Glutathione Metabolism in Patients with Non-Small Cell Lung Cancers

Sarah L. Blair, Paul Heerdt, Sonu Sachar, Amir Aboihoda, Steven Hochwald, Huiming Cheng, and Michael Burt

ABSTRACT

Non-small cell lung cancer (NSCLC) is the leading cause of cancer death in the United States. Because NSCLC is highly chemoresistant, it is usually not treatable. Altered glutathione (GSH) metabolism is thought to be one major mechanism of chemoresistance, and GSH levels are reported to be elevated in NSCLC. The main objective of this study is to delineate the potential mechanisms involved in elevation of tissue GSH, including extraction from the circulation by NSCLC. Twenty consecutive patients with NSCLC were enrolled. At the time of lobectomy, pulmonary artery and vein were identified, and blood flow was measured by an electromagnetic probe. Subsequently, blood samples were drawn from pulmonary artery, the vein draining the tumor-bearing lobe, and a normal lobe. Immediately after lobectomy, tumor and lung specimens were snap frozen. NSCLC tumor specimens had higher levels of GSH compared with lung tissue (20.8 ± 9.4 versus 11.6 ± 3.0 nmol/mg protein, respectively; P < 0.05). The tumor demonstrated higher activity of the enzyme γ-glutamyl transpeptidase, a membrane-bound enzyme involved in transmembrane uptake of GSH, than lung tissue (41.3 ± 24.4 versus 22.4 ± 12.3 units/mg protein, respectively; P < 0.05). Also, the tumor-bearing lobe showed elevated extraction of GSH and two of its component amino acids compared with lung tissue (GSH uptake: 0.60 ± 0.67 versus 0.20 ± 0.40 μM/min, respectively; P < 0.05). NSCLC tumors are able to extract circulating GSH and its constituent amino acids to synthesize intracellular GSH. Increased activity of γ-glutamyl transpeptidase may be one mechanism underlying increased GSH uptake by NSCLC.

INTRODUCTION

Lung cancer is the leading cause of cancer-related deaths and causes more than 125,000 deaths annually in the United States alone. Because most patients are diagnosed in advanced stages of the disease, the overall 5-year survival is only 15%. NSCLC comprises 80% of the primary lung cancers. At present, complete surgical resection remains the only hope for cure, with chemotherapy or radiotherapy having minimal impact on survival.

GSH metabolism has been postulated to contribute to resistance in radiation and chemotherapy in a wide variety of neoplastic tissues (1, 2). GSH is a tripeptide formed from the amino acids glutamine, cysteine, and glycine, and is the most prevalent intracellular free radical scavenger. GGTP is a membrane-bound enzyme that hydrolyzes the γ-glutamyl bond of glutathione and transfers the γ-glutamyl amino acid into the cell where it is resynthesized into GSH (3, 4). GSH and its related enzymes have been shown to be elevated in several human cell lines, including NSCLC cells (5–7). Furthermore, our laboratory has demonstrated that mammalian tumors are able to use increased amounts of circulating GSH and that this heightened cellular extraction may be mediated through the increased activity of the enzyme GGTP (8).

Received 6/20/96; accepted 10/31/96.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom requests for reprints should be addressed, at Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021. Phone: (212) 639-8026; Fax: (212) 717-3124.
2 The abbreviations used are: NSCLC, non-small cell lung cancer; GSH, glutathione; GGTP, γ-glutamyl transpeptidase; TPV, tumor pulmonary vein; NPV, normal pulmonary vein; GST, glutathione S-transferase.

MATERIALS AND METHODS

Clinical Material. Twenty consecutive patients with presumptive or biopsy-proven NSCLC, scheduled to undergo curative lobectomy, were entered in this study after signing of informed consent. There were 12 women and 8 men with an average age of 62.5 ± 10.9 years. Eighteen patients had a smoking history, and two patients had preoperative chemotherapy. The procedures followed in this study were in accordance with the ethical standards of the Committee on Human Experimentation at Memorial Sloan-Kettering Cancer Center and were based on an Institutional Review Board protocol approved by the Department of Health and Human Services.

Experimental Design. Surgical dissection for the planned lobectomy was performed in a standard fashion. The pulmonary artery and vein pertaining to the tumor-bearing lobe and normal (tumor-free) lobe were identified and isolated. An electromagnetic flow probe [models SF430, SF435, or SF445, Carolina Medical Instruments, Inc., King, NC; Ref. 9] was placed around the pulmonary artery and/or the draining pulmonary vein from the corresponding lobe. In the first five patients, we studied flow through both the artery and vein and found no difference. Subsequently, flow was measured only from the pulmonary vein. If an adequate probe fit could not be attained, the patient was excluded from the study. Blood samples were drawn in heparinized syringes from the main pulmonary artery, a branch of the pulmonary vein draining the tumor-bearing lobe (TPV) and a branch of the vein draining a tumor-free lobe (NPV). Blood samples were immediately centrifuged (Fisher Scientific, Pittsburgh, PA) at 9000 rpm for 2 min. A portion of the supernatant was immediately deproteinized with cold 10% trichloroacetic acid in preparation for GSH analysis. The remainder of the supernatant was aliquoted for subsequent glucose, lactate, and amino acid measurements and stored at −80°C. Lobectomy was completed, and the resected lobe was transferred to a side table where the surgeon carefully excised a sample of tumor tissue and adjacent normal lung from the lobectomy specimen. To minimize contamination of tumor from lung, a sample of tumor tissue was removed from the center of the in situ tumor. The specimens were then snap frozen in liquid nitrogen and stored at −80°C.

Tissue extraction was defined as the pulmonary arterial concentration − pulmonary venous concentration multiplied by the blood flow:

\[ \text{Extraction} = (\text{Conc}_{\text{in}} - \text{Conc}_{\text{ven}})Q. \]

where Q was measured in liters/min.

GSH Measurement. Reduced GSH was measured by the method described previously by Hassan and Hilf (10). Briefly, 50 mg of each tissue specimen were homogenized on ice for 30 s (Polytron, Kinotia, Luzern-Schweiz, Switzerland) in 1 ml of 5% trichloroacetic acid. Previously deproteinized plasma samples and the freshly prepared tissue homogenates were centrifuged at 3000 × g for 15 min. Two hundred μl of each supernatant were mixed with 1.7 ml of phosphate buffer [sodium phosphate 0.1 M/EDTA 5 mM (pH 8.0)] and 100 μl of O-phthaldehyde (1 mg/ml in absolute methanol). After a 15-min dark incubation, the sample fluorescence was read at 350 nm excitation and 420 nm emission. The concentration of GSH was determined using a known standard curve. All measurements were performed in duplicate.

γ-Glutamyl Transpeptidase Analysis. Tumor and normal lung tissue were homogenized (Polytron) in PBS, and the homogenates were used to determine GGTP activity by means of spectrophotometric assay. The reaction was performed at 37°C for 20 min and used l-γ-glutamyl-p-nitroanilide (Sigma, St. Louis, MO) as the substrate and glycyglycine as the acceptor (11). One unit of GGTP is defined as that amount of activity that liberates 1 nmol amino acid constituents across in situ primary lung cancer and surrounding normal lung; and (b) to investigate the possible mechanisms involved in elevation of tissue GSH levels in NSCLC.
of p-nitroaniline per minute, measured at 540 nm. An aliquot of the homogenate was used to determine protein concentration by a modified Folin method (12).

Analysis of Amino Acids, Glucose, and Lactate. Plasma-free amino acid concentration was determined by high-pressure liquid chromatography using Pico-tag (Waters, Milford, MA) method as described previously (13). Briefly, plasma or known standard solution was mixed in a 1:1 ratio with internal standard solution (methanol: 1 M sodium acetate: triethylamine:water:phenylisothiocyanate in a ratio of 7:1:1:1 by volume) and dried under vacuum. Twenty μl of the derivatization reagent (methanol: 1 M sodium acetate: triethylamine:water:phenylisothiocyanate in a ratio of 7:1:1:1 by volume) was added to each tube and incubated for 20 min at room temperature. After complete drying, the sample was diluted with 100 μl of Pico-tag diluent (5 mM Na2HPO4 (pH 7.4) mixed with acetoni trite (ratio of 95:5 by volume). The solution was then injected into high-pressure liquid chromatography apparatus. The system consisted of Waters pump 510, Autosampler 717, and Temperature Control Module. Detection was performed with Waters 486 tunable absorbodetector at 254 nm (Waters). Data were collected and calculated by Millennium version 3.10 program (Waters). Ten μl of sample were injected onto the column (Pico.tag amino acid analysis column, 3.9 X 300 mm, Waters) in a column heater insert using a dual solvent system and a flow rate of 1 ml/min. Eluent A consisted of 0.13 M sodium acetate trihydrate and acetonitrile in a ratio of 47:3. Eluent B consisted of acetonitrile and Milli-Q quality water in a ratio of 40:60. The gradient was as described previously. Retention times for peaks of glutamine and glycine were taken from the branch of the pulmonary artery, TPV, and NPV. Extraction was defined as the amount of substance extracted from the tumor tissue divided by the total amount of substrate available in the blood. Extraction was measured in liters/mm.

RESULTS

Tumor Characteristics. Pathological stage and histology are shown in Table 1. The mean tumor size was 3.77 ± 1.9 cm, corresponding with an average volume of 155 ± 220 cm³. Blood flow was equivalent between the tumor-bearing lobe (0.75 ± 0.3 liter/min) and the normal lobe (0.74 ± 0.4 liter/min).

Substrate Flux. Tumor extracted higher amounts of glucose than normal lung; however, the difference was not significant (Table 2). Furthermore, the amount of glucose extracted by the tumor-bearing lobe correlated linearly with the tumor volume (R² = 0.88, P < 0.001; Fig. 1). Tumor released more lactate (~4.7 ± 7.1 mg/mm) than lung (0.65 ± 9.0 mg/min), and this difference approached significance (P = 0.069; Table 2).

Amino Acid Flux. Tumor extracted significantly higher amounts of glutamine and glycine than normal lung. There was no significant difference in cysteine uptake between tumor and normal lung (Table 2).

Tissue GSH and GGTP Levels. Tumor tissue contained significantly higher levels of GSH (20.8 ± 9.4 nmol/mg protein) compared with lung tissue (11.6 ± 3.0 nmol/mg protein, P < 0.05; Table 1). The tumor, also, had significantly higher activity of the enzyme GGTP (41.9 ± 26.4 versus 22.4 ± 12.3 units/mg protein, P < 0.05; Table 1). There was no correlation between tumor size and tissue level of GSH.

GSH Extraction. The tumor-bearing lobe demonstrated higher extraction of GSH than the tumor-free lobe (0.60 ± 0.67 μM/min versus 0.20 ± 0.40 μM/min, P < 0.05; Table 1).

DISCUSSION

Other investigators have demonstrated high extraction of several energy substrates in various types of human tumors. Norton et al. (16) have shown that the tumor extracts significantly higher amounts of glucose than normal lung. In this study, we have demonstrated that the tumor extracts significantly higher amounts of amino acids than normal lung. These findings are consistent with previous studies, which have shown that the tumor extracts significantly higher amounts of amino acids than normal lung.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Type*</th>
<th>Stage</th>
<th>GSH tumor nM/mg protein</th>
<th>GSH lung nM/mg protein</th>
<th>GGTP tumor units/mg protein</th>
<th>GGTP lung units/mg protein</th>
<th>Extraction tumor μM/min</th>
<th>Extraction lung μM/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>Adeno</td>
<td>T1NO</td>
<td>10.4</td>
<td>13.2</td>
<td>17.7</td>
<td>12.2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>Squam</td>
<td>T2NO</td>
<td>13.5</td>
<td>11.2</td>
<td>15.7</td>
<td>0.43</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>Bronch</td>
<td>T2NO</td>
<td>20.3</td>
<td>11.1</td>
<td>19.6</td>
<td>18.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>Neuroend</td>
<td>T3NO</td>
<td>30.5</td>
<td>13.1</td>
<td>31.3</td>
<td>28.2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>Bronch</td>
<td>T3NO</td>
<td>19.6</td>
<td>13</td>
<td>33.1</td>
<td>42.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>Adeno</td>
<td>T2NO</td>
<td>9.4</td>
<td>10.3</td>
<td>24.1</td>
<td>18.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>Squam</td>
<td>T3NO</td>
<td>34.4</td>
<td>17.1</td>
<td>119</td>
<td>10.8</td>
<td>2.1</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>Adeno</td>
<td>T3NO</td>
<td>15.3</td>
<td>12.0</td>
<td>45.6</td>
<td>30.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>Squam</td>
<td>T3NO</td>
<td>28.4</td>
<td>8.3</td>
<td>59.3</td>
<td>16.9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>Squam</td>
<td>T1NO</td>
<td>17.1</td>
<td>18.0</td>
<td>15.8</td>
<td>14.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>Adeno</td>
<td>T2NO</td>
<td>12.8</td>
<td>9.7</td>
<td>42.3</td>
<td>5.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>Adeno</td>
<td>T1NO</td>
<td>27.8</td>
<td>8.2</td>
<td>64.6</td>
<td>13.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>Large</td>
<td>T2NO</td>
<td>15.0</td>
<td>13.0</td>
<td>29.6</td>
<td>31.0</td>
<td>0.68</td>
<td>0.98</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>Squam</td>
<td>T1NO</td>
<td>14.6</td>
<td>16.7</td>
<td>20.5</td>
<td>23.9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>Adeno</td>
<td>T2NO</td>
<td>41.4</td>
<td>8.4</td>
<td>39.4</td>
<td>24.6</td>
<td>1.15</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>F</td>
<td>Adeno</td>
<td>T3NO</td>
<td>15.0</td>
<td>8.5</td>
<td>40.6</td>
<td>44.0</td>
<td>0.49</td>
<td>0.19</td>
</tr>
<tr>
<td>17</td>
<td>M</td>
<td>Adeno</td>
<td>T2NO</td>
<td>26.6</td>
<td>8.6</td>
<td>38.7</td>
<td>20.0</td>
<td>0.69</td>
<td>0</td>
</tr>
<tr>
<td>18</td>
<td>F</td>
<td>Neuroend</td>
<td>T2NO</td>
<td>5.4</td>
<td>8.7</td>
<td>37.6</td>
<td>45.5</td>
<td>0.90</td>
<td>1.45</td>
</tr>
<tr>
<td>19</td>
<td>F</td>
<td>Squam</td>
<td>T2NO</td>
<td>35.6</td>
<td>6.5</td>
<td>28.0</td>
<td>23.6</td>
<td>0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>20</td>
<td>F</td>
<td>Adeno</td>
<td>T2NO</td>
<td>23.4</td>
<td>13.0</td>
<td>31.3</td>
<td>27.7</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Adeno, adenocarcinoma; Squam, squamous cell carcinoma; Bronch, bronchialveolar carcinoma; Large, large cell carcinoma.
showed high glucose extraction across sarcoma-bearing limbs in humans. Similarly, Holm et al. (17) demonstrated that in situ colon carcinomas were highly active in glucose metabolism and released high levels of lactate into the circulation (18). These studies reproducibly illustrated the heightened metabolic state of tumor tissue and introduced several novel methodologies allowing measurement of in vivo substrate utilization across tumor bed. Our model provides an opportunity for direct measurement of blood flow to tumor-bearing and normal tissue and creates a unique setting to investigate substrate flux across tumor bed. Because each lobe of the lung has a separate blood supply, we were able to directly compare the metabolic activity of a tumor-bearing lobe to a tumor-free lobe.

To study the metabolic activity of the primary lung cancer, we studied the flux of two energy substrates, i.e., glucose and lactate, across the tumor bed. We showed a positive correlation between tumor size and glucose uptake. We did not observe a significant difference in glucose uptake or lactate release between tumor-bearing and normal lung; however, there was a trend that the tumor-bearing lobe extracted more glucose and released more lactate. One possible explanation for these findings is the relatively small size of the tumors studied in this experiment. Larger differences could be expected in bulkier tumors, as has been reported previously for other types of tumors.

There is a paucity of information about the in vivo metabolism of GSH in human lung and primary lung cancer. Animal data suggest that normal lung is capable of effectively using the circulating GSH, and the majority of this extraction is mediated by transmembrane transporters. As reported previously, our experiments show significantly higher levels of GSH in NSCLC compared with lung. Also, we demonstrated that tumor-bearing lung is capable of extracting higher amounts of GSH from the pulmonary circulation compared with normal lung. These findings are substantiated by the observation of elevated GGTP enzyme activity in the primary lung cancer, potentially a mechanism for intracellular trafficking of circulating GSH. D.L-Buthionine-S,R sulfoximine is a peptide with a low toxicity profile and a known inhibitor of GSH synthesis through inhibition of the rate-limiting enzyme γ-glutamyl synthetase. This agent has been shown in vitro to increase chemotherapeutic cytotoxicity in NSCLC and may represent an important addition to the treatment of NSCLC (26).

In summary, the current study demonstrated higher GSH levels and GGTP enzyme activity in NSCLC compared with lung. Also, we showed increased extraction of GSH and its amino acid constituents from the pulmonary circulation by the tumor compared with normal lung. The elevation of the GGTP enzyme activity may be one mechanism underlying the increased tissue GSH levels in NSCLC. Further study is necessary to assess the role of GSH modulation in increasing the efficacy of medical management of NSCLC.

REFERENCES

Glutathione Metabolism in Patients with Non-Small Cell Lung Cancers

Sarah L. Blair, Paul Heerdt, Sonu Sachar, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/57/1/152

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.