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Thoracic tumor effects on plasmatic coagulation: Role of hemeoxygenase-1

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ABSTRACT

Objectives: Lung cancer is an important health threat worldwide, and is associated with a 3.8–13.9% incidence of thrombophilia. Of interest, patients with lung tumors have been noted to have an increase in endogenous carbon monoxide production *via* upregulation of hemeoxygenase-1 activity. Given that it has been demonstrated that carbon monoxide enhances plasmatic coagulation *in vitro* and *in vivo via* formation of carboxyhemefibrinogen, we sought to determine if patients with thoracic tumors undergoing lung resection/pneumonectomy had an increase in endogenous carbon monoxide and concurrent plasmatic hypercoagulability.

Materials and methods: Nonsmoking patients with thoracic tumors ($n = 19$) had preoperative carboxyhemoglobin (a measure of carbon monoxide production) determined, and a thromboelastometric method to assess citrated plasma coagulation kinetics and the formation of carboxyhemefibrinogen was utilized. Thoracic tumor patient coagulation kinetics was compared with normal subject ($n = 30$) plasma samples.

Results and conclusion: Patients with thoracic tumors were determined to have an abnormally increased carboxyhemoglobin concentration of $2.1 \pm 0.6\%$, indicative of hemeoxygenase-1 upregulation. It was found that 84% of thoracic tumor patients had plasma clot strength that exceeded the 95% confidence interval value observed in normal subjects, and 44% of this hypercoagulable subgroup had carboxyhemefibrinogen formation. Future investigation of the role played by plasmatic hypercoagulability and hemeoxygenase-1 derived carboxyhemefibrinogen in the pathogenesis of thoracic tumor related thrombophilia is warranted.

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1. Introduction

It has been estimated that 1.6 million people were diagnosed with lung cancer worldwide in 2008, with nearly 1.4 million deaths in patients with lung cancer occurring in the same year [1]. In the United States alone, it is expected that nearly 230,000 diagnoses of lung cancer will be made and nearly 160,000 deaths from lung cancer will occur in 2013 [2]. Lung cancer is associated with significant thrombophilia, with a venous thromboembolism incidence of 3.8–13.9% [3,4]. Investigations of etiologies of hypercoagulability associated with lung cancer have revealed that these patients increased concentrations of circulating microparticles with attached tissue factor activity [5,6], enhancing intravascular thrombin generation. Further, significant increases in circulating fibrinogen have been documented in patients with lung cancer, with average values varying from 400 to 500 mg/dl values [7,8] and occasionally exceeding 800 mg/dl [8]. Taken as a whole, important source of increased intravascular thrombin generation as well as increased fibrinogen concentration have been identified as sources of thrombophilia in the setting of lung cancer.

Of interest, endogenous carbon monoxide (CO) production is increased in lung cancer patients [9–11], perhaps in part by direct endogenous synthesis by lung cancer cells [12] and also secondary to cancer associated hemolytic anemia [13] *via* upregulation of hemoxygenase-1 (HO-1) activity [12,14]. HO-1 is solely responsible for endogenous production of CO during the catabolism of heme [14]. Importantly, increases in plasmatic CO concentration enhance coagulation *via* interaction with a heme group(s) attached to fibrinogen in humans *in vitro* and rabbits *in vivo* [15–18]. Thus, lung cancer associated upregulation of HO-1 could contribute to plasmatic hypercoagulation.

The goals of the present investigation were to determine the incidence of HO-1 upregulation and CO mediated plasmatic

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hypercoagulability in patients with either primary or metastatic thoracic tumors undergoing lung resection. These goals were achieved by determination of carboxyhemoglobin (COHb) concentrations obtained from routine arterial blood gas analyses and *in vitro* determination of the formation of carboxyhemefibrinogen (COHF) as previously described [18,19].

2. Methods

2.1. Normal subject and cancer patient plasma

2.1.1. Normal subjects

Normal subject citrated plasma obtained (George King Bio-Medical, Overland Park, KS, USA) anticoagulated with sodium citrate was utilized for experimentation. A standard lot of 30 individuals (15 males, 15 females; mean age 34 years, with range of 19–56 years) were utilized. All normal subjects were verified to be without blood borne disease (*e.g.*, hepatitis), not pregnant, and nonsmokers as per the vendor's specifications. The rationale for using plasma of this nature is that standard, plasma-based tests of coagulation (*e.g.*, prothrombin time, activated prothrombin time, fibrinogen concentration, coagulation factor activities) commonly have 95% confidence interval values in clinical pathology laboratories within hospitals/ambulatory clinics established with such material. Indeed, that is the express commercial purpose of the manufacturing of such normal subject plasma lots, as most facilities do not have the resources or time to insure that their normal reference population is actually disease/medication free. Further, by using the same lot of plasma for reference between hospitals, a greater standardization of "normality" is realized, so that comparison of patient population sample values across institutions is more reasonable.

2.1.2. Cancer patients

Our protocol (#12-0179-04) was approved by the University of Arizona Institutional Review Board. Patients with thoracic tumors scheduled for tumor resection, aged 18–80 years of age that were nonsmokers, were recruited for this investigation. The patients had no history of inherited bleeding disorder and were not being administered anticoagulant or medications. After written consent was obtained and induction of anesthesia, but before operation, COHb concentration values (GEM Premier 4000, Instrumentation Laboratory, Bedford, MA, USA) were obtained from the first routine arterial blood gas analysis. Further, whole blood (15 ml) was obtained from the patients' indwelling arterial catheter and anticoagulated with sodium citrate (9 parts blood to 1 part 0.105 M sodium citrate) and subsequently centrifuged at 3000xg for 15 min at room temperature. Plasma was decanted, aliquoted and stored at −80 °C prior to experimentation.

2.2. Thromboelastometric COHF assay

These analyses were performed with a ROTEM® *delta* hemostasis system (Tem Innovations GmbH, Munich, Germany) generously provided by the manufacturer. Additional plasma from the same set of thirty normal subjects was used for this series of experiments. All disposable cups/pins and reagents were also provided by Tem Innovations. Plasma was rapidly thawed at 37 °C on the day of experimentation. The final volume of all plasma samples was 339.4 μl. Sample composition consisted of 306 μl of plasma, 10 μl of ex-tem® (a tissue factor-containing reagent) diluted 1:15 with a proprietary buffer, 3.4 μl of dH_2O or CORM-2 (carbon monoxide releasing molecule-2, tricarbonyldichlororuthenium (II) dimer, 100 μM final concentration; Sigma–Aldrich, Saint Louis, MO, USA), and finally 20 μl 200 mM $CaCl_2$ as previously described [18]. This dilution of ex-tem® was used as coagulation kinetics with this mixture in plasma resulted in clot growth velocity and final clot strength similar to that observed with activation by 1:1000 tissue factor agent as described previously (data not shown). The complete sample was mixed by pipette once, and the reaction commenced at 37 °C. The amplitude was recorded at 15 min, with values converted to *G* (dynes/cm^2). This modification of our COHF assay was designed to identify COHF by a resistance to hypercoagulation after exposure to exogenous CO that would be secondary to endogenous CO already present and bound to fibrinogen-associated heme groups [19]. Using this method, we subsequently described hypercoagulability as a *G* value > 95% confidence interval value of the normal subjects data set; further, we defined the presence of COHF as a % increase in *G* secondary to CORM-2 that was ≤ the 33rd percentile value increase in *G* observed in normal subject plasma.

2.3. Statistical analyses and graphics

Demographic and COHb data are presented as mean ± SD. *G*-based data are presented as raw values, 95% confidence interval value, and 33% percentile values as indicated previously. Confidence intervals were determined with a commercially available statistical program (SigmaStat 3.1, Systat Software, Inc., San Jose, CA, USA). Graphics were generated with a commercially available program (OrigenPro 7.5, OrigenLab Corporation, Northampton, MA, USA).

3. Results

Twenty patients that underwent thoracic tumor surgery were recruited into the study; one was *post hoc* excluded as pathological examination demonstrated that the tumor was fungal disease and not malignancy. Of the 19 patients remaining, the average age was 65 ± 9 years.

With regard to elastic modulus, the 95% confidence interval value for clot strength in normal subjects was 1579 dynes/cm^2 and the 33rd percentile increase in *G* secondary to CORM-2 addition (based on individual, not group mean responses) was 77%. Thus, for a thoracic tumor patient to be considered hypercoagulable, the elastic modulus had to exceed 1579 dynes/cm^2; similarly, for COHF to be considered present, the 33rd percentile CORM-2 induced increase in *G* value had to be ≤77%.

Table 1
Clinical characteristics and plasma coagulation of lung thoracic tumor patients.

Patient	Age	COHb	Pathology	Coagulation
1	68	1.7	Invasive adenocarcinoma	Hyper/COHF
2	67	1.3	Carcinoid	Hyper/COHF
3	54	2.1	Squamous carcinoma *in situ*	Hyper
4	58	2.1	Metastatic colon adenocarcinoma	Hyper/COHF
5	66	3.7	Non small cell adenocarcinoma	Hyper
6	61	3.0	Metastatic liver carcinoma	Hyper/COHF
7	52	1.3	Metastatic breast carcinoma	Hyper/COHF
8	62	1.8	Metastatic renal cell carcinoma	Hyper
9	76	1.8	Metastatic renal cell carcinoma	Hyper
10	61	2.2	Squamous cell carcinoma	Hyper
11	76	2.2	Mesothelioma	Hyper/COHF
12	67	2.2	Mesothelioma	Normal
13	72	1.7	Squamous cell carcinoma	Hyper
14	51	1.7	Metastatic cholangiocarcinoma	Hyper
15	66	2.0	Adenosquamous cell carcinoma	Hyper
16	75	1.8	Large cell neuroendocrine carcinoma	Hyper
17	74	2.3	Metastatic melanoma	Normal
18	78	2.2	Squamous cell carcinoma	Hyper/COHF
19	53	1.9	Mesothelioma	Normal/COHF

COHb, % carboxyhemoglobin; tumors were assessed by our pathology department; normal = ≤95% confidence value of normal subject clot strength; Hyper = > 95% confidence value of normal clot strength; COHF, carboxyhemefibrinogen present.

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