

Procedure-related pulmonary hypertension in patients with hepatocellular carcinoma undergoing percutaneous ethanol injection—Role of ethanol, hemolysis, asymmetric dimethylarginine, and the nitric oxide system

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Objective: Local percutaneous tumor ablation with ethanol injection (PEI) under general anesthesia is an established therapy for patients with advanced nonresectable hepatocellular carcinoma (HCC). There are reports of sudden hypotension immediately after PEI therapy, which was attributed to an acute increase in pulmonary vascular resistance. The aim of our study was to objectively confirm pulmonary hypertension by right heart catheterization and to evaluate biochemical markers with relevance to the pulmonary circulation.

Design: Cross-sectional clinical study.

Setting: Interventional Gastroenterology in a tertiary care center.

Patients: We studied 40 patients with HCC who underwent percutaneous ethanol injection under general anesthesia for the treatment of nonresectable HCC.

Results: In patients with HCC, PEI leads to a significant increase in pulmonary arterial pressure. Concomitantly, free hemoglobin and blood ethanol level significantly increased, whereas

the L-arginine/asymmetric dimethylarginine ratio, a parameter of nitric oxide (NO) production capacity, and nitrite, a marker of NO synthesis, significantly decreased.

Conclusion: Procedure-related pulmonary hypertension in patients undergoing PEI is multifactorial. Plasma concentrations of the NO precursor L-arginine are reduced by arginase released from lysed erythrocytes, a condition further exacerbated by the increased concentrations of symmetric dimethylarginine, which may compete with the cellular uptake of L-arginine. The result would be reduced synthesis of NO, the concentration of which would be further decreased extracellularly through free hemoglobin. Predictably, the result would be severe endothelial dysfunction and pulmonary hypertension in patients undergoing PEI. These mechanisms might also be relevant in other states of (sudden) hemolysis. (*Crit Care Med* 2009; 37:000–000)

KEY WORDS: asymmetric dimethylarginine; nitric oxide; free hemoglobin; hemolysis; hepatocellular carcinoma; ethanol; ultrasound

Local percutaneous tumor ablation with ethanol injection (PEI) is an established therapy for patients with advanced nonresectable hepatocellular carcinoma (HCC) (1). This technique was introduced >20 years ago by Ebara et al (2) for HCC

smaller than 3 cm. Livraghi et al expanded PEI to treat larger HCC using a single-session procedure under general anesthesia. Although the overall mortality associated with percutaneous ethanol injection is low, given the severity of the underlying disease, acute complications include bleeding, hemoglobinuria, fever, and inebriation. Furthermore, there are reports of sudden hypotension immediately after PEI therapy (3), which was attributed to an acute increase in pulmonary vascular resistance (4). Neither the magnitude nor the mechanisms of this procedure-related pulmonary hypertension have been investigated yet.

The aim of our study was to evaluate biochemical markers with relevance to the pulmonary circulation. These included (i) asymmetric dimethylarginine (ADMA), the most potent endogenous inhibitor of nitric oxide (NO) synthase

known to increase pulmonary vascular resistance (5); (ii) free hemoglobin, which is known to scavenge NO; (iii) ethanol, which is known to induce pulmonary artery vasoconstriction in men (6); and (iv) nitrite as a surrogate for NO production. Furthermore, we aimed to measure pulmonary vascular resistance by pulmonary artery catheterization.

SUBJECTS AND METHODS

Participants and Protocols. The study protocol was approved by the local Ethics Committee, and all participants gave informed consent. We recruited 40 whites (age 68.0 ± 9.0 years; 7 women; weight 73.4 ± 14.7 kg) with advanced HCC, who underwent single-session PEI in general anesthesia. PEI was aimed at complete tumor destruction within a single session by an adequate dose of 95% ethanol up to 100 mL (average ethanol dose 43.5 ± 21.6 mL). Eleven patients were randomly selected to undergo right heart catheter

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examination during the time of PEI. Blood samples for measurement of plasma ADMA, ethanol, free hemoglobin, nitrite, and routine chemistry as well as complete blood count were carefully drawn before and after ethanol injection without the application of a tourniquet, using a large bore venous access. Free hemoglobin was immediately measured. All other blood samples were immediately cooled on ice and centrifuged at 1500g and 4°C for 10 minutes. Supernatants were stored in 1-mL aliquots at -80°C until further use.

Measurements and Calculations. Plasma concentrations of ADMA, L-arginine, and symmetric dimethylarginine (SDMA) were measured applying a recently developed liquid chromatography-mass spectrometry method described elsewhere (7), with the following specifications—L-arginine: sensitivity 0.4 μmol/L at peak/noise ratio of 3, intra-assay variation 4.5% (n = 10), interassay variation 4.7% (n = 6); ADMA: sensitivity 0.02 μmol/L at peak/noise ratio of 3, intra-assay variation 5.5% (n = 10), interassay variation 7.7% (n = 6); SDMA: sensitivity 0.01 μmol/L at peak/noise ratio of 3, intra-assay variation 3.9% (n = 10), and interassay variation 4.9% (n = 6). Nitrite and nitrate in plasma were measured by gas chromatography-mass spectrometry as described (8). Precision (relative SD, %) of the method ranged between 0.2% and 1.3% for nitrite and 0.1% and 4.3% for nitrate. Accuracy of the method was 103% (QC2) and 96.1% (QC3) for nitrite and 108% (QC2) and 104% (QC3) for nitrate.

Measurement of free hemoglobin was manually performed by spectrophotometric analysis of human plasma using three wavelengths (577, 562, and 602 nm). All other measurements were done with routine laboratory tests using certified assay methods.

Statistical Analysis. We used SPSS for statistical analysis (SPSS 11.51 for Windows). The normality of data distribution was confirmed with the Shapiro-Wilk test. A paired Student's *t* test was used to compare the intraindividual pre-PEI and post-PEI biochemical parameters and cardiovascular parameters obtained during the right heart catheter experiments. The significance level was set at *p* < 0.05.

RESULTS

In patients with HCC, PEI leads to a significant increase in pulmonary arterial pressure (PAP) (Fig. 1). Although the mean arterial blood pressure significantly increased, comparing the average mean arterial blood pressure before and after the first ethanol injection (78.7 ± 16.8 mm Hg vs. 86.2 ± 17.9 mm Hg, *p* < 0.01), without a change in heart rate (65.0 ± 11.8 bpm vs. 64.2 ± 18.8 bpm, not significant), there was no difference in blood pressure comparing mean arterial blood pressure before the first etha-

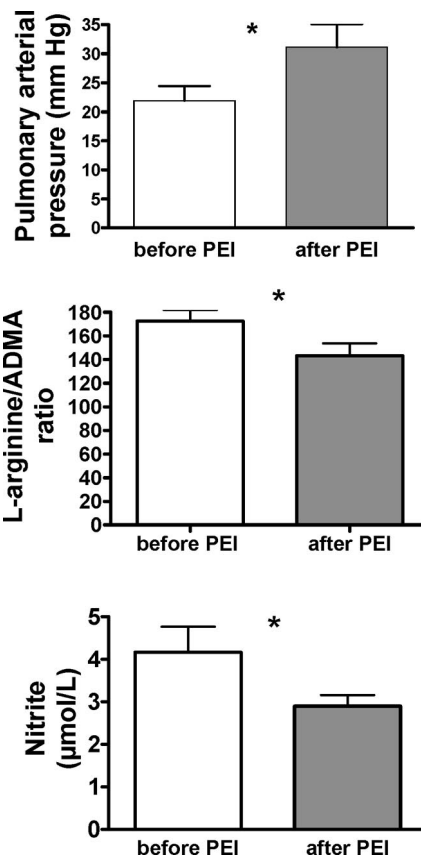


Figure 1. Mean pulmonary arterial pressure before and after percutaneous ethanol installation (PEI) in 11 patients (**p* < 0.001). L-Arginine/asymmetric dimethylarginine (ADMA) ratio and nitrite plasma levels before and after PEI in 40 patients (**p* < 0.001).

nol injection and after the last ethanol injection (78.7 ± 16.8 mm Hg vs. 78.1 ± 17.9 mm Hg, not significant); however, the heart rate tended to increase (65.0 ± 11.8 bpm vs. 69.9 ± 16.5 bpm, not significant). Free hemoglobin and blood ethanol level significantly increased, whereas the L-arginine/ADMA ratio, a parameter of NO production capacity, and nitrite, a marker of NO synthesis, significantly decreased (Fig. 1). There was a correlation between the change in PAP and the change in ADMA (*r* = .768; *p* < 0.05). The association between delta PAP and delta SDMA was even stronger (*r* = .846; *p* < 0.01). Major laboratory parameters that changed significantly are provided in Table 1.

DISCUSSION

The major novel findings of the current study are (1) PAP increases during PEI; (2) ethanol and free hemoglobin, i.e., substances known to increase pulmo-

nary vascular resistance, increase on PEI treatment; (3) circulating nitrite, an indicator of endothelium-derived NO, decreases on PEI treatment; and (4) L-arginine decreases, although ADMA levels remain unchanged on PEI, suggesting elevation of the inhibitory potency of ADMA toward NO synthase.

Potential Mechanisms of PEI-Induced Pulmonary Hypertension

Ethanol. The sudden entry of moderate to large doses of ethanol through the hepatic veins could be one reason for the pulmonary vasoconstriction observed in this study. In dogs, acute intravenous administration of 0.5–1.5 g/kg of absolute ethanol increased pulmonary vascular resistance and decreased right ventricular systolic function (9). A study in healthy volunteers showed a significant increase in pulmonary vascular resistance 30 minutes after the oral ingestion of 0.5 g/kg ethanol (diluted to 15 vol%), with return to normal values after 60 minutes (6). Because the dose of ethanol in our patients was 0.52 g/kg and led to an average blood ethanol level of 0.5 g/kg, it is conceivable that ethanol significantly contributed to the pulmonary hypertension we observed in this study.

Hemolysis. The increase in free hemoglobin in blood, as observed in our patients, can cause pulmonary hypertension through several mechanisms as it has been recently reviewed for pulmonary hypertension associated with hemolytic anemia (10). Free hemoglobin is a potent scavenger of NO and is known to cause vasoconstriction. Nitrite and S-nitrosohemoglobin in intact red blood cells are considered important sources of NO bioactivity in the human circulation. The decrease in plasma nitrite concentration seen in our study may have resulted from scavenging of NO released from both the endothelium and erythrocytes. The net result of these processes would be decreased NO bioavailability in the circulation.

Hemolysis also releases into plasma erythrocytic arginase activity, which hydrolyses L-arginine to L-ornithine, thus decreasing the concentration of L-arginine, which is the substrate for NO synthesis (11). Although we did not directly measure arginase activity, we observed a significant reduction in L-arginine plasma concentration, leading to a decreased L-arginine/ADMA molar ratio. It

Table 1. Laboratory parameters before and after PEI

	Before PEI	After PEI	<i>p</i>
Ethanol (g/kg)	0.01 ± 0.01	0.52 ± 0.30	<0.001
Free hemoglobin (mg/L)	113 ± 235	711 ± 708	<0.001
Creatinine (μmol/L)	66.0 ± 14.7	70.4 ± 14.7	<0.05
Aspartate transaminase (U/L)	23.7 ± 16.9	39.1 ± 18.4	<0.001
Alanine transaminase (U/L)	21.8 ± 18.5	28.3 ± 19.5	0.005
GGT (U/L)	89.4 ± 73.1	83.9 ± 69.4	0.005
CHE (kU/L)	3.49 ± 1.47	2.93 ± 1.07	<0.001
Lactate dehydrogenase (U/L)	188 ± 40	198 ± 35	0.005
Creatine kinase (U/L)	27.00 ± 14.26	29.78 ± 13.58	<0.001
Lactate (mmol/L)	1.53 ± 0.60	2.21 ± 0.94	0.05
White blood cells (1000/μL)	5.04 ± 1.99	3.76 ± 1.71	0.05
Platelets (1000/μL)	125.2 ± 63.7	95.9 ± 47.2	0.005
Asymmetric dimethylarginine (μmol/L)	0.51 ± 0.12	0.53 ± 0.11	NS
L-Arginine (μmol/L)	85.3 ± 21.0	73.7 ± 23.8	<0.001
Nitrite (μmol/L)	4.17 ± 0.61	2.90 ± 0.26	<0.05
Nitrate (μmol/L)	38.6 ± 2.9	35.4 ± 2.1	NS
Symmetric dimethylarginine (μmol/L)	0.79 ± 0.31	0.87 ± 0.29	<0.005

PEI, percutaneous tumor ablation with ethanol injection; NS, not significant.

is of note that hemolysis interferes with the measurement of other laboratory parameters leading to an overestimation/increase of alanine transaminase, aspartate transaminase, creatine kinase, and lactate dehydrogenase (12).

ADMA and SDMA. Apart from ethanol and free hemoglobin, there are other mechanisms to consider for pulmonary hypertension. Several preclinical and clinical studies (5) have documented that the concentrations of ADMA are elevated in pulmonary hypertension. Furthermore, acute infusions of ADMA in healthy volunteers lead to a significant increase of pulmonary vascular resistance (5). Interestingly, in our study, mean ADMA did not increase during PEI treatment. However, the change in ADMA was correlated to the change in PAP. SDMA, the structural isomer of ADMA, increased significantly and the change in SDMA was tightly correlated to the change in PAP. This might be of pathophysiologic relevance because SDMA competes with L-arginine for entry into the cell via the y^+ transporter system that is colocalized with NO synthase in the endothelial caveolae.

We wish to point out important limitations of our study; first, the lack of complete hemodynamic data ethically deemed to require an unnecessary extension of anesthesia time because the time from the first ethanol injection to the end of the last injection was on average 17 ±

13 minutes. In other words, an invasive hemodynamic monitoring would have consumed >50% of the actual procedure time. Second, due to the nature of the study, we lack invasive hemodynamic data for the majority of our patients.

In summary, it is likely that the procedure-related pulmonary hypertension in patients undergoing PEI is multifactorial. Plasma concentrations of the NO precursor L-arginine are reduced by arginase released from lysed erythrocytes, a condition further exacerbated by the increased concentrations of SDMA, which may compete with the cellular uptake of L-arginine. The result would be reduced synthesis of NO, the concentration of which would be further decreased extracellularly through free hemoglobin. Predictably, the result would be severe endothelial dysfunction and pulmonary hypertension in patients undergoing PEI. These mechanisms might also be relevant in other states of (sudden) hemolysis, highlighting the potential importance of our findings. Finally, understanding the pathophysiology of hemolysis-related pulmonary hypertension will help to devise therapeutic strategies, especially in acute procedure-related pulmonary hypertension.

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REFERENCES

1. Livraghi T, Benedini V, Lazzaroni S, et al: Long term results of single session percutaneous ethanol injection in patients with large hepatocellular carcinoma. *Cancer* 1998; 83:48–57
2. Ebara M, Kita K, Yoshikawa M, et al: [Non-vascular interventional radiology—Percutaneous ethanol injection (PEI) in hepatocellular carcinoma smaller than 3 cm in diameter]. *Gan To Kagaku Ryoho* 1989; 16: 3311–3318
3. Gelczer RK, Charboneau JW, Hussain S, et al: Complications of percutaneous ethanol ablation. *J Ultrasound Med* 1998; 17:531–533
4. Naik B, Lobato E, Urdaneta F: Acute cardiovascular instability during percutaneous ethanol injection of a hepatocellular carcinoma under general anesthesia. *Anesthesiology* 2004; 100:1307–1308
5. Kielstein JT, Bode-Boeger SM, Hesse G, et al: Asymmetrical dimethylarginine in idiopathic pulmonary arterial hypertension. *Arterioscler Thromb Vasc Biol* 2005; 25:1414–1418
6. Koskinen P, Kupari M, Nieminen MS, et al: Effects of alcohol on systemic and pulmonary hemodynamics in normal humans. *Clin Cardiol* 1986; 9:479–482
7. Martens-Lobenhoffer J, Bode-Boeger SM: Simultaneous detection of arginine, asymmetric dimethylarginine, symmetric dimethylarginine and citrulline in human plasma and urine applying liquid chromatography–mass spectrometry with very straightforward sample preparation. *J Chromatogr B* 2003; 798: 231–239
8. Tsikas D: Simultaneous derivatization and quantification of the nitric oxide metabolites nitrite and nitrate in biological fluids by gas chromatography/mass spectrometry. *Anal Chem* 2000; 72:4064–4072
9. Kettunen R, Timisjarvi J, Saukko P: The acute dose-related effects of ethanol on right ventricular function in anesthetized dogs. *Alcohol* 1992; 9:149–153
10. Barnett CF, Hsue PY, Machado RF: Pulmonary hypertension: An increasingly recognized complication of hereditary hemolytic anemias and HIV infection. *JAMA* 2008; 299: 324–331
11. Morris CR, Kato GJ, Poljakovic M, et al: Dysregulated arginine metabolism, hemolysis-associated pulmonary hypertension, and mortality in sickle cell disease. *JAMA* 2005; 294:81–90
12. Lippi G, Salvagno GL, Montagnana M, et al: Influence of hemolysis on routine clinical chemistry testing. *Clin Chem Lab Med* 2006; 44:311–316