Postoperative Heme Oxygenase Induction and Carbon Monoxide Production as a Novel Method to Assess Hepatic Regeneration and Predict Hepatic Related Morbidity After Partial Hepatectomy

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Protocol Summary

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Protocol Title:	Postoperative Heme Oxygenase Induction and Carbon Monoxide Production as a Novel Method to Assess Hepatic Regeneration and Predict Hepatic Related Morbidity After Partial Hepatectomy
Study Rationale:	The goal of this proposal is to elucidate the role of HO-1 induction in hepatic regeneration after partial hepatectomy (PH). There is a growing body of evidence that HO-1 induction through CO production has an important role in cellular protection and regeneration. To test this concept, we will monitor endogenous CO production in patients who undergo PH and analyze the relationship between CO production and hepatic regeneration. Using this approach we will test the following two hypotheses: (1) HO-1 induction after PH is proportional to the extent of the surgical resection, and (2) failure to appropriately induce HO-1 is associated with impaired hepatic regeneration.
Study Objectives:	Specific Aim 1: To monitor the extent of HO-1 induction (by measuring endogenous CO production) in relationship to the extent of hepatic resection 1.1 Monitor CO in exhaled air of patients before liver resection, at 4 hours and 24 hours after PH 1.2 Monitor carboxyhemoglobin(COHb) in the arterial blood gas of patients before liver resection at 4 hours and 24 hours after PH 1.3 Analyze the relationship between CO production and the extent of hepatic resection assessed by computed scans liver volumetric study at one and three months after PH, size and weight of the resected specimen and operative report. Specific Aim 2: To analyze the relationship between HO-1 induction and the quality of post-hepatectomy liver regeneration after PH

	2.1 Analyze the relationship between CO production and the quantity and quality of hepatic regeneration assessed by computed scans liver volumetric study at one and three months after PH and liver function tests after PH. 2.2 Determine the relation between postoperative liver related morbidity in patients undergoing PH and the extent of postoperative HO-1 induction
Study Design:	Study design: Aim 1.1: The monitoring of exhaled CO is based on previous report of assessing exhaled CO in septic patients. Exhaled CO will be determined with an infrared CO analyzer with a sensitivity of 0.1 ppm at 4 hours and 24 hours after PH Aim 1.2: Arterial blood gas (ABG) will be collected from patients before liver resection, at 4 hours and 24 hours after PH. The ABG will be obtained through an arterial line usually placed as part of perioperative monitoring of the patient hemodynamic. This model was found accurate in reflecting HO-1 induction in previous study in patient receiving liver transplantation. Aim 1.3: FLR will be calculated through liver volumetric study from a pre-resectional CT scan. The operative note will be checked for operative details regarding the number of resected hepatic segments and pathological examination of the specimen will allow accurate weight of the resected specimen. According to the resected amount of hepatic parenchyma, three groups of patients will be created (<25%, 25-50% and >50% PH) history of smoking and intraoperative blood transfusion will be reported for each patient. Procedures and Methods - Protocol: For collecting exhaled air specimen, each patient will be instructed to exhale completely, and then

	take a deep breath, hold it for 20 seconds, and breathe out through D shaped flatpak mouth piece attached to CO analyzer - Equipment: A Micro+ CO monitor (Bedfont Scientific Ltd, Wales, England) will be used to analyze CO level in a single breath in exhaled air. The device will be used by applying Anti-bacterial filter and one-way valve for increased infection control. The device has adjustable breath-hold countdown timer for patients who are unable to hold their breath. Calibration will be performed every 6 months as per manufacture recommendation. The device is able to detect and analyze CO concentration within a range of: 0-250 ppm with a sensitivity of 0.1 ppm and accuracy of ±2% in < 20 seconds. Direct evaluation of the COHb level will be performed using an arterial blood gas analyzer (ABL 800 Flex, Radiometer, Copenhagen) and will be reported as % of the total Hb as well as calculated amount in mg/mL. - Assessment of postoperative liver function and regeneration: Liver volumetry will be measured using image J software downloaded from http://rsbweb.nih.gov/ij/. The accuracy of this method was reported in a prior study. Volumetric study will be performed based on CT scan studies obtained as standard of care preoperatively before each liver resection and at one and three months after each resection. Collected data will be reported as percentage compared to the preoperative hepatic volume. Specific Aim 2: To analyze the relationship between HO-1 induction and the quality of post-hepatectomy liver regeneration

	2.1 Analyze the relationship between CO production and the quantity and quality of hepatic regeneration assessed by computed scans liver volumetric study at one and three months after PH and liver function tests. 2.2 Determine the relation between hepatic related morbidity in patients undergoing partial hepatectomy and their ability to induce HO-1 Study design: Aim 2.1: As CO is uniquely produced by HO reaction; its level was previously validated to check HO-1 induction. Checking this level and comparing outcome of the previously created three groups of patients (<25%, 25-50% and >50% PH) according to their ability to induce HO-1 after PH. Liver regeneration will be evaluated by radiological criteria at one and three months after PH as described above as well as laboratory criteria including liver function tests. Aim 2.2: HO-1 induction was found to offer beneficial protective effect in different clinical and surgical occasions. In this setting of PH, surgical complications in the perioperative (30 days) period graded by Clavien-Dindo Classification will be reported and analyzed regarding its relation with the ability to induce HO-1. Procedures and Methods: Liver function tests routinely checked on daily basis till normalization will be monitored. This includes serum protein, Albumin, liver enzymes (AST and ALT), alkaline phosphatase and bilirubin level. The duration needed to this normalization will be checked as well as the trend of each of these laboratory parameters. Hepatic morbidity will be evaluated using the criteria used by the international study group of liver surgery being normalization of the INR and bilirubin by POD#5 while the severity

	of post hepatectomy liver failure is graded based on its impact on clinical management. Grade A post hepatectomy liver failure requires no change of the patient's clinical management. The clinical management of patients with grade B post hepatectomy liver failure deviates from the regular course but does not require invasive therapy. The need for invasive treatment defines grade C post hepatectomy liver failure. The Clavien-Dindo classification will be used to document surgical outcome of patients after PH. Analyses: All results will be collected in Excel Document. Analyses of groups based on the extent of liver resection and CO production. Results of different subgroups will be analyzed; comparison between groups will be performed by fisher test, paired and unpaired student t test, main Whitney test or ANOVA with repeated measurements when appropriate. Significance is defined as $p<0.05$. Potential problems and alternative strategy: It is possible that HO-1 induction varies according to preoperative and intraoperative factors beside the patient own ability to induce HO-1. The most important known other factors are smoking and intraoperative blood transfusion will be reported in the study, however, other factors may exist and influence the result of this study. Preoperative factors may include preoperative use of chemotherapy and the presence of underlying liver cirrhosis. Intraoperative factors may include operative time and surgical technique (open or minimally invasive). In case of significance variance between patient s in the same group (same percentage of PH) Subgroup analysis including different variants (preoperative chemotherapy, perioperative

	blood transfusion and open versus minimally invasive technique) will be used to assess the effect of these factors.
Study Population:	125 participants enrolled locally; 125 enrolled worldwide
Inclusion Criteria:	1-Patients plans to have partial liver resection for primary liver pathology or metastatic disease 2-Patients able to comprehend and willing to sign the written consent form
Exclusion Criteria:	1-Patients with age less than 18 years 2-Patients identified as part of the vulnerable populations as defined in the current application, except women with child bearing potential with negative pregnancy test
Study Treatment:	For Aim 1, after the patient's procedure, they will have to exhale into the device listed for collecting exhaled air specimen. For Aim 2, the patient's blood samples that are routinely collected after a hepatectomy will be used for analyzing serum protein, Albumin, AST and ALT, alkaline phosphatase, and bilirubin level. The duration of an individual's participation will be about 3 months +/- 2 weeks.
Study Procedures and Assessments:	Aim 1: Procedures and Methods - Protocol: For collecting exhaled air specimen, each patient will be instructed to exhale completely, and then take a deep breath, hold it for 20 seconds, and breathe out through D shaped flatpak mouth piece attached to CO analyzer - Equipment: A Micro+ CO monitor (Bedfont Scientific Ltd, Wales, England) will be used to analyze CO level in a single breath in exhaled air. The device will be used by applying Anti-bacterial filter and one-way valve for increased infection control. The device has adjustable breath-hold

	countdown timer for patients who are unable to hold their breath. Calibration will be performed every 6 months as per manufacture recommendation. The device is able to detect and analyze CO concentration within a range of: 0-250 ppm with a sensitivity of 0.1 ppm and accuracy of ±2% in < 20 seconds. Direct evaluation of the COHb level will be performed using an arterial blood gas analyzer (ABL 800 Flex, Radiometer, Copenhagen) and will be reported as % of the total Hb as well as calculated amount in mg/mL. - Assessment of postoperative liver function and regeneration: Liver volumetry will be measured using image J software downloaded from http://rsbweb.nih.gov/ij/. The accuracy of this method was reported in a prior study. Volumetric study will be performed based on CT scan studies obtained as standard of care preoperatively before each liver resection and at one and three months after each resection. Collected data will be reported as percentage compared to the preoperative hepatic volume. Aim 2: Procedures and Methods: Liver function tests routinely checked on daily basis till normalization will be monitored. This includes serum protein, Albumin, liver enzymes (AST and ALT), alkaline phosphatase and bilirubin level. The duration needed to this normalization will be checked as well as the trend of each of these laboratory parameters. Hepatic morbidity will be evaluated using the criteria used by the international study group of liver surgery being normalization of the INR and bilirubin by POD#5 while the severity

	of post hepatectomy liver failure is graded based on its impact on clinical management. Grade A post hepatectomy liver failure requires no change of the patient's clinical management. The clinical management of patients with grade B post hepatectomy liver failure deviates from the regular course but does not require invasive therapy. The need for invasive treatment defines grade C post hepatectomy liver failure. The Clavien-Dindo classification will be used to document surgical outcome of patients after PH.
Study Endpoints:	Aim 1: -CO output/production based on extent of liver resection, to be collected at 4 hrs and 24 hrs after PH -CT scans looking at hepatic regeneration and one and three months after PH and liver function tests
Statistical Analyses:	Aim 1 Analyses: All results will be collected in Excel Document. Analyses of groups based on the extent of liver resection and CO production. Results of different subgroups will be analyzed; comparison between groups will be performed by fisher test, paired and unpaired student t test, main Whitney test or ANOVA with repeated measurements when appropriate. Significance is defined as $p<0.05$. Aim 2 Analyses: All results will be collected in Excel Document. Analyses of groups based on the extent of liver resection and CO production. Results of different subgroups will be analyzed; comparison between groups will be performed by fisher test, paired and unpaired student t test, main Whitney test or ANOVA with repeated measurements when appropriate. Significance is defined as $p<0.05$.
Study Duration:	15 years

1. INTRODUCTION

Inducible Heme Oxygenase (HO - 1), an enzyme critical for heme catabolism, is also involved in cellular response to oxidative stress. We have previously shown that induction of HO-1 expression in response to limb ischemia confers pulmonary cellular protection against oxidative stress suggesting a systemic factor; however, the mechanism of this protection is unclear. Our follow-up study showing that carbon monoxide (CO) the product of heme catabolism by HO-1, can mimic this protection suggests that CO itself may be the important active molecule. Recent animal work has independently shown that HO-1 induction may also influence cellular regeneration in the liver following partial hepatectomy. Independently, CO has emerged as growth-promoting signal molecule with anti-apoptotic effect that protects hepatocytes from hypoglycemia - induced cytotoxicity. Further, administration of CO significantly improved the survival of mice after initiation of fulminant hepatitis and enhances rapid and early hepatocytes proliferation after partial hepatectomy, when mice lacking functional HO-1 were unable to mount an appropriate regenerative response. Collectively, these data suggest that that the heme oxygenase - CO offers either direct or indirect protection against cellular injury and may promote regeneration in the liver. In this proposal, we will directly test the hypothesis that HO-1 induction is critical to hepatic regeneration after partial hepatectomy. We will further test the relevance of this observation in human patients undergoing partial hepatectomy by measuring CO production and correlating this to parameters of liver regeneration. To achieve objective, we will pursue two specific aims: (1) we will monitor the extent of HO-1 induction (by measuring endogenous CO production) in relationship to the extent of hepatic resection (2) we will assess the production of CO in patients following liver resection and correlate these findings to the rate of liver regeneration and liver function. This research will establish a framework for understanding the role of HO-1 induction in liver regeneration and will provide a foundation for developing more effective approaches in enhancing hepatic regeneration and preventing liver failure after partial hepatectomy.

2. STUDY OBJECTIVE

The goal of this proposal is to elucidate the role of HO-1 induction in hepatic regeneration after partial hepatectomy (PH). There is a growing body of evidence that HO-1 induction through CO production has an important role in cellular protection and regeneration. To test this concept, we will monitor endogenous CO production in patients who undergo PH and analyze the relationship between CO production and hepatic regeneration. Using this approach, we will test the following two hypotheses: (1) HO-1 induction after PH is proportional to the extent of the surgical resection, and (2) failure to appropriately induce HO-1 is associated with impaired hepatic regeneration.

3. INVESTIGATIONAL PLAN

This is a University of Maryland Medical System multi-site study being done at University of Maryland Medical Center and UM Baltimore Washington Medical Center.

The study will monitor carbon monoxide production in patients undergoing liver resection and aims to identify the relationship between CO production and recovery after this resection.

Carbon monoxide will be checked from arterial blood gas obtained routinely as a part of the postoperative care as well as from the exhaled air of the patient through a CO detector commercially available and used as smokerlyzer helping people to stop smoking. The results of the surgical resection will be collected from the patient routinely ordered tests after liver resection including pathology of the resected part of the liver, laboratory and radiology tests.

The study duration for each individual patient is approximately 3 months +/- 2 weeks.

4. STUDY TREATMENT

Subjects who met all inclusion and exclusion criteria according to the data collected from medical records will have their carbon monoxide checked from arterial blood gas obtained as part of routine post-operative care, as well as from exhaled air measured through a CO detector. This study involves prospective data collection that does no interfere with treatment plan of participants.

5. STUDY POPULATION

The populations to be included are as follows:

Gender: Female, Male

Ages: 18-88 years (adult)

Race/Ethnicity: All Races Included

Languages: English

No vulnerable populations are being targeted.

5.1 Inclusion Criteria

-Patient plans to have partial liver resection for primary liver pathology or metastatic disease

-Patients is able to comprehend and willing to sign the written consent form

5.2 Exclusion Criteria

-Patients with age less than 18 years

-Patients identified as part of the vulnerable populations as defined in the current application, except women with child bearing potential with negative pregnancy test

6. STUDY VISITS

Patients planned to undergo partial hepatectomy for primary or metastatic hepatic pathology will be screened for eligibility to participate in this study. Objectives, methods of data

collection, and analysis will be discussed. After answering all patient questions and explaining the patient rights including refusal to participate, the patient will be offered to participate in the study. Patients who will accept to participate will be asked to sign the participation consent form. It is a prospective data collection without interference with the treatment plan.

For Aim 1, CO will be monitored before a patient's liver resection, and at 4- and 24-hours post-resection.

For Aim 2, CT scans of liver volume will be performed at one and three months post-hepatectomy, as well as liver function tests PH.

6.1 Withdrawal of Participants:

The PI can remove a participant from the research study without the participants approval. Possible reasons include failure to follow instructions, or if the PI decides that the research study is no longer in the participant's best interest.

During the study period, the patient can ask to withdraw from the study without that decision interfering with his/her treatment plan.

For this purpose, a letter addressed to the PI or the provider in charge of follow up is needed. The Physician will inform the PI who will withdraw the patient information from the database.

If the study is terminated for any other reason, the PI will be entitled to send a notification letter to all participants.

The doctor/PI will speak to the participant about the withdrawal and the participant will have a chance to ask questions if this were to happen.

7. STUDY ASSESSMENTS

This study is a prospective data collection and thus poses no associated health risks.

7.1 Measuring CO by exhaled air using a smokerlyzer device pre-liver resection, and at 4- and 24-hours post resection

7.2 Measuring carboxyhemoglobin (COHb) in arterial blood gas pre-liver resection, and at 4 and 24 hours post resection

7.3 CT scan at 1 and 3 months post liver resection

8. STUDY ETHICAL CONSIDERATIONS

The investigator agrees that the study will be conducted according to Good Clinical Practice principles of the ICH E6 (R1), ISO 14155 and the principles of the World Medical Association Declaration of Helsinki 1964 (including all amendments and Notes of Clarification). The Investigator will conduct all aspects of this study in accordance with all national, state, and local laws or regulations.

9. ADMINISTRATIVE CONSIDERATIONS

All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain subject confidentiality. All records will be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the subject (or the subject's guardian), except as necessary for the FDA (or other international regulatory agency), or the IRB/IEC.

The Investigator and all employees and coworkers involved with this study may not disclose or use for any purpose other than performance of the study, any data, record, or other unpublished confidential information disclosed to those individuals for the purpose of the study.

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