III or IV) disease with the exception of one patient (Patient 2) who had T2 carcinoma of the oropharynx (see Table 1). No patients were diabetic. The protocol for blood sampling was approved by the Human Subjects Committee, University of Washington, Seattle, and informed consent was obtained. Specimens were obtained from patients while anesthetized with a variety of general anesthetics for composite resection of their tumors as the primary mode of therapy. Fluid replacement often contained lactate, but infusion rates varied. Only Patient 2 received glucose containing fluids. Prior to surgery, patients were allowed nothing by mouth overnight. The patients receiving TPN prior to therapy had TPN tapered during the week prior to their procedure. Patient 8 received TPN for 3 weeks whereas Patient 10 received therapy for only 2 weeks. During the procedure, all patients were monitored with radial artery cannula, from which a mixed arterial sample was obtained at the same time as venous samples.

Sampling Technique. The sampling procedure was carried out in the course of the standard radical neck dissection (7). Generally the superior and inferior aspects of the jugular vein were ligated before sampling as diagramed in Fig. 1. This ligation was performed to minimize back flow of blood through these large valveless veins and to contain spread of tumor cells which might occur during manipulation of the specimen with further dissections. Standard oncological surgical principals were maintained while procuring each specimen.

Heparinized venous blood samples were obtained during surgical excision of tumors by aspiration of veins large enough to accept a 19-gauge needle after central drainage was clamped or by collecting the blood from the cut end of small veins and allowing them to bleed into the container. Great care was taken to ensure accuracy of the sample collection. All venous samples were collected before ligation of the arterial blood supply. Additional venous samples were obtained from three patients, two of which sampled the superior vena cava via a central venous pressure monitor system, and one of which sampled the internal jugular on the ipsilateral side after major feeding vessels from the tumor had been ligated. These "somatic vein" samples were processed similarly. All samples once collected and determined to be anatomically satisfactory were assayed and reported here. Venous samples were graded for later review according to the following schedule and as diagramed in Fig. 1. Since samples of type I allowed considerable "normal" tissue blood flow contribution, they were excluded from this study: (I) an ipsilateral jugular vein in continuity with tumor; (II) a proximal vein draining the general area of the tumor mass; (III) a vein not directly observed to be draining tumor, but tumor could be palpated on a medial mucosal surface, and the vein appeared to drain in this area; (IV) a vein observed to be directly draining the tumor mass. Many venous blood samples collected were discovered to be unsuitable during the course of the operation and were not processed because of prolonged stasis in veins prior to sampling (because proximal ligatures had been applied). Such samples would not accurately represent the contribution of the tumor to the AV difference. Often further dissection indicated that a specimen had potential contributions from collateral vessels not observed at the time it was procured. These samples were not included. Less than one of four surgical procedures yielded suitable samples for the above reasons. Blood samples were treated as follows. A portion of iced, heparinized blood was diluted 1:1 with water. An amount of 70% perchloric acid was added to yield 6%.
CANCER NUTRIENT UPTAKE IN VIVO

Table 1 Selected head and neck cancer patients' nutritional and tumor status

<table>
<thead>
<tr>
<th>Patient</th>
<th>Venous sample code</th>
<th>Preoperative TPN</th>
<th>Height (cm)</th>
<th>Wt (kg)</th>
<th>Estimated wt. loss (kg)</th>
<th>Tumor Site</th>
<th>Tumor Stage</th>
<th>Tumor Site</th>
<th>Tumor Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IV</td>
<td>-</td>
<td>175</td>
<td>65.9</td>
<td>4.5</td>
<td>Tongue</td>
<td>T₂N₂M₂</td>
<td>III</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>IV</td>
<td>-</td>
<td>185</td>
<td>84.8</td>
<td>6.8</td>
<td>Oropharynx</td>
<td>T₂N₂M₂</td>
<td>III</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>IV</td>
<td>-</td>
<td>180</td>
<td>69.5</td>
<td>-</td>
<td>Glottic larynx</td>
<td>T₂N₂M₂</td>
<td>III</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>II</td>
<td>-</td>
<td>170</td>
<td>70</td>
<td>-</td>
<td>Unknown primary</td>
<td>T₂N₂M₂</td>
<td>III</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>II</td>
<td>-</td>
<td>172</td>
<td>74.6</td>
<td>-</td>
<td>Unknown primary</td>
<td>T₂N₂M₂</td>
<td>III</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>IV</td>
<td>-</td>
<td>178</td>
<td>69.1</td>
<td>-</td>
<td>Supraglottic larynx</td>
<td>T₂N₂M₂</td>
<td>III</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>II</td>
<td>-</td>
<td>157</td>
<td>44.4</td>
<td>-</td>
<td>Supraglottic larynx</td>
<td>T₂N₂M₂</td>
<td>III</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>IV</td>
<td>+</td>
<td>155</td>
<td>38.8</td>
<td>9.2</td>
<td>Transglottic larynx</td>
<td>T₂N₂M₂</td>
<td>III</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>III</td>
<td>-</td>
<td>180</td>
<td>70.9</td>
<td>13.6</td>
<td>Floor of mouth</td>
<td>T₂N₂M₂</td>
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<td></td>
</tr>
<tr>
<td>10</td>
<td>IV</td>
<td>+</td>
<td>169</td>
<td>50.7</td>
<td>15.9</td>
<td>Transglottic larynx</td>
<td>T₂N₂M₂</td>
<td>III</td>
<td></td>
</tr>
</tbody>
</table>

* See text and Fig. 1 for the proximity of the vein to the tumor mass.
* TPN was tapered prior to surgery.
* Well (W), moderately well (M), or poorly (P) differentiated squamous carcinomas.
* Received intraoperative glucose.

Table 2 shows the whole blood arterial and tumor venous glucose, lactate, pyruvate, \( \beta \)-hydroxybutyrate, and acetoacetate concentrations from the 10 patients examined. In these patients blood collections were also made from a deep central vein. All tumors showed substantial glucose uptake. In contrast, the AV differences for lactate and pyruvate were either positive, negative, or zero. With the exception of two samples (Patients 1 and 9), the lactate concentrations in the tumor venous blood were close to 2 mM. In Patients 9 and 10, no lactate uptake or release was observed.

All tumors studied also took up \( \beta \)-hydroxybutyrate and acetoacetate but in varying amounts. The tumor in Patients 4 and 8 took up substantial amounts of acetoacetate. Patient 8 was markedly cachectic and had a long history of weight loss prior to surgery. Despite total parenteral nutrition prior to surgery, he had not gained weight. His tumor was large enough to require emergency tracheostomy for airway management, and definitive surgical treatment of his tumor was delayed for several weeks because of significant nutritional depletion and a hemorrhage from the upper gastrointestinal tract. Patients 4, 8, and 9 had the highest arterial acetoacetate concentrations and the greatest tumor acetoacetate AV differences. Ketone body uptake by human tumors appears to be proportional to the rate of supply, as in rat tumors (3). On the other hand, \( \beta \)-hydroxybutyrate uptake was less than expected.

Table 3 shows change in glucose, lactate, \( \beta \)-hydroxybutyrate, and acetoacetate concentrations in the patients studied and the percentage of change from the arterial level. It is interesting to note that the highest negative glucose difference (Patient 8) is not the highest positive lactate difference (Patient 4). In Patient 2, who received i.v. glucose supplement, the highest glucose concentration does not yield the highest glucose AV difference. High arterial glucose concentrations have been shown to be deleterious to tumor metabolism (6). Also, glucose AV difference across normal tissues is always higher than that observed for the tumors. This contrasts to the observation for hepatomas grown on rat epigastric pedicles, where glucose uptake was directly related to glucose concentrations (2). The percentage of the AV difference of the various metabolites and the relative concentrations are approximately equal in both the rat hepatoma model and the head and neck cancer patients. The extraction of metabolites relates to the vasculature surface area, the time the blood sends in contact with the tumor cells (blood flow), as well as the intracellular metabolism concentration. Tumor metabolite utilization, therefore, may vary considerably depending upon the host and tumor metabolism. Table 3 also shows that although the amount of uptake of acetoacetate was small, the proportion extracted was quite high, as incoming levels of acetoacetate were relatively low. The average uptake of acetoacetate was 40% of that in the arterial blood, as opposed to only 26.7% for \( \beta \)-hydroxybutyrate.

Fig. 2 shows a plot of the paired AV samples with tumor vein lactate level on the abscissa plotted against the arterial lactate level on the ordinate. Those samples which lie above the dotted line demonstrate net lactate production and those below it show lactate acid uptake. The clustering of the tumor lactate acid level between 1.5 and 2.5 mM can be readily appreciated. The lactate acid concentration leaving these tumors was
Table 3 Whole blood metabolite arteriovenous differences collected across selected head and neck tumor bearing patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Glucose</th>
<th>β-Hydroxybutyrate</th>
<th>Acetoacetate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AV diff</td>
<td>% extracted</td>
<td>AV diff</td>
</tr>
<tr>
<td>1</td>
<td>0.18</td>
<td>0.04</td>
<td>77</td>
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<tr>
<td>2</td>
<td>0.26</td>
<td>0.02</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>0.06</td>
<td>0.03</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>0.21</td>
<td>0.15</td>
<td>21</td>
</tr>
<tr>
<td>5</td>
<td>0.03</td>
<td>0.09</td>
<td>56</td>
</tr>
<tr>
<td>6</td>
<td>0.05</td>
<td>0.03</td>
<td>37</td>
</tr>
<tr>
<td>7</td>
<td>0.08</td>
<td>0.07</td>
<td>63</td>
</tr>
<tr>
<td>8</td>
<td>0.10</td>
<td>0.25</td>
<td>43</td>
</tr>
<tr>
<td>9</td>
<td>0.11</td>
<td>0.17</td>
<td>29</td>
</tr>
<tr>
<td>10</td>
<td>0.70</td>
<td>0.04</td>
<td>50</td>
</tr>
</tbody>
</table>

Av. % extracted ± SD 15.2 ± 9.15 26.7 ± 19.5 40.6 ± 17

Fig. 2. Arterial versus venous lactate levels in selected patients with head and neck squamous carcinoma.

reasonably constant at about 2 mM. These results compare favorably with results observed in rats and suggest that the tumor venous lactate concentration may represent the tumor intracellular lactate concentration in humans as well as in rats (5). Most importantly, high lactic acid concentrations were not observed in the venous blood draining any of these human tumors.

DISCUSSION

We have observed a heterogeneous group of head and neck cancers by measuring the AV differences of metabolites across the tumor bed taking as much care as possible to minimize the contributions of blood draining nontumorous areas. We observe glucose as well as ketone body uptake. In contrast to traditional opinions, we observed a variable gradient for lactate.

In recent years, the metabolism of glucose has been examined in some detail in cancer patients. Bennegard et al. (9) assessed glucose as well as lipid and amino acid metabolism in patients with loss of 7–20% of their normal body weight. They identified a difference in glucose uptake measured across the lower extremity in cancer patients. Significantly, cancer patients showed no important uptake of glucose across the extremity after an overnight fast, in contrast to other groups of either normal patients or patients who had lost weight for other reasons. Bennegard et al. (9) further studied malnourishment in cancer patients and identified an increased glucose demand characteristic in eight patients including two head and neck cancer patients and one with esophageal carcinoma. They thought that this increased glucose demand contributed to the patients’ weight loss. In addition, increased lactate turnover contributed
to the whole body glucose flux in metastatic cancer patients. It has been calculated that the elevated glucose turnover may impose an energy cost of up to 40% of increased energy expenditure in metastatic cancer (9, 10).

Holroyde et al. (11) studied weight loss in 12 patients with colo-rectal cancer and identified significant elevated rates of glucose production and recycling. They showed a significant delayed clearance of glucose in a blunted insulin secretory responsiveness and observed some significant metabolic heterogeneity despite a uniform tissue diagnosis. Previously, Holroyde et al. (12) identified increased rates of lactate production in a similar group with a moderately increased rate of lactate oxidation and an increased percentage of glucose derived from lactate. In fact, general glucose intolerance in cachectic patients has been identified and reviewed in a variety of studies (13). Hyperalimentation may also alter glucose metabolism. Interestingly, the results of altered glucose metabolism in patients with colorectal cancer are not correlated with overall tumor burden. One patient treated successfully with chemotherapy has shown a decrease in lactate production, while nonresponding patients showed no change (11). The few reports of lactic acidosis include patients with tumors of epithelial origin (bronchogenic carcinoma), who have significant liver metastasis. It seems likely that concurrent increased lactate production and decreased lactate catabolism contribute to this phenomenon (14). Our results suggest that the tumor may not be the source of elevated blood lactic acid concentration. The lactic acidosis of cancer would then have some cause other than tumor lactate production. Indeed tumors can take up lactate if it is increased in serum.

Substrate utilization across tumor-bearing limbs has been measured (6). Soft tissue sarcoma patients showed a significant difference in the uptake between the tumor-bearing limb and control limb, but this was not true for osteogenic sarcoma patients. Arterial plasma glucose was again elevated in all patients studied, and the tumors appeared to release free fatty acids into the bloodstream. Our study significantly differs from the sarcoma study by sampling blood close to the tumor source and eliminating the contribution by normal tissues as much as possible. Other reviews have shown carbohydrate metabolism to be frequently abnormal in sarcoma patients (6) and in normal tissues of patients with esophageal squamous carcinoma (15).

Ketone body metabolism has been shown to be an important feature of substrate for synthesis for neurolipids and cell lines obtained from gliomas and neuroblastomas (16). Little additional information is available regarding the metabolism of ketone bodies in fasted cancer patients or their tissues. In considering the metabolism of acetyl-CoA, Tisdale (17) points out that although hyperlipidemia is a common feature in cancer patients, ketosis does not occur. Further studies are required to determine if the ketone bodies are oxidized or contribute carbon to cell growth in human tumors.

The human tumors studied here appeared to be more metabolically heterogeneous with regard to nutrient uptake than transplanted rat tumors (2, 3). However, the tumors observed in this study all arose spontaneously and have not been adapted by either serial transplantation or tissue culture; thus they may lack the biochemical homogeneity often seen in an established tumor line. It is possible that the ligation of veins in the course of the radical neck dissection may contribute to changes we observe in metabolite concentrations. Also, the environments of the tumors were different; each patient had nutritional, immunological, and physical individuality. It is perhaps not surprising, therefore, that a wide spectrum of tumor metabolic activities would be observed. We also were unable to control the type of anesthesia and the glucose infusion rates in these patients because they were dictated by the cardiac, hepatic, and fluid needs of their respective surgical procedures. Despite these constraints, there are many similarities between the observations made here and in the rat tumor model. First, all of the tumors take up glucose and the ketone bodies. This may be the first direct demonstration of glucose and ketone body uptake by human tumors in vivo. As a general rule, the tumors observed produced lactate. Two patients had a negative lactate AV gradient. This small study group does not permit interpretation of the significance of the finding of these study situations where lactate was not produced. Still it appeared that lactate production is not necessarily associated with every tumor. In Patient 10, tumor glucose utilization occurred but no change in lactic acid was observed. Similar patterns of arterial versus tumor vein lactic acid levels were observed in rats. Although we do not yet know the fate of the carbons utilized, it seems reasonable to assume that they are used for both energy and macromolecule synthesis.

In the last decade, the provision of adequate nutrition to head and neck cancer patients has become an important and accepted adjunct to successful surgical outcome (18, 19). Changes in the nutritional support of the host may favor changes in the tumor growth. To our knowledge, there are no previously reported direct select in vivo measurements of tumor nutrient uptake in humans. Further determinations must be made before the influence of host cachexia and/or total parenteral nutrition of the tumor can be assessed.

REFERENCES


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