

Electrolytic liver ablation is not associated with evidence of a systemic inflammatory response syndrome

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Background: Local ablation has been proposed for treatment of liver tumours. Cryoshock, a variant of the systemic inflammatory response syndrome (SIRS), is a potentially fatal complication of cryoablation caused by systemic release of necrotic breakdown products from ablated liver. The proinflammatory cytokines tissue necrosis factor (TNF) α and interleukin (IL) 1 are important mediators of this response. This study assessed the risk of SIRS complicating electrolytic liver ablation by measuring circulating levels of inflammatory cytokines, other inflammatory markers and clinical markers of organ function.

Methods: Electrolytic liver ablation was performed in 16 pigs and four pigs served as controls. Platelet count, and serum levels of urea, creatinine, liver enzymes, C-reactive protein (CRP), TNF- α and IL-1 β were measured before treatment and for 72 h after the procedure.

Results: There were significant dose-related increases in CRP and alanine aminotransferase levels with liver electrolysis. There was no significant derangement in renal function or platelet count following ablation. A rise in serum TNF- α and IL-1 β levels was not associated with liver electrolysis.

Conclusion: There was no evidence of organ failure or significantly raised levels of proinflammatory cytokines as a result of liver electrolysis, suggesting that this is a safe procedure for liver ablation.

Paper accepted 22 September 2003

Published online 18 December 2003 in Wiley InterScience (www.bjs.co.uk). DOI: 10.1002/bjs.4400

Introduction

A number of local ablative methods have been proposed for the treatment of irresectable primary and secondary liver cancers¹. Cryotherapy and radiofrequency ablation (RFA) have produced promising results, although complications from overtreatment and undertreatment have been reported. These include incomplete ablation in areas adjacent to blood vessels owing to the temperature modulation effect of blood flow², haemorrhage as a result of damage to major blood vessels³ and cryoshock⁴.

Cryoshock may occur after cryoablation of liver; it is a syndrome characterized by multiple organ failure, thrombocytopenia and severe coagulopathy⁴. In clinical practice, the cryoshock syndrome occurs after 1 per cent of hepatic cryoablations, and is associated with a mortality rate of 35 per cent⁴. Experimental⁵ and clinical⁶ studies have identified raised levels of the cytokines interleukin (IL) 6 and tumour necrosis factor (TNF) α following hepatic cryotherapy. Postoperative plasma concentrations of IL-6 and TNF- α were significantly associated with the duration of hepatic freezing and ablation volume⁶. IL-6 and TNF- α ,

along with IL-1, are important mediators of the systemic inflammatory response syndrome (SIRS) and it is likely that cryoshock represents a variant of this syndrome.

Electrolytic ablation has recently been proposed as an alternative to thermal ablation for the treatment of colorectal liver metastases⁷. Electrolytic ablation is a non-thermal treatment that involves passing low-level direct current between electrodes inserted into the tissue. The resulting decomposition of interstitial fluid leads to the production of cytotoxic products, such as hydroxide ions and chlorine gas, and in localized changes in tissue pH. The dose of electrolysis is a unit of charge (coulombs), and is the product of the level of current and the time over which the current is delivered. Previous studies have shown that liver electrolysis results in localized tissue necrosis, the volume of which is proportional to the dose delivered⁸.

Experimental studies have identified possible safety advantages of electrolytic ablation adjacent to major blood vessels, with reports of complete ablation up to blood vessel walls without damage to vessels⁸. There are no reports of a SIRS-like phenomenon complicating electrolysis despite

its widespread clinical use in China for the treatment of a wide variety of malignancies⁹. The authors' initial clinical experience in a small group of patients suggested that morbidity was minimal following liver electrolysis¹⁰.

This study investigated the risk of a cryoshock-like phenomenon complicating liver electrolysis by measuring levels of cytokine mediators of SIRS as well as clinical markers of organ function.

Materials and methods

Approval was obtained from the Animal Ethics Committees of the North Western Adelaide Health Service, University of Adelaide and the Institute of Medical and Veterinary Sciences. Twenty healthy juvenile female specific pathogen-free White pigs, each weighing approximately 30 kg, were used. All pigs underwent general anaesthesia and laparotomy. Four 6-Fr electrolysis catheters (Cordis Webster, Diamond Bar, California, USA) were inserted into the left paramedian lobe of the liver at a spacing of 18 mm. Each catheter had two active 4-mm platinum electrodes 18 mm apart, which were assigned as anode and cathode such that adjacent electrodes were of opposite polarity. The electrodes were connected to a direct current generator (ECU 100; Söring GmbH Medizintechnik, Quickborn, Germany) capable of delivering a preset charge at a constant current of 200 mA. Pigs were randomly assigned to receive doses of 0, 400, 600, 800 or 1000 coulombs (C); there were four pigs in each electrolysis group and four control pigs. Treatment times ranged from 35 min for 400 C up to 90 min for 1000 C; controls had electrodes inserted for 35 min but no current was passed.

Immediately before laparotomy the right femoral vein was cannulated with a PVC line via a small groin incision. This line was then tunnelled subcutaneously and fixed on the pig's back. The line was used to collect blood before laparotomy, at the completion of the operation, and then at 1, 4, 24, 48 and 72 h after surgery. Oxygen saturation, end-tidal carbon dioxide, respiratory rate and heart rate were monitored during the operation. Pig behaviour, appetite, thirst, bowel habit and urine output were all observed before and after the procedure on a daily basis. Pigs were killed 3 days after surgery and the liver was harvested. The abdominal and thoracic cavities were examined to determine the presence of any pathology.

Blood samples were sent to a clinical pathology laboratory (Institute of Medical and Veterinary Science, Adelaide, South Australia, Australia) for measurement of platelet count, and serum levels of electrolytes, urea, creatinine, liver enzymes and C-reactive protein (CRP). Additional blood samples were centrifuged and serum

was stored at -80°C for later batch cytokine assays. Serum IL-1 β (the circulating form of IL-1) and TNF- α concentrations were measured by means of solid-phase sandwich enzyme-linked immunosorbent assays, using kits specific for swine IL-1 β and TNF- α (BioSource International, Camarillo, California, USA).

Three-dimensional measurements of ablation lesion length, width and height were recorded from the pathological specimens using a Vernier calliper device. Volumes of necrosis were calculated using a formula for volume of an ellipse ($V = \frac{4}{3}\pi \times r_1 \times r_2 \times r_3$).

Statistical analysis was performed using a repeated measures analysis (SPSS[®] version 11.0; SPSS, Chicago, Illinois, USA). Preoperative measurements were compared with measurements at each time interval after surgery. Controls were compared with each electrolysis group individually and all groups combined. The interactions of dose and time, and of treatment and time were assessed for each variable. $P < 0.050$ was considered significant for comparison of single time points. For multiple simultaneous comparisons the Bonferroni t statistic was used; for $\alpha = 0.05$, a significance level of α/k was set, where k is the number of simultaneous tests.

Results

Laparotomy and electrolytic liver ablation was performed successfully in all pigs. The pigs recovered uneventfully over the 3 days after operation with no clinical signs of organ failure. By the third day all pigs were well with normal behaviour and feeding habits. Intra-abdominal adhesions to the surface of the liver at the site of electrode insertion were identified at autopsy. Ablation volume in the electrolysis group ranged from 26.4 to 99.3 (mean 57.2) cm^3 .

Liver enzymes

Alanine aminotransferase (ALT) levels increased after surgery in both electrolysis and control groups (Fig. 1). Levels were significantly increased at 24 h after treatment, returning to preoperative levels within 48 h. The serum ALT level was significantly higher ($P = 0.027$) in the electrolysis group than in the control group at 24 h. Overall, there was a significant effect of dose on ALT levels ($P = 0.009$).

The serum alkaline phosphatase (ALP) concentration increased in all animals after operation, peaking at 24 h, but falling to preoperative levels or lower within 48 h of surgery (Fig. 2). The ALP level was significantly greater in the combined electrolysis groups than in controls at 24 h ($P = 0.036$). There was no overall dose effect on serum ALP levels ($P = 0.557$).

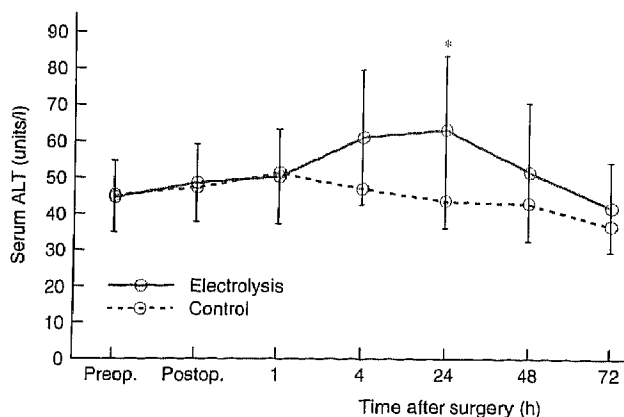


Fig. 1 Mean(s.d.) serum alanine aminotransferase (ALT) levels in electrolysis and control groups. Preop., before laparotomy; postop., at completion of surgery. * $P = 0.027$ versus control (repeated measures analysis)

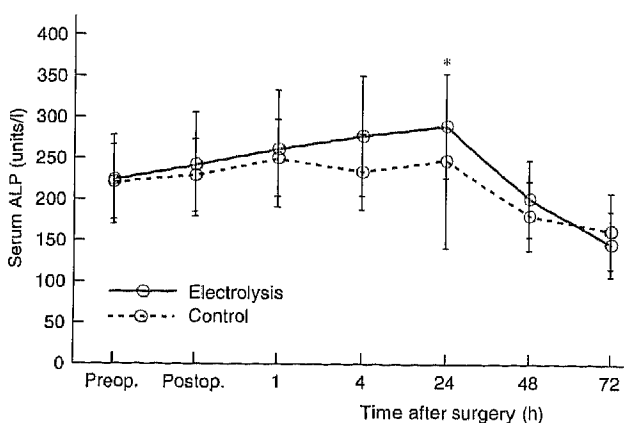


Fig. 2 Mean(s.d.) serum alkaline phosphatase (ALP) levels in electrolysis and control groups. Preop., before laparotomy; postop., at completion of surgery. * $P = 0.036$ versus control (repeated measures analysis)

Platelets

Platelet counts after surgery in control animals did not alter significantly from preoperative levels. Only in the 400-C and 800-C treatment groups did the platelet counts decrease significantly below preoperative levels. Comparison of the control group with all electrolysis groups combined revealed a significant effect of electrolysis on platelet counts immediately after surgery ($P = 0.035$), and at 1 h ($P = 0.033$) and 4 h ($P = 0.011$) after operation (Fig. 3). When the effect of treatment was assessed over the whole follow-up period the difference between electrolysis and control groups was not significant ($P = 0.075$). There was no significant effect of dose on platelet counts ($P = 0.224$).

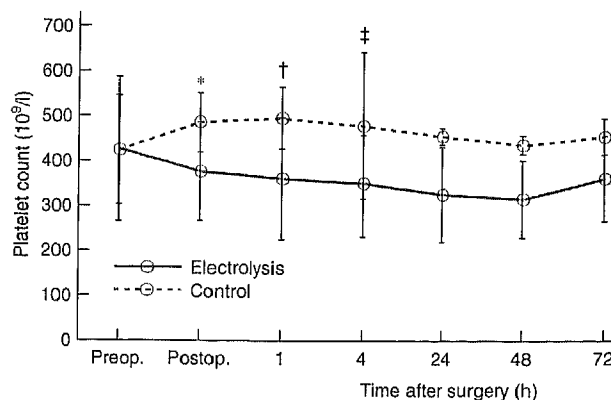


Fig. 3 Mean(s.d.) platelet count in electrolysis and control groups. Preop., before laparotomy; postop., at completion of surgery. * $P = 0.035$, † $P = 0.033$, ‡ $P = 0.011$ versus control (repeated measures analysis)

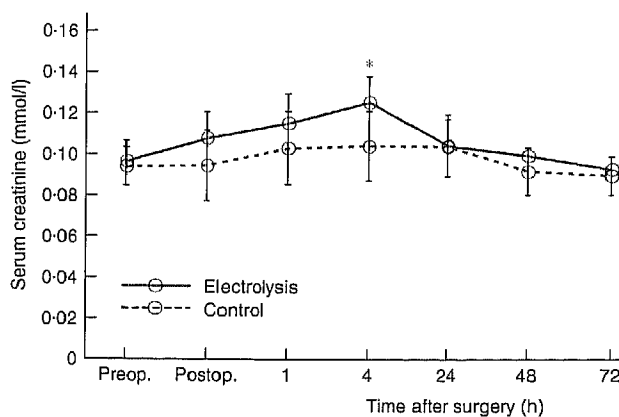


Fig. 4 Mean(s.d.) serum creatinine levels in electrolysis and control groups. Preop., before laparotomy; postop., at completion of surgery. * $P = 0.015$ versus control (repeated measures analysis)

Renal function

Serum creatinine levels rose slightly after surgery in the control group, reaching significance only at 24 h, and returning to preoperative levels within 48 h (Fig. 4). In the electrolysis group creatinine levels were significantly raised above preoperative levels immediately after the procedure and at 1, 4 and 24 h after surgery, but had returned to preoperative levels by 48 h. There was a significant difference between electrolysis and control groups at 4 h only ($P = 0.015$). Overall there was no significant interaction between dose ($P = 0.382$) or treatment ($P = 0.142$) and serum creatinine levels.

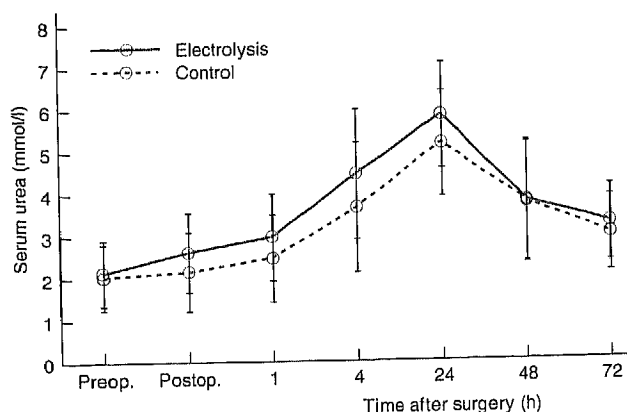


Fig. 5 Mean(s.d.) serum urea levels in electrolysis and control groups. Preop., before laparotomy; postop., at completion of surgery

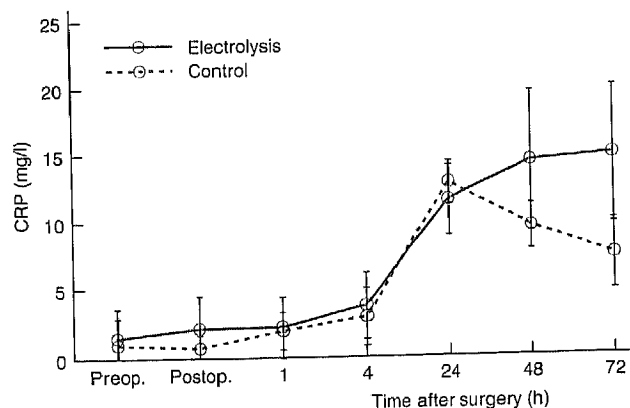


Fig. 6 Mean(s.d.) serum C-reactive protein (CRP) levels in electrolysis and control groups. Preop., before laparotomy; postop., at completion of surgery

Serum urea levels peaked at 24 h in both electrolysis and control groups (Fig. 5). By 72 h urea levels had dropped but were still significantly higher than preoperative values. There was no significant difference between electrolysis and control groups over the follow-up period, and no interaction between dose or treatment and serum urea levels.

C-reactive protein

The CRP level was significantly raised from preoperative levels between 24 and 72 h after surgery in both groups (Fig. 6). The level peaked at 24 h in control animals but remained increased at 72 h in the electrolysis group. When combined electrolysis groups were compared with controls,

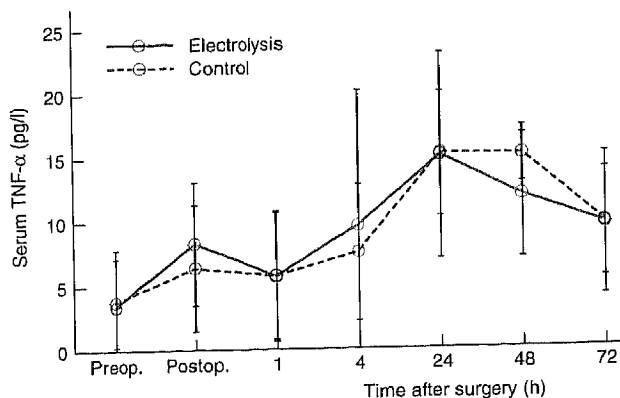


Fig. 7 Mean(s.d.) serum tumour necrosis factor (TNF) α levels in electrolysis and control groups. Preop., before laparotomy; postop., at completion of surgery

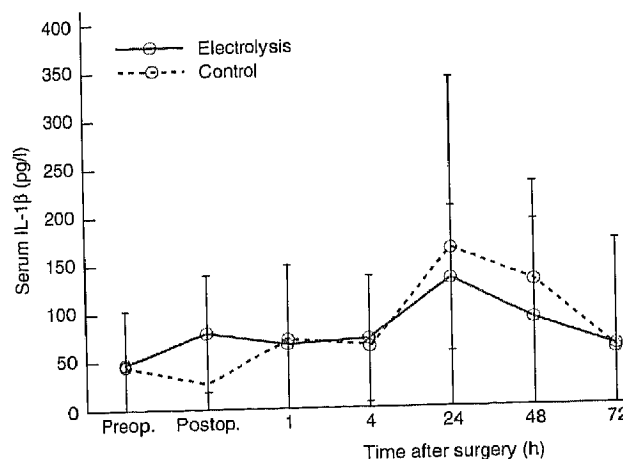


Fig. 8 Mean(s.d.) serum interleukin (IL) 1 β levels in electrolysis and control groups. Preop., before laparotomy; postop., at completion of surgery

the interaction between treatment and CRP approached significance ($P = 0.059$). There was a significant interaction between dose and CRP concentration ($P = 0.007$).

Cytokines

TNF- α levels appeared to peak at 24 h after surgery in both electrolysis and control groups (Fig. 7). In controls, TNF- α levels were significantly raised above preoperative levels between 24 and 72 h after surgery. In electrolysis groups, the levels were significantly raised compared with preoperative levels at all postoperative time points. When control and electrolysis groups were compared there was no significant difference in TNF- α levels. Overall there

was no interaction between dose ($P = 0.970$) or treatment ($P = 0.844$) and levels of TNF- α .

IL-1 β levels also peaked at 24 h after surgery. Compared with preoperative values, IL-1 β levels were significantly increased at 24 and 48 h in both electrolysis and control groups. There was no significant difference in IL-1 β levels between controls and all electrolysis groups combined (Fig. 8). There was no significant interaction between dose ($P = 0.322$) or treatment ($P = 0.219$) and IL-1 β levels.

Discussion

Systemic inflammatory activation leading to profound shock with multiple organ failure is an important complication of solid organ cryoablation⁴. The breakdown of necrotic tissue after cryoablation may be responsible for the pathophysiological changes seen after cryosurgery¹¹. IL-1, IL-6 and TNF- α are the likely cytokine mediators of this syndrome⁶.

In the present study, serum IL-1 β and TNF- α concentrations were increased in both electrolysis and control groups, peaking at around 24 h after surgery. However, there was no significant difference in cytokine levels between groups, suggesting that the increase was a result of surgery and not of the electrolytic treatment. Serum IL-6 levels could not be assessed as a suitable quantitative assay for swine IL-6 was not available.

The magnitude of the rise in TNF- α and IL-6 concentrations following hepatic cryoablation is related to ablation dose⁶. No such dose relationship was found in the present study. The doses of electrolysis (400–1000 C) used in this study are consistent with those used in clinical practice. Fosh *et al.*¹⁰ used doses of 300–1500 C in adults undergoing liver electrolysis for the treatment of colorectal liver metastases and hepatocellular carcinoma. The ablation volumes achieved in the present study ranged from 26.36 to 99.32 (mean 57.22) cm³. Although ablation volume as a percentage of liver volume was not assessed in this study, the authors have previously measured liver volumes of 652–1120 cm³ in female specific pathogen-free White pigs of a similar size (28–34 kg)¹¹.

Although tissue necrosis is the end result of all forms of ablative treatment, a specific shock syndrome has been reported only for cryoablation. In rats undergoing 35 per cent liver ablation using RFA there was no evidence of acute lung inflammation at 24 h or a raised serum TNF- α concentration within 6 h¹², whereas in rats after 35 per cent liver cryoablation there was a mortality rate of 45 per cent at 24 h associated with acute lung injury¹³. Nuclear factor (NF) κ B is a transcription complex factor that regulates transcription of TNF- α , and IL-1, IL-2, IL-6 and IL-8¹⁴.

Cryoablation and acute lung injury were associated with a raised serum level of TNF- α and NF- κ B activation in liver and lung^{12,13}. However, ablation of 35 per cent of rat liver by RFA was not associated with an increased TNF- α concentration and NF- κ B activation in the first 6 h after ablation¹².

Electron microscopy of liver sections after cryoablation showed intact cytoplasmic organelles with disruption of the hepatocyte plasma membrane¹². In contrast, in RFA-treated tissue there was destruction of intracytoplasmic organelles with relatively intact hepatocyte plasma membranes¹². The electron microscopic appearance of electrolytic ablation lesions has not been reported in the literature.

In this study, local liver necrosis and inflammation were evidenced by a moderate rise in ALT and ALP concentrations, greater in electrolysis than control groups, peaking at 24 h and returning to normal within 48 h. Histological studies of pig liver tissue following electrolytic ablation demonstrate sharply demarcated areas of coagulative necrosis with only a thin marginal rim of inflammatory response⁸. Coagulation of blood vessels less than 1 mm in diameter on the periphery of the ablation lesion was also noted⁸. It is hypothesized that this forms a 'cocoon' around the lesion, which prevents the rapid egress of breakdown products into the systemic circulation, thus reducing the risk of systemic inflammatory activation.

Although the serum creatinine level was transiently increased after surgery in both electrolysis and control groups, there was a proportionately greater rise in the level of urea suggesting that this probably resulted from mild dehydration rather than renal failure. Renal function was normal in all pigs at 72 h after surgery.

There appeared to be a fall in platelet count after electrolysis in the immediate postoperative period, returning to normal levels with 72 h. There was no dose effect, and overall the interaction between treatment and platelet counts was not significant. Given the relatively small number of animals in this study, further studies are needed to clarify the importance of this finding.

This study was a short-term follow-up of liver electrolysis at clinically relevant doses in a large animal model. Increases in CRP and ALT levels were associated with electrolytic treatment and with dose of electrolysis delivered. There was no evidence of significant derangement in renal or hepatic function. Levels of the proinflammatory cytokines TNF- α and IL-1 β were transiently increased after surgery, but this was not associated specifically with electrolysis. The present findings suggest that electrolytic liver ablation is a safe procedure.

Acknowledgements

This work was supported by funding from The Queen Elizabeth Hospital Research Foundation and the Anti-Cancer Foundation of South Australia.

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