PAPER

Hepatic Cryoablation–Induced Acute Lung Injury

Pulmonary Hemodynamic and Permeability Effects in a Sheep Model

William C. Chapman, MD; Jacob P. Debelak, MS; Timothy S. Blackwell, MD; Kim A. Gainer, BA; John W. Christman, MD; C. Wright Pinson, MD; Kenneth L. Brigham, MD; Richard E. Parker, PhD

Hypothesis: Hepatic cryoablation of 30% to 35% or more of liver parenchyma in a sheep model results in eicosanoid and nuclear factor–κB (NF-κB)–mediated changes in pulmonary hemodynamics and lung permeability.

Setting: Laboratory.

Interventions: At initial thoracotomy, catheters were placed in the main pulmonary artery, left atrium, right carotid artery, and efferent duct of the caudal mediastinal lymph node for subsequent monitoring in adult sheep. After a 1- to 2-week period of recovery, animals underwent laparotomy and left-lobe cryoablation (approximately 35% by volume) with subsequent awake monitoring and on postoperative days 1 to 3.

Main Outcome Measures: Cryoablation-induced lung permeability and hemodynamic changes were compared with baseline values in sheep that underwent instrumentation. Similarly handled sheep underwent resection of a similar volume of hepatic parenchyma or had pulmonary artery pressure increases induced by mechanical left atrial obstruction. Activation of NF-κB was assessed with electrophoretic mobility shift assay, and serum thromboxane levels were measured with mass spectroscopy.

Results: Cryoablation resulted in acutely increased mean pulmonary (20 to 35 cm water) and systemic pressures, which returned to baseline at 24 hours with no change in cardiac output. Serum thromboxane levels increased 30 minutes after cryoablation (9-fold) and returned to baseline at 24 hours. Activation of NF-κB was present in liver and lung tissue by 30 minutes after cryoablation. Lung lymph–plasma protein clearance markedly exceeded the expected increase from pulmonary pressures alone, and increased lymph–plasma protein ratio persisted after pulmonary artery pressures normalized. Similar changes were not associated with 35% hepatic resection.

Conclusions: This study demonstrates that 35% hepatic cryoablation results in an acute but transient increase in pulmonary artery pressure that may be mediated by increased thromboxane levels. Increases in pulmonary capillary permeability are not accounted for by pressure changes alone, and may be a result of NF-κB–mediated inflammatory mechanisms. These data show that cryosurgery causes pathophysiological changes similar to those observed with endotoxin and other systemic inflammatory stimuli.

Arch Surg. 2000;135:667-673

From the Divisions of Hepatobiliary Surgery and Liver Transplantation (Drs Chapman and Pinson and Mr Debelak) and Allergy, Pulmonary, and Critical Care Medicine (Drs Blackwell, Christman, Brigham, and Parker and Ms Gainer), Vanderbilt University Medical Center, Nashville, Tenn.

LIVER RESECTION remains the standard treatment for primary and selected metastatic tumors in the liver. Recently, hepatic cryoablation has been used to ablate tumors that are otherwise unresectable. This technique involves circulation of liquid nitrogen through metallic probes placed on the surface or into the center of the tumor. A cycle of rapid freezing followed by gradual thawing of the tumor and surrounding hepatic parenchyma induces cell death by a variety of mechanisms, including internal freezing, cell membrane rupture, solute-solvent shifts, and hypoxic cell death from small-vessel obliteration.[1-3] This therapy appears to result in tumor ablation and patient survival comparable with that of hepatic resection[4-10] in selected single-center series.

Interestingly, while cryoablation of small areas of the liver is usually well tolerated with minimal adverse effects, major complications have been noted when more than 30% to 35% of the liver volume has been treated with cryoablation.[1,2] Complications reported in this setting have included thrombocytopenia and disseminated intravascular coagulation, renal failure, hepatic failure, and adult respiratory distress syndrome (ARDS).[11] These complications are now well recognized and have been described as a *cryoshock phenomenon*,[12] although the responsible mechanisms for multisystem injury in this setting have not been well defined.

Kupffer cells within the liver represent the largest pool of mononuclear

MATERIALS AND METHODS

SURGICAL PROCEDURES

Adult yearling sheep (20-35 kg) underwent initial instrumentation at thoracotomy as previously described.[14] In brief, after induction of general anesthesia, catheters were placed in the main pulmonary artery, left atrium, right carotid artery, and efferent lymph duct of the caudal mediastinal lymph node and tunneled subcutaneously to the back for subsequent monitoring and access. Animals were allowed a 7- to 14-day period of recovery, during which they received food and water ad libitum.

On the day of planned intervention, a 2-hour baseline collection and monitoring period was established. During this time, continuous hemodynamic monitoring was performed with data acquisition via pressure transducers connected to a computer with data capture at 10-second intervals. Pulmonary lymph flow collections were performed at 15-minute intervals, with aliquots placed on ice and frozen for subsequent analysis. Blood samples were collected for blood gas analysis and serum protein determination as described below.

After baseline assessment, a midline laparotomy was performed with the animal under general anesthesia. Cryoablation (n = 5) was performed with a combination of trocar probe placement (8 mm) and surface ablation with a disk probe (Cryotech LC System 2000; Cryogenic Technology Ltd, Derbyshire, England) with careful isolation of adjacent structures, including the gastrointestinal tract, to avoid inadvertent organ injury. One freeze-thaw cycle of the left lobe was induced, encompassing approximately 35% (by volume) of the liver, with careful maintenance of normothermia during the freeze-thaw period. After completion of the freeze-thaw cycle, warmed isotonic sodium chloride solution (200 mL) was instilled into the abdominal cavity, and the peritoneum, fascia, and skin were closed in layers.

After the animal emerged from general anesthesia, monitoring was initiated and continued for 6 hours after cryoablation and for 4 hours on subsequent postoperative days 1 through 4. Arterial blood gas sampling and plasma, pulmonary, and lymph samples were collected for total protein measurements and thromboxane determinations as outlined below.

In separate studies, liver resection of a comparable portion of the left lobe (approximately 35% by volume) was performed in the sheep previously subjected to instrumentation. This was performed with the use of 0-chromic catgut sutures placed in a horizontal mattress fashion and with finger fracture and minimal blood loss. Postresection monitoring and lymph and plasma samples were collected for subsequent analysis, as in animals undergoing cryoablation.

To determine the relationship between pulmonary artery pressure and lung lymph flow in this model, similarly handled sheep underwent placement of a Foley balloon in the left atrium with sequential inflation at steady state. In these animals, increased left atrial pressure resulted in elevations of pulmonary arterial systolic, diastolic, and mean pressures and increased plasma clearance.

To investigate NF-κB activation in the sheep in response to cryoablation, separate animals underwent a similar ablation procedure (approximately 35% liver ablation, left lobe), with collection of tissue and blood samples at baseline and at 30 minutes and 1, 2, and 3 hours after cryoablation. Liver samples were taken from the nonablated tissue, freeze-clamped, and stored at −70°C for subsequent analysis. Peripheral lung tissue samples were collected in a similar fashion. Serum samples were collected at similar time points for assessment of thromboxane levels.

phagocytic cells in the body (80%- 90%) and are known to produce cytokine and eicosanoid mediators. It is possible that hepatic injury or dysfunction (with Kupffer cell activation) may play a primary role in systemic inflammatory response and the development of ARDS. Blackwell et al,[13] in a study from our laboratory, recently reported the induction of systemic inflammatory response and overproduction of nuclear factor–κB (NF-κB)–dependent cytokines and subsequent neutrophilic lung injury in a rat model of cryoablation. The current study was undertaken to investigate the pulmonary hemodynamic and permeability changes resulting from cryoablation with the use of a model of capillary permeability changes that used unanesthetized sheep subjected to long-term instrumentation.

RESULTS

Sheep subjected to instrumentation were able to undergo cryoablation, with 1 postcryoablation death in this group (20% mortality). This single animal was noted to have hyperventilation and failed to fully recover from the operative procedure. At autopsy, no evidence of intra-abdominal hemorrhage, liver fracture, or other technical complication was noted. The remaining animals were able to tolerate the instrumentation and cryoablation procedure without notable complications.

Hepatic cryoablation resulted in transiently increased pulmonary artery pressures, which returned to baseline within 24 hours of the cryoablation (**Figure 1**). There was a transient increase in systemic mean pressures that normalized by 24 hours after the cryoablation procedure (**Figure 2**). There were no significant changes in pulmonary or systemic arterial pressures after hepatic resection (data not shown). Arterial blood gas assessment demonstrated a significant decrease in Pa_{O_2} in the immediate postcryoablation period (**Figure 3**).

Hepatic cryoablation was associated with markedly increased lung microvascular lymph flow (**Figure 4**) immediately after cryoablation that persisted for 48 hours after this procedure. There was a significant increase in lung lymph–plasma protein clearance after cryoablation (**Figure 5**).

Analysis of normalized lung lymph flow rates and plasma clearance (**Figure 6**) demonstrated a marked increase in pulmonary lung lymph–plasma protein ratios, which were not accounted for by increased vascular pressures alone.

Plasma thromboxane levels were significantly elevated immediately after cryoablation (**Figure 7**) but

LUNG LYMPH AND SERUM PROTEIN DETERMINATIONS

Total protein concentrations were measured via a temperature-compensated refractometer (American Optical Corp, Keene, NH), specially calibrated for measurement of sheep plasma and lung lymph.

NUCLEAR PROTEIN EXTRACTION

Nuclear protein extracts were prepared from liver and lung tissue by the method of Deryckere and Gannon,[15] with protein content determined by the method of Bradford,[16] as we have previously reported.[13]

ASSAYS

Electrophoretic mobility shift assays for NF-κB were performed as previously reported by Blackwell and Christman.[17]

Thromboxane B_2 levels were determined by methods previously described.[18,19] After isolation of serum or plasma, a standard 11-dehydrothromboxane B_2–D_4 (Cayman Chemical, Ann Arbor, Mich) was prepared by adding 100 µL of water and 2 µL of 11-dehydrothromboxane B_2–D_4 (1 ng/µL) to a microcentrifuge tube. For the serum samples, 100 µL was added to the 100 µL of water, and for the plasma samples, 200 µL was added. Each sample and standard was allowed to come to room temperature for 30 minutes, and then a drop of 10% formic acid and 1 mL of ethyl acetate were added to each tube and vortexed. The standard and samples were centrifuged and the top layer was transferred to a reactive vial. Each tube was placed under a stream of dry (anhydrous) nitrogen to evaporate the solvent and recover or crystallize out the compounds dissolved; then 100 µL of 0.5% methoxyamine in pyridine was added to both samples and standard. These were allowed to incubate overnight at room temperature or for 1 hour at 40°C. After the incubation period, the tubes were again dried under nitrogen. Once dried, the pentafluorobenzyl ester was formed with 40 µL of 10% pentafluorobenzyl bromide and 20 µL diisopropylethylamine, and the samples and standards were allowed to stand in a 37°C water bath for 20 minutes. After the incubation period, the samples and standards were dried under nitrogen. The samples and standards were redissolved with 40 µL of methanol, loaded into a silica gel thin-layer chromatography plate, and developed in a mobile phase with the use of a 98% ethyl acetate, 2% methanol solvent system. The corresponding area around the standard (double bands at approximately 70 mm) was scraped and extracted with 1 mL of ethyl acetate. The samples were also extracted from the silica by eluting with 1 mL of ethyl acetate. After the extraction, the samples and standard were reacted with 20 µL *N*,*O*-bis-trimethylsilyl-trifluoroacetamide and 10 µL of *N*,*N*-dimethylformamide, and they were allowed to stand at room temperature for 1 hour. Derivatization was completed by formation of the trimethylsilylether derivative. The silation mixture was evaporated and 20 µL n-undecane was added to each tube to redissolve the samples and standards. The 11-dehydrothromboxane B_2–D_4 in each sample was quantitated by gas chromatograph interfaced with mass spectrophotometer in negative ion–chemical ionization mode monitoring ions 614 and 618 for endogenous thromboxane B_2 and the tetradeuterated internal standard.

STATISTICAL ANALYSIS

Data are expressed as mean ± SE. Comparisons were made in sheep undergoing cryoablation (baseline vs postcryoablation measures) by paired *t* test or 1-way analysis of variance with Student-Newman-Keuls test for differences between multiple time points. We assumed significance for *P* values $<.05$.

returned to baseline by 24 hours after the procedure. Similar increases were not observed after liver resection.

Hepatic cryoablation induced significant NF-κB activation in this model (**Figure 8**). Minimal basal NF-κB activation was detected in liver and lung; however, there was significant activation of NF-κB in nuclear extracts from sheep liver and lung tissues at 30 minutes, 1 hour, and 2 hours after 35% hepatic cryoablation (Figure 8). Activation of NF-κB was most prominent in liver at each time point. The specificity of detected binding on electrophoretic mobility shift assay is shown by cold and nonspecific competition. The addition of 50 ng of specific NF-κB oligonucleotide markedly diminished the NF-κB bands (cold competition), while the addition of 50 ng of unlabeled oligonucleotide that did not contain the NF-κB motif (nonspecific competition) did not affect NF-κB binding. These findings authenticate the reliability of the bands identified as NF-κB on electrophoretic mobility shift assay.

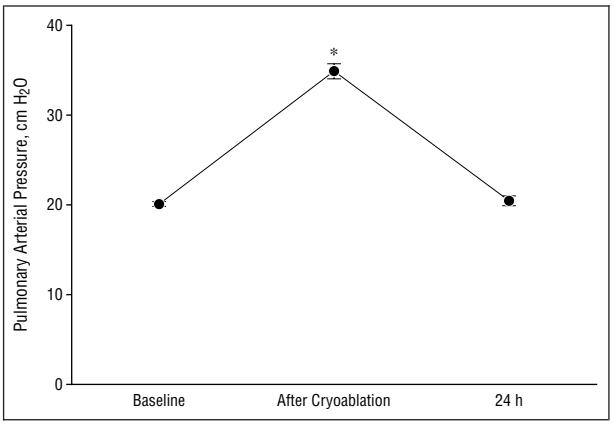

Figure 1. *Mean pulmonary artery pressure before and after hepatic cryoablation. Cryoablation transiently increased pulmonary artery pressures, with return to baseline by 24 hours and beyond. Asterisk indicates* $P<.05$.

COMMENT

Cryotherapy of hepatic tumors is performed by inducing a cycle of rapid freezing followed by gradual thawing of the tumor and surrounding hepatic parenchyma that produces tissue infarction and tumor involution. This technique is thought to induce cell death by a variety of mechanisms.[2,3] Interestingly, while cryoablation of small areas of the liver is usually well tolerated with few adverse effects, major complications have been noted when larger volumes (≥35% of total liver volume) have been treated.[3,6,11] Major pulmonary complications, including

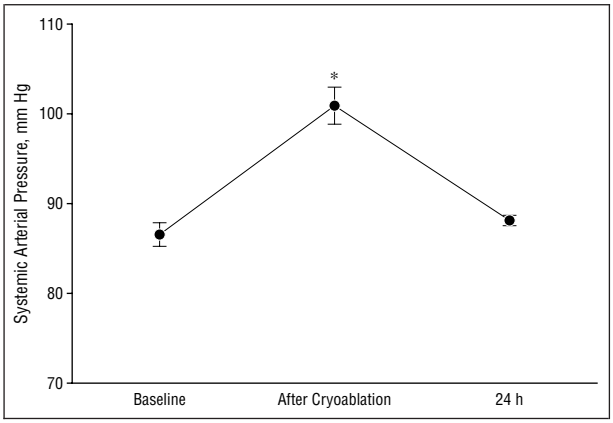

Figure 2. *Mean systemic arterial pressure before and after hepatic cryoablation. Cryoablation transiently increased systemic pressures, with return to baseline by 24 hours and beyond. Asterisk indicates* P<.05.

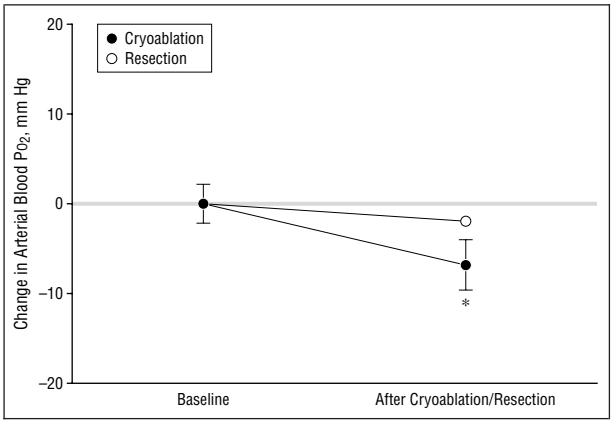

Figure 3. *Change in arterial blood Po_2 after hepatic cryoablation or resection. There was a decrease in Pao_2 values immediately after cryoablation. Asterisk indicates* P<.05.

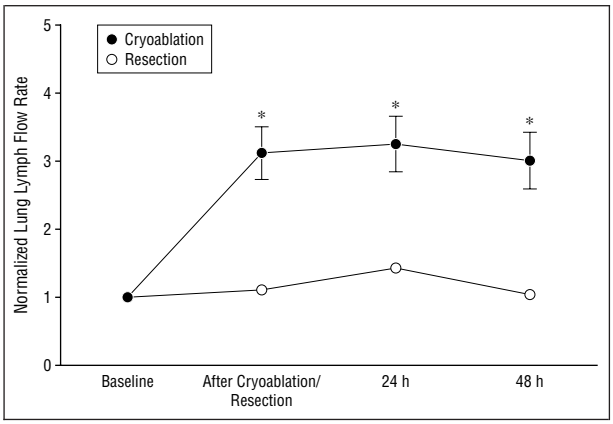

Figure 4. *Effects of hepatic cryoablation or resection on lung lymph flow rate. Cryoablation induced an immediate increase in lung lymph flow rates that persisted for 48 hours. Asterisks indicate* P<.05.

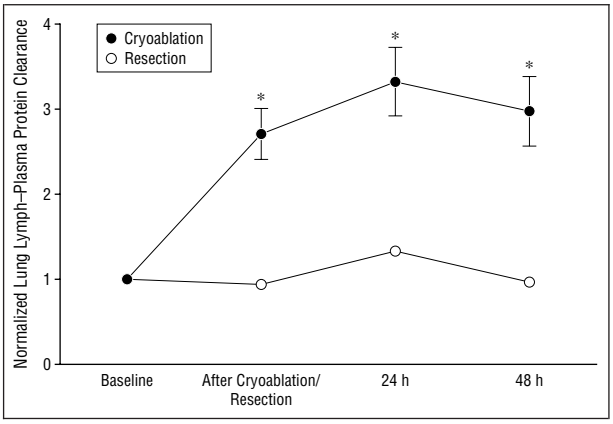

Figure 5. *Effects of hepatic cryoablation or resection on lung lymph–plasma protein clearance. Cryoablation was associated with a significantly increased lymphatic plasma protein clearance that persisted for 48 hours. Asterisks indicate* P<.05.

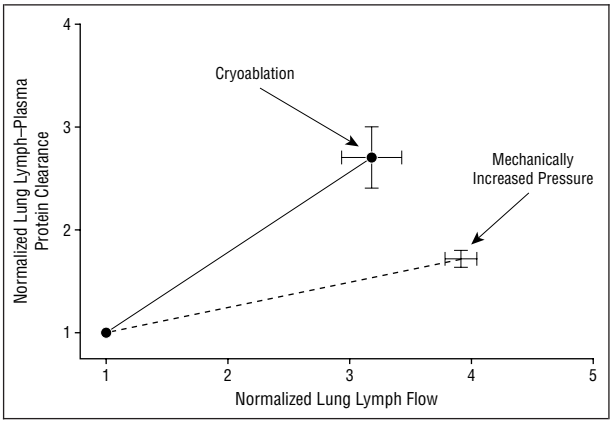

Figure 6. *Comparison of plasma protein clearance in sheep with mechanically increased pulmonary pressure and animals undergoing hepatic cryoablation. Hepatic cryoablation appears to increase lung microvascular permeability to a greater degree than occurs with increased pressure changes alone.*

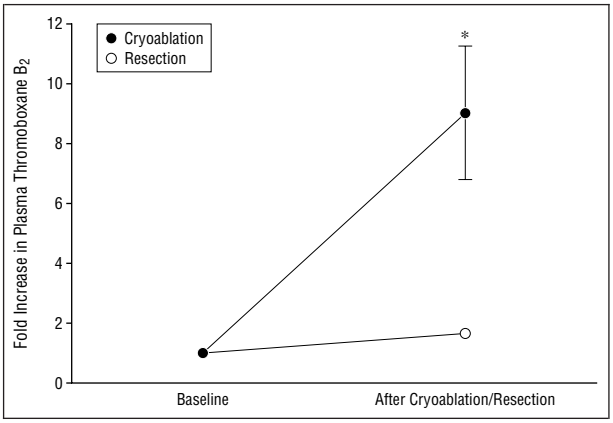

Figure 7. *Effects of hepatic cryoablation or resection on plasma thromboxane B_2 levels. Cryoablation resulted in significantly elevated plasma thromboxane levels immediately after the procedure, with a return to a baseline by 24 hours. Similar changes were not associated with hepatic resection. Asterisk indicates* P<.05.

the development of ARDS, have been noted after hepatic cryoablation[11] but have not received substantial attention and the mechanisms have not been well defined. Previous data from our laboratory[13,20,21] have shown that hepatic injury in a rat model induces gene expression of the cytokines tumor necrosis factor α and macrophage inflammatory protein-2, and this cytokine activation is associated with NF-κB activation in both the liver and lung. Furthermore, our animal model of acute liver injury is associated with severe neutrophilic lung injury. A recent report by Seifert et al[22] confirmed the re-

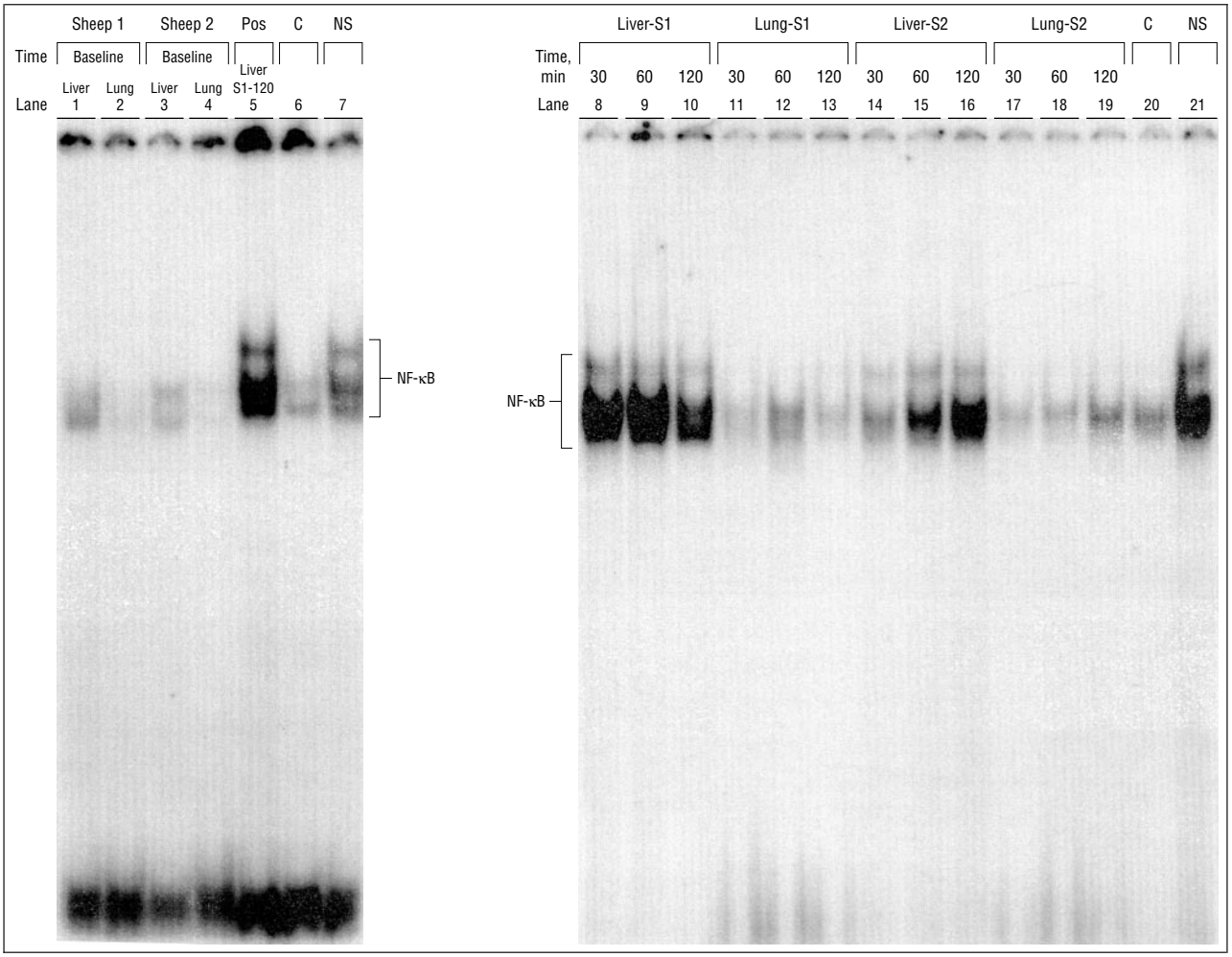

Figure 8. *Electrophoretic mobility shift assay shows the time course of activation of nuclear factor–κB (NF-κB) in nuclear extracts from sheep tissue after 35% hepatic cryoablation. Tissue samples from 2 sheep are shown at baseline (left) and at 30 minutes, 1 hour, and 2 hours after cryoablation (right). Minimal activation is present in the liver or lung at baseline. However, significant activation of NF-κB is present by 30 minutes in the liver and to a lesser degree in the lung, and remains activated at the 1- and 2-hour time points. Lane 5 represents a positive (Pos) result from the liver at 2 hours after cryoablation. In lanes 6 and 20, addition of 50 ng of unlabeled, specific NF-κB oligonucleotide markedly diminished all 3 bands (cold competition [C]). The addition of 50 ng of unlabeled oligonucleotide that does not contain an NF-κB motif (nonspecific competition [NS]) did not affect NF-κB binding (lanes 7 and 21).*

lease of tumor necrosis factor α and interleukin 6 after hepatic cryoablation in their rat model.

In the current study of cryoablation in the instrumented sheep model, hepatic cryoablation resulted in transiently increased pulmonary artery pressures that returned to baseline within 24 hours of the cryoablation. There was also a transient increase in systemic mean arterial pressures that normalized by 24 hours after the cryoablation procedure. Arterial blood gas assessment demonstrated a slight decrease in P_{O_2}. These hemodynamic effects were transient and did not appear to be associated with major adverse effects.

Of interest, however, hepatic cryoablation was associated with markedly increased lung microvascular lymph flow (Figure 4) and a significant increase in lung lymph–plasma protein clearance immediately after cryoablation that persisted for 48 hours after this procedure. Analysis of normalized lung lymph flow rates and plasma clearance (Figure 6) demonstrated a significant increase in pulmonary lung lymph–plasma protein ratios, which were not accounted for by increased vascular pressures alone. While increases in pulmonary artery pressures can increase lung lymph–plasma protein clearance, the early increases in pulmonary pressures alone were insufficient to account for the increased clearance that we observed in this study. Furthermore, pulmonary pressures returned to baseline within 24 hours, yet the increased lung lymph–protein clearance persisted for 48 hours after cryoablation. The reasons for the apparent increased capillary permeability in this model are unknown, but we speculate that this may be related to acute lung inflammation mediated in part by NF-κB–dependent cytokine up-regulation.

The hemodynamic and permeability responses of sheep to liver cryoablation are similar to that during endotoxemia.[14] Both have initial pulmonary arterial hypertension (with concomitant increases in circulating thomboxane B_2 levels) and increased pulmonary microvascular permeability as indicated by large increases in protein-rich lung lymph flow rate. One interesting difference was the prolonged (>48 hours) apparent increased permeability phase after liver cryoablation. Normally, endotoxin-induced increased lung microvascular permeability returns to normal values 12 to 18

hours after the endotoxin challenge. It is possible that the prolonged permeability phase is related to the severely injured or necrotic liver tissue that is left in place after cryoablation.

While the mechanism for increased complications after cryoablation of 35% or more of liver volume has not been fully characterized, it is likely not caused by direct parenchymal loss, since up to 70% of hepatic parenchyma can be safely resected without risks of liver failure.[23,24] A newer ablative modality, radiofrequency ablation, has not been associated with multisystem injury in clinical studies.[25,26] Radiofrequency ablation is currently limited to small-diameter (3-cm maximum) ablation zones because of technological limitations, so it remains unclear whether the lack of systemic injury is related to small ablation volumes or to a differing effect of a "heat-kill" technique compared with the "freeze-kill" method of cryoablation.

The true incidence of multisystem injury after cryoablation is unknown and difficult to quantitate precisely given the heterogeneous nature of patients being treated, differing tumor types, surgeon experience, and use with other therapies, including surgical resection and radiofrequency ablation. In an attempt to more accurately assess this phenomenon, Seifert and Morris[12] conducted a survey of 299 worldwide hepatobiliary centers performing cryoablation. Forty-five percent of the centers responded and reported an observation of severe multisystem injury in 21 (1.0%) of 2173 patients treated with cryoablation. Although infrequent, the cryoshock phenomenon accounted for 6 (18%) of the 33 deaths in this report. While there are limitations to the validity of survey data such as this report, the study does document the recognized problem of multisystem injury after cryoablation and confirms the need to avoid large-volume cryoablation whenever possible.

Freezing small portions of liver has been shown, in certain circumstances, to yield results equivalent to those of resection, and complications in this setting appear to be minimal. For larger-volume ablations, however, it may not be surprising that the presence of once frozen, then necrotic tissue in the body would elicit a marked physiological response. We have identified 2 factors, thromboxane production and NF-κB activation, that are up-regulated after cryosurgery and may mediate the downstream pathophysiological effects. Future studies will investigate whether inhibition of the production of NF-κB–dependent inflammatory mediators can attenuate the lung injury observed after cryoablation.

This study was supported in part by the US Department of Veterans Affairs, Washington, DC, through Merit Funding and by grant R01 HL63204-01 from the National Institutes of Health, Bethesda, Md.

Presented at the 107th Scientific Session of the Western Surgical Association, Santa Fe, NM, November 15, 1999.

Reprints: William C. Chapman, MD, Department of Hepatobiliary Surgery and Liver Transplantation, Vanderbilt University Medical Center, 801 Oxford House, Nashville, TN 37232-4753 (e-mail: will.chapman@surgery.mc.vanderbilt.edu).

REFERENCES

1. Onik GM, Atkinson D, Zemel R, Weaver ML. Cryosurgery of liver cancer. *Semin Surg Oncol.* 1993;9:309-317.
2. Stewart GJ, Preketes A, Horton M, Ross WB, Morris DL. Hepatic cryotherapy: double-freeze cycles achieve greater hepatocellular injury in man. *Cryobiology.* 1995;32:215-219.
3. Brown NJ, Gbjayjoo P, Reed MWR. Effect of cryosurgery on liver blood flow. *Br J Cancer.* 1993;68:10-12.
4. Kane RA. Ultrasound-guided hepatic cryosurgery for tumor ablation. *Semin Interv Radiol.* 1993;10:132-142.
5. Ravikumar TS, Kane R, Cady B, Jenkins R, et al. A 5-year study of cryosurgery in the treatment of liver tumors. *Arch Surg.* 1991;126:1520-1524.
6. Horton MD, Warlters AV, Dilley AV, Clingan PR, Morris DL. Survival after hepatic cryotherapy for hepatic metastases [abstract]. *Br J Surg.* 1992;79:A452.
7. Weaver ML, Atkinson DP, Zemel R. Hepatic cryosurgery in treating colorectal metastases. *Cancer.* 1995;76:210-214.
8. Shafir M, Shapiro R, Sung M, et al. Cryoablation of unresectable malignant liver tumors. *Am J Surg.* 1996;171:27-31.
9. Seifert JK, Morris DL. Prognostic factors after cryotherapy for hepatic metastases from colorectal cancer. *Ann Surg.* 1998;228:201-208.
10. Korpan NN. Hepatic cryosurgery for liver metastases: long-term follow-up. *Ann Surg.* 1997;225:193-201.
11. Haddad FF, Chapman WC, Blair TK, Wright JK, Pinson CW. Clinical experience with cryosurgery for advanced hepatobiliary tumors. *J Surg Res.* 1998;75:103-108.
12. Seifert JK, Morris DL. World survey on the complications of hepatic and prostate cryotherapy. *World J Surg.* 1999;23:109-114.
13. Blackwell TS, Debelak JP, Venkatakrishnan A, et al. Acute lung injury after hepatic cryoablation: correlation with NF-κB activation and cytokine production. *Surgery.* 1999;126:518-526.
14. Parker RE. Sheep as an animal model of endotoxemia-induced diffuse lung injury. In: Cantor J, ed. *CRC Handbook of Animal Models of Pulmonary Disease.* Vol. 1. Boca Raton, Fla: CRC Press LLC; 1989:31-45.
15. Deryckere F, Gannon F. A one-hour minipreparation technique for extraction of DNA-binding proteins from animal tissues. *Biotechniques.* 1994;16:405.
16. Bradford M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* 1976; 72:248-254.
17. Blackwell TS, Christman JW. The role of nuclear factor-κB in cytokine gene regulation. *Am J Respir Cell Mol Biol.* 1997;17:3-9.
18. Catella F, Healy D, Lawson JA, Fitzgerald GA. 11-Dehydrothrombozane B2: a quantitative index of thromboxane A2 formation in the human circulation. *Proc Natl Acad Sci U S A.* 1986;83:5861-5865.
19. Fitzgerald GA, Lawson J, Blair IA, Brash AR. Analysis of urinary metabolites of thromboxane and prostacyline by negative-ion chemical-ionization gas chromatography/mass spectrometry. *Adv Prostaglandin Thromboxane Leukot Res.* 1985;15:87-90.
20. Debelak J, Washington K, Blackwell T, et al. Pulmonary histopathologic findings associated with hepatic cryoablation: role of NF-κB dependent cytokine production and severe lung injury [abstract]. *Gastroenterology.* 1999;116:A1202.
21. Debelak JP, Blackwell TS, Venkatakrishnan A, et al. Attenuation of NKκB dependent cytokine activation following hepatic cryoablation [abstract]. *Hepatology.* 1998;28:254A.
22. Seifert JK, Finlay I, Armstrong N, Morris DL. Thrombocytopenia and cytokine release following hepatic cryosurgery [abstract]. *Aust N Z J Surg.* 1998;68:526.
23. Bradley AL, Chapman WC, Wright JK, et al. Surgical experience with hepatic colorectal metastasis. *Am Surg.* 1999;65:560-567.
24. Fong Y, Blumgart LH. Surgical options in the treatment of hepatic metastases from colorectal cancer. *Curr Probl Surg.* 1995;32:333-421.
25. Curley SA, Izzo F, Delrio P, et al. Radiofrequency ablation of unresectable primary and metastatic hepatic malignancies: results in 123 patients. *Ann Surg.* 1999; 230:1-11.
26. Siperstein AE, Rogers SJ, Hansen PD, Gitomirsky A. Laparoscopic thermal ablation of hepatic neuroendocrine tumor metastases. *Surgery.* 1997;122:1147-1155.

DISCUSSION

H. Gill Cryer, MD, Los Angeles, Calif: Dr Chapman and his colleagues designed an experiment to determine the mechanism of the development of major pulmonary complications and ARDS in patients undergoing large tumor cryoablation similar to the patient that Dr Bilchek recently told us should not be repeated. The reason is that these patients developed a severe systemic inflammatory response syndrome, ARDS, and multiple organ failure. In this experiment Dr Chapman and his colleagues have shown that a 35% liver cryoablation in sheep results in a pulmonary capillary leak that persists for 48 hours and is associated with an increase in plasma thromboxane levels and increased expression of the proinflammatory transcription factor NF-κB in both the liver and lung. These

results are consistent with a systemic inflammatory response. However, the overall clinical insult to these sheep was relatively mild. There was no hyperdynamic cardiac response. There was only a mild decrease in P_{O_2}, and, at least from reading the manuscript and listening to the presentation, it did not seem that these sheep really developed a severe pulmonary injury. Did this model really result in a pulmonary inflammatory insult? Do you have histopathologic evidence such as PMN [neutrophil] infiltration or alveolar injury?

In trauma patients who have retained injured liver, which is similar to this experimental model, the liver injury represents an increased risk factor for the development of ARDS and multiple organ failure. In some trauma patients, the pulmonary injury seems to be a graded response that is mild in some patients and devastating in others, but begins very early after the injury. Do you believe that your model represents a mild form of ARDS beginning early after injury?

In other trauma patients, ARDS seems to develop a little bit later. These patients have been shown to have circulating PMNs that are primed to have an exaggerated activation response to a second stimulus. Do you think that perhaps in this model the reason that your sheep did not have a major pulmonary injury is that the initial injury resulted in a priming state of the neutrophils without activation? Do you have any evidence of the primed state of those neutrophils?

The importance of this study is that these animals did not develop a severe pulmonary injury when they should have. How can we duplicate that in our patients? It's really quite an interesting phenomenon.

Are there any agents that you think might ablate the increase in pulmonary capillary leak, such as pentoxifylline or lisofylline?

Lazar J. Greenfield, MD, Ann Arbor, Mich: It wasn't clear whether there was actually an increase in pulmonary vascular resistance in these animals. In other words, what happened to the cardiac output? Second, the hypothesis is based on a simulation of the destruction of tumor tissue, and since you were destroying normal liver tissue, do you think you really achieved a comparable injury? One of the potential differences would be the fact that there are inflammatory cells associated with tumor infiltration, and their destruction could very well produce some of the same effects that you measured.

Finally, a lot of the changes that you show could be induced by a simple infusion of thrombin. I wonder if there were any coagulation studies in these animals and also would echo the concern about the histology and whether or not there were any demonstrated inflammatory changes.

Ernest E. Moore, MD, Denver, Colo: As suggested by Dr Cryer, this may have profound implications that apply to other patient groups. If indeed the mechanism parallels the common phenomenon of ARDS following multiple inciting events, then you should be able to document neutrophil infiltration, up-regulation of ICAM-1 [intercellular adhesion molecule-1], lavage IL-8, etc, in the lungs. Moreover, if this mechanism is confirmed, you may have an opportunity to arrest the process by using preablation steroids or perhaps a small bolus of hypertonic saline to nullify the inflammatory process. Additionally, the elevation of thromboxane is reminiscent of models employing infrarenal aortic ischemia/reperfusion (I/R). In that regard, have you attempted vascular isolation of the liver prior to ablation to see if I/R is a contributing mechanism that may augment the lung injury?

Steven C. Stain, MD, Pasadena, Calif: In the manuscript you noted the Kupffer cells in the liver to be the largest source of macrophages in the body. If you have a nonhepatic insult, ie, a burn injury, pancreatitis, are the Kuppfer cells also activated as they are in NF-κB activation in the hepatic insult to the liver?

Dr Chapman: In response to several of the questions that were raised today about the true inflammation and presence of neutrophilic lung injury in this model, we have not and did not in this study look at neutrophilic lung infiltration. Now, because this is a chronic instrumented model, those animals are past their phase of changes that we observed by the time they are sacrificed, so we didn't have tissue samples out at that stage. However, we looked at this and reported in the journal *Surgery* just a couple of months ago our experience with a similar model in a rat model with 35% cryoablation in which we do see and do demonstrate a severe neutrophilic lung injury in that setting.

Why are there less serious ongoing manifestations of injury in this model? Several reviewers, Dr Greenfield and Dr Cryer, raised this question. One of our animals, I didn't mention it in the presentation, did die in the postcryo period while it was undergoing management and assessment. We didn't demonstrate any technical problem in that animal. These animals in some ways do demonstrate less severe manifestations of lung injury than one might expect in this model. I can't answer the question as to exactly why more severe manifestations weren't noted in these animals.

A question was raised about pulmonary vascular resistance. Cardiac output was not altered in these animals at any point during their postcryo evaluation phase.

Dr Moore raised a question about this model and as a model perhaps for other investigations of multisystem injury, particularly where hepatic injury may be a factor. We certainly think this model does have some application in other settings. We have looked at the use of TNF-α polyclonal antibody therapy in a rat model and additionally have looked at the induction of endotoxin tolerance in that setting and have shown a decrease in the lung inflammation with pretreatment prior to cryoablation. We have not conducted any intervention strategies in this particular sheep model, but those are studies that we do have planned for future investigations.

Dr Stain raised a question about the role of Kupffer cell as a large site of fixed macrophages in the body. Your point is one that the liver may play a significant role in many other conditions that result in multisystem injury, and so from that standpoint this model may be useful in other settings as well.