Original Article

Electrochemical Treatment: An Investigation of Dose-Response Relationships Using an Isolated Liver Perfusion Model

Ralf Czymek, Dorothea Dinter, Stephan Löffler, Maximilian Gebhard[2], Tilman Laubert,
Andreas Lubienski[1], Hans-Peter Bruch, Andreas Schmidt

Departments of Surgery, [1]Radiology, [2]Institute of Pathology, University of Luebeck Medical School, Ratzeburger Allee 160, Luebeck, Germany

Address for correspondence:
Dr. Ralf Czymek,
Department of Surgery,
University of Luebeck Medical School, Ratzeburger Allee 160,
D-23538 Luebeck, Germany.
E-mail: ralf_czymek@web.de

ABSTRACT

Background/Aim: Ablative techniques such as radiofrequency ablation or non-thermal electrochemical treatment (ECT) are used to manage unresectable liver metastases. Although ECT is not affected by the cooling effect from adjacent vessels, there is a paucity of data available on ECT. **Materials and Methods:** We used porcine livers to establish an organ model with portal venous and hepatic arterial blood flow for a standardized analysis of the relationship between dose (electric charge) and response (volume of necrosis). **Results:** This model allowed us to study pressure-controlled perfusion of portal venous and hepatic arterial circulation in the absence of a capillary leak. A specially designed guiding template helped us place platinum electrodes at reproducible locations. With two electrodes, there was a linear relationship between charges of no more than 200 C and necrosis. The relationship was logarithmic at charges of 400-600 C. Larger electrode spacing led to a significant increase in necrosis. We measured pH values of 0.9 (range: 0.6-1.3) at the anode and 12.6 (range: 11.6-13.4) at the cathode. **Conclusions:** Using a perfusion model, we established an experimental design that allowed us to study ECT in the liver of large animals without experiments on living animals. An electrode template helped us improve the standardized analysis of dose-response relationships. ECT created reproducible and sharply demarcated areas of necrosis, the size of which depended on the charge delivered as well as on the number and spacing of electrodes. Doses higher than 600 C require longer treatment times but do not increase the area of necrosis (logarithmic dose-response relationship).

Key Words: Ablation, coulomb, dose-response relationship, electrochemical treatment, liver, perfusion model

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In many patients with malignant liver lesions, curative resection is no longer possible at the time of diagnosis.[1-4] Affected patients are potential candidates for non-resective techniques such as radiofrequency ablation (RFA) and electrochemical treatment (ECT). The latter involves the placement of two or more electrodes into tissue and the continuous delivery of direct current between the electrodes. An anti-tumor effect of this direct current therapy was reported for the first time in the late 19th century.[5] Landmark work on the use and application of this procedure was, however, performed by Bjoern Nordenstroem, a Swedish professor and radiologist. In 1978 he used this procedure to treat malignant tumors of the lung.[6-9] ECT has been used on various animal species in an experimental setting and on different h0075man organ systems in a clinical therapeutic setting.[10-12] It is reported to be associated with few complications and to effectively destroy tumor tissue. ECT causes ion and pH changes and thus induces coagulative and colliquative necrosis in the area of electrode application. Since ECT is a non-thermal procedure and is not affected by the cooling effect from adjacent major vessels, which limits the effectiveness of RFA, ECT has conceivable advantages. Although basic research into the mode of action of ECT started as early as the 1970s, data available in the literature is limited and heterogeneous. For example, the relationship between the electric charge delivered and the response induced is controversially discussed. Whereas a number of authors[13,14] report a linear dose-response relationship, others[15] describe a logarithmic relationship. The objective

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of this study was to establish an experimental design that helps us reduce animal use and allows us to investigate the relationship between dose (in coulombs) and response (volume of necrosis) in the liver.

MATERIALS AND METHODS

Porcine livers were obtained from a nearby slaughterhouse. Three to five minutes after the animals were killed, the livers were perfused through the portal vein and the hepatic artery with ten liters of a heparinized isotonic electrolyte solution (Ringer's solution with 2000 units of heparin per liter) until no blood components were macroscopically detected in the effluent perfusate.

The livers were cooled and transported to the hospital laboratory where they were inserted into a closed perfusion system. The common hepatic artery was connected to a dialysis machine (Hospal Dasco BSM-22SC, Medolla, Italy), which was used to regulate arterial circulation. A gear pump (ISMATEC ISM 405A, Glattbrugg, Switzerland) regulated portal venous circulation and provided a continuous flow. Manometric pressure control and pump settings allowed us to adapt both inflows to the physiological parameters of the target organ (arterial pressure: 110-130 mmHg, portal venous pressure: 15-20 mmHg). During the experiments, a specimen was placed in a Plexiglas box and immersed in Ringer's solution to prevent its surface from drying out. After the caudal end of the vena cava had been closed by suture, the perfusate was passed through the vena cava and reached two gear pumps with two circulation thermostats (Haake, Type 001-4202/ 001-7992, Berlin, Germany; LKB 2219 Multitemp II, Bromme, Sweden), which were used for coarse and fine temperature control. An oxygenator (Maquet, Jostra Quadrox Safeline®, Hirrlingen, Germany) was used to enrich the temperature-controlled perfusate with oxygen before the fluid was returned either to the portal venous side or the arterial side (dialysis machine) of the circulation.

In order to investigate the mode of action of ECT, we used a direct current generator (ECU 300, Soering Medizintechnik, Quickborn, Germany) and platinum electrodes with a diameter of 1 mm.

The extent of necrosis that is induced by ECT depends on the applied electric charge, which is the product of electric current (in amperes) and time (in seconds) and is expressed in coulombs (C). One coulomb is defined as the quantity of electricity carried by a current of one ampere in one second ($1C = 1A \times 1s$). The electric current was ramped up to its preset level over a period of five seconds.

In our study, we delivered electric charges of 50, 100, 200, 400 and 600 coulombs in order to evaluate dose-response relationships. Since we used constant levels of electric current (50 mA) and voltage (25 V), we applied the current for different lengths of time that were calculated using the aforementioned formula. A guiding template, which had been designed for these experiments, helped us place the electrodes at different locations in peripheral liver tissue and investigate dose-response relationships [Figure 1].

During ECT, pH values were measured using an Orion 3-Star Plus Portable pH Meter (Thermo Fisher Scientific, Waltham, United States). The sensor had a diameter of 2 mm.

Necrotic tissue specimens were fixed in buffered formalin for 48 h and then placed into embedding cassettes. They were stained according to a standard hematoxylin and eosin protocol and analyzed histologically at the Institute of Pathology of the University of Luebeck Medical School.

RESULTS

Liver harvesting and perfusion model

When we studied the dead liver tissue that we obtained from a butcher, we managed to produce a sufficient and reproducible flow of current between anode and cathode only when the electrodes were separated by no more than 1.5-2 cm. When we used fresh organs from a slaughterhouse and placed them into a perfusion system, we were able to achieve a substantial movement of ions when the electrodes were separated by a distance of more than 12 cm without causing dysfunction of the direct current generator.

We investigated dose-response relationships for ECT in a total of 41 porcine livers with a mean weight of 2120 g ± 472 g (range: 1495-2495 g). When the specimens were collected from the slaughterhouse, they had a mean

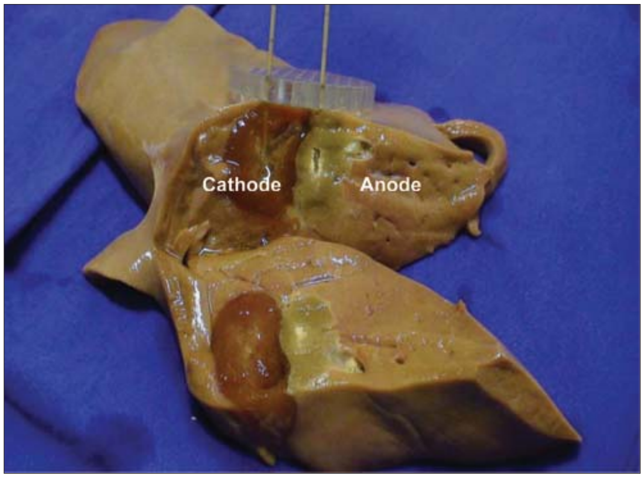

Figure 1: Macroscopic specimen after electrochemical treatment. One anode and one cathode had been inserted into peripheral liver tissue with a new template for reproducible electrode placement

temperature of 38.1°C ± 0.8°C (range: 37.8-39.2°C).

In order to verify the functioning of the entire system, we examined four porcine livers with a mean weight of 2017 g ± 287 g (range: 1788-2312 g). During these pretests, we were able to prove that the preparation of the freshly harvested livers allowed us to investigate the organs within the perfusion system without problems. For portal venous circulation, we used a portal venous pressure of 15-20 mmHg by adjusting the flow rate with the aid of roller pumps. The dialysis machine, which regulated hepatic arterial circulation, was adjusted to maintain a mean arterial pressure of 110-130 mmHg. Both pressures were continuously monitored. During the experiments, which lasted several hours, pump settings remained constant and pressures did not change. Doppler ultrasonography confirmed perfusion of the liver including peripheral portions of the organ.

Dose-response relationships for electrochemical treatment with two electrodes

For investigating dose-response relationships in isolated perfused porcine livers, we examined a total of 22 livers with a mean weight of 2085 ± 247 g (range: 1495-2300 g) in the perfusion model. Mean post-interventional weight was 2111 g ± 238 g (range: 1513-2356 g). This result shows that there was no significant increase in weight during the experiments. Both manometrically measured pressures remained constant. There was no increased intrahepatic resistance. The histological examination revealed the absence of intracellular and interstitial edema in non-ablated liver tissue. We interpreted these findings as indirect evidence of an intact structure of the liver and the absence of a major capillary leak.

We performed a total of 60 ablation procedures in the peripheral tissue of the 22 livers. In each case, one anode and one cathode were inserted and electric charges of 50, 100, 200, 400 and 600 coulombs were delivered 12 times. Since we used constant levels of electric current (50 mA) and voltage (25 V), treatment time ranged between 17 mins (at 50 C), 33 mins (at 100 C), 66 mins (at 200 C), 132 mins (at 400 C) and 198 mins (at 600 C).

Of the 12 procedures that we performed using five different amounts of electric charge, four procedures were performed with electrodes spaced 1 cm apart, four with electrodes spaced 2 cm apart, and four with electrodes spaced 4 cm apart. The results are presented in Figures 2-4.

We measured the cylindrically shaped areas of necrosis macroscopically and determined the volumes of necrosis. The areas of ablations were then analyzed histologically. The macroscopically detectable margins of necrosis were confirmed microscopically. The ablated tissue was sharply demarcated from intact liver tissue. This morphological feature was present in all specimens. The area of necrosis at the anode appeared paler in color than the lesion at the cathode.

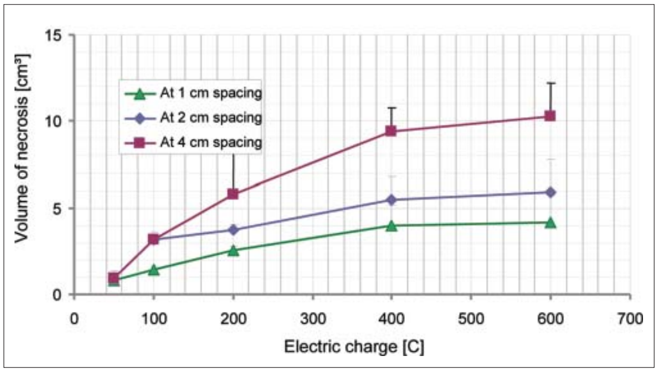

Figure 2: Volumes of necrosis at the anode as a function of electric charges delivered at different electrode spacings

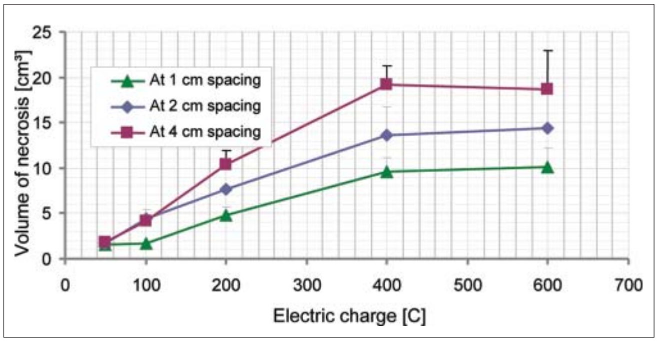

Figure 3: Volumes of necrosis at the cathode as a function of electric charges delivered at different electrode spacings

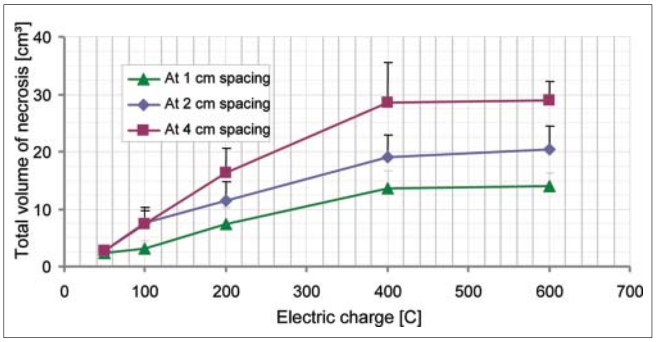

Figure 4: Total volumes of necrosis at the anode and cathode as a function of electric charges delivered at different electrode spacings

Hematoxylin and eosin staining demonstrated histologically different areas of necrosis around the two electrodes. In the region around the cathode, cell nuclei were not stained as a result of a loss of biochemical function. No cell membranes were detected. As a result of cell lysis and sinusoidal obstruction, histology revealed the homogenization of (light) cytoplasm and the loss of the normal lobular structure.

By contrast, the cytoplasm in the necrotic area around the anode was found to be strongly eosinophilic. The morphological structure of the cell nuclei was still intact and the cell membranes were clearly visible. The macroscopically observed sharp demarcation was confirmed both in the transition zone between necrotic tissue at the anode and peripheral tissue and between necrotic tissue at the cathode and the surrounding intact liver tissue. An eosinophilic zone was detected between the anode and the cathode where the areas of necrosis came into contact with each other [Figure 5].

When the electrodes were separated by no more than 2 cm, the areas of coagulative and colliquative necrosis came into direct contact with each other. Complete cell lysis was seen in the entire area [Figure 1].

An analysis of the results obtained at the anode showed that larger electrode spacing (1 cm, 2 cm and 4 cm) was associated with a significant increase in the volume of necrosis induced. The higher the charge delivered (50 C, 100 C, 200 C, 400 C, and 600 C), the larger was the size of the area of ablation. When an electric charge of no more than 200 coulombs was delivered, the volume of necrosis increased in a linear fashion. When an electric charge of more than 200 coulombs was applied, the volume of necrosis increased in a logarithmic manner [Figure 2].

Likewise, an analysis of the results obtained at the cathode showed that the volume of colliquative necrosis increased in a logarithmic fashion with higher doses of coulombs. Larger electrode spacing was again associated with an increase in the size of the areas of ablation. A comparison of the zones of necrosis at the anode and the cathode showed that the coagulation defects at the anode were invariably smaller than the lesions at the cathode.

When the effects of ECT at the anode and at the cathode were added and total volumes of necrosis were determined [Figure 3], we obtained ablation areas of potential clinical usefulness.

When we consider ECT times and the resultant total volumes of necrosis, the targeted destruction of a liver volume of approximately 20 cm^3 appears to be realistic when one anode and one cathode are used [Figure 4].

The distance from the anode or the cathode was the decisive factor on which the pH value and thus the destructive effect of ECT depended. We measured pH values between 0.9 (range: 0.6-1.3) at the anode and 12.6 at the cathode (range: 11.6-13.4).

The various amounts of electric charge did not cause significant differences in pH at the various distances from the electrode. At distances of up to 1.5 cm from the electrode, low electric charges (50 C) induced pH changes similar to those caused by higher charges [Figures 6 and 7].

Dose-response relationships for electrochemical treatment with four electrodes

Since four electrodes can be connected to each of the three treatment channels of an ECU 300 direct current generator for the ablation of liver tissue, we modified the experimental design after the first series of experiments and inserted two anodes and two cathodes instead of one anode and one cathode. The electrodes were placed into the tissue in such a way that the anodes were located on the ends of each of the two diagonals of a square and the cathodes were located on the other ends of the diagonals. Under these conditions, we placed 19 livers with a mean weight of 2165g ± 392 g (range: 1683-2495g) into the perfusion model and investigated dose-response relationships. Mean post-interventional weight was 2198 ± 377g (range: 1702-2566 g). This result shows that there was no significant increase in weight during the experiments. The histological examination revealed the absence of intracellular and interstitial edema in non-ablated liver tissue. We performed a total of 48 ablation procedures in the peripheral tissue of the 19 livers. In each case, electric charges of 50, 100, 200, and 400 coulombs were delivered 12 times. Since we used constant levels of electric current (50 mA) and voltage (25 V), the procedures lasted for 17 mins (at 50 C), 33 mins (at 100 C), 66 mins (at 200 C), or 132 mins (at 400 C).

Of the 12 procedures that we performed using four different amounts of electric charge, four procedures were performed with electrodes spaced 1 cm apart, four with electrodes spaced 2 cm apart, and four with electrodes spaced 4 cm apart. An analysis of the specimens showed that areas of necrosis had been created symmetrically around the four electrodes. Macroscopic and histological findings were consistent with those obtained with two electrodes. Areas of total necrosis with sharply demarcated zones of transition between necrotic and normal liver tissue were again created by electrolysis. Coagulative necrosis was found at the anodes. Colliquative necrosis was seen at the cathodes.

In contrast to our experiments with two electrodes, larger electrode spacing (1 cm, 2 cm and 4 cm) did not significantly influence the size of the resulting areas of necrosis. The higher the electric charge delivered (50 C, 100 C, 200 C and 400 C), the larger was the size of the area of ablation in all experiments. When an electric charge of no more than 200 coulombs was delivered, the volume of necrosis increased in a linear fashion. When an electric charge of more than 200 coulombs was applied, the volume of necrosis increased in a logarithmic manner.

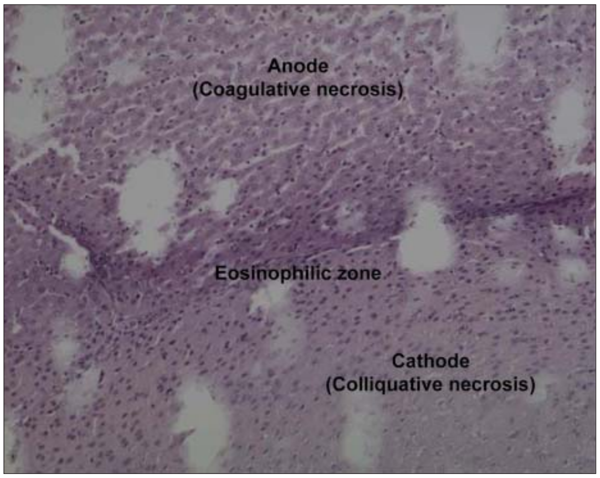

Figure 5: Histological appearance of the transition zone between anode and cathode. An eosinophilic zone is visible after staining with hematoxylin and eosin (magnification: ×100)

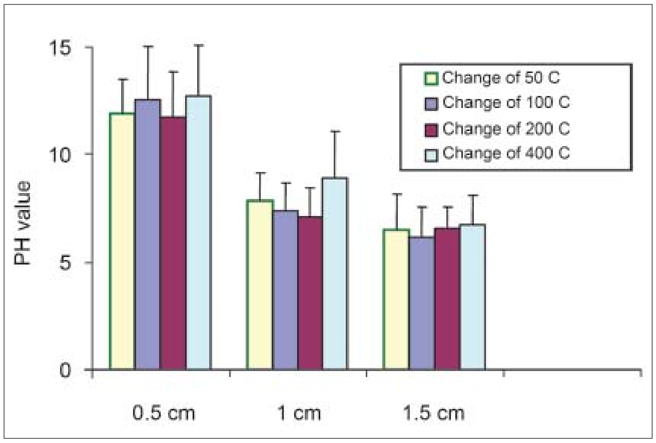

Figure 6: PH values measured at different distances from the cathode after the delivery of different electric charges (in coulombs)

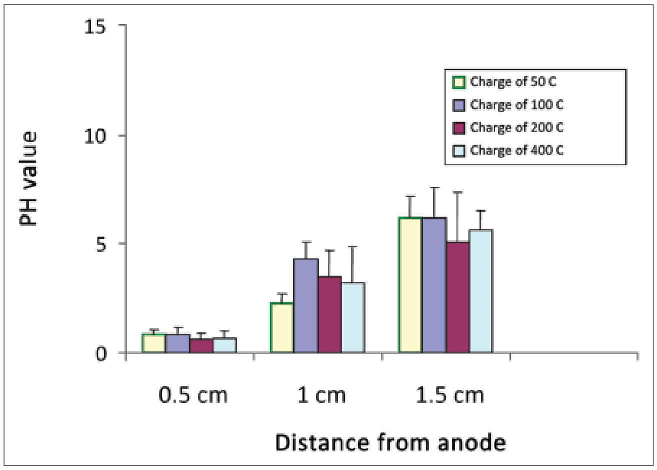

Figure 7: PH values measured at different distances from the anode after the delivery of different electric charges (in coulombs)

A comparison of the various zones of necrosis showed again that the necrotic areas at the anodes were invariably smaller than those at the cathodes.

When we compared the results of the two-electrode experiments with those of the four-electrode experiments, we found that the area of ablation created by four electrodes was significantly larger than that produced by two electrodes. The area of ablation was almost twice as large.

DISCUSSION

With improved surgical techniques and technical advances in the field of liver surgery, the indications for the surgical resection of malignant liver lesions have broadened considerably in recent decades.

For a variety of patient-specific or tumor-related causes, however, a large proportion of patients with intrahepatic malignancies are unsuitable for resection and are therefore treated by local ablation. Electrochemical treatment (ECT) is a form of ablation that currently does not play a major role in Western industrialized countries. By contrast, ECT has become increasingly popular in other regions of the world. Following the groundbreaking work of Nordenstroem,[6-9] clinical studies were conducted especially in China where research groups in all parts of the country applied electrochemical treatment. Xin Yu-Ling and co-workers reported their experience according to more than 10,000 patients in a clinical setting.[16,17] The mechanisms of direct current therapy, however, are still not fully understood.

Ethical considerations as well as financial and logistic requirements that are associated with experiments on living animals are major challenges confronting researchers with an interest in this area. The objective of our effort was therefore to establish an experimental design that allows us to reflect and verify basic aspects of ECT and at the same time to reduce or replace animal use. For this reason, we used the perfusion model based on the work of Bitsch *et al.*,[18] and Lubienski *et al.*[19]

In the field of transplantation medicine, organs from a donor must sometimes be preserved and transported for several hours under hypothermic conditions before they are transplanted into the recipient. Organ function was reported to be intact even after one hour of warm ischemia and four hours of normothermic extracorporeal liver perfusion.[20] In our study, freshly harvested organs were transported for a period of approximately 30 mins under hypothermic conditions before they were used for the experiments under normothermic conditions. Organ function was therefore assumed to be intact. This was confirmed by indirect parameters such as a constant organ weight (no major capillary leak), constant perfusion pressures and a histologically intact tissue structure. Of particular relevance to our experimental design are the results reported by Li *et al.*,[21] who showed that ECT destroyed both normal and malignant cells in a similar fashion.

In the absence of perfusion, it was impossible to produce a sufficient flow of current in liver tissue. This suggests that the establishment of an organ model permitting the direct *ex vivo* use and perfusion of a liver is a minimum requirement for an evaluation of ECT. To our knowledge, the perfusion model presented here is the first in the literature to investigate ECT.

In previous studies or publications on ECT, electrodes were inserted into the target organ without a mechanism for the fine adjustment of electrode positions. This approach is associated with two problems. Firstly, the sites where the electrodes perforate the liver capsule can be identified on the liver surface with relatively high accuracy, but only an approximate placement of the electrodes can be achieved within the deeper liver parenchyma at the site of electrode application. Even under careful ultrasonographic control, it is impossible to place anodes and cathodes in a soft organ in an exactly parallel fashion. When four or more electrodes are used, these sources of error lead to even stronger limitations of an analysis of the effects of ECT. Secondly, the stability of surrounding tissue decreases during the course of the experiments. This loss of stability played a major role in the pretests of this study. The process of ECT leads to the formation of areas of local necrosis, especially in edematous tissue at the cathode and thus causes a softening of the tissue. The resulting loss of support causes the electrodes to become loose and to change their predetermined position. For this reason, we developed a guiding template [Figure 1] with holes evenly spaced 5 mm apart, which not only allowed us to exactly place the electrodes at predetermined positions but also provided lateral guidance so that the electrodes maintained their parallel position in the deeper liver parenchyma during the entire period of ECT. This newly developed template improved the reproducibility of the experiments and results.

Most authors conduct ECT experiments on the basis of the values given by Nordenstroem for relevant parameters such as electric charge, electric current, voltage, and electrode spacing and on the basis of the clinical experience obtained by Chinese workers. Nordenstroem initially proposed the application of 100 coulombs per cm of tumor diameter.[7] In China, Xin *et al.*,[16] modified Nordenstroem's method and delivered 30-100 C per cm of tumor diameter in everyday clinical practice. Ren *et al.*,[22] applied ECT with doses of coulombs that depended on tumor volume and not tumor diameter. They found that there was no clear scientific evidence to support the application of 100 C, which was an amount that was commonly used. Apart from this data, there are a few studies that address systematic dose-response relationships for ECT in small animals. Schauble *et al.*,[23] applied low levels of electric current (3, 0.5 and 0.001 mA) for the treatment of melanomas in hamsters over a period of four days. They used stainless steel electrodes. While Samuelsson *et al.*, used copper electrodes and investigated lung metastases in a rat model,[24] most research groups prefer platinum electrodes with a diameter of 0.5 mm,[22] 0.7 mm[25] or 1.0 mm, the latter of which was also used in our study. Since the data that is available on the materials, electric charges, voltages, and electric currents used in the ECT of a variety of species is highly heterogeneous, we performed our own measurements using the isolated porcine liver perfusion model presented here. The vast majority of studies on ECT were performed on small animals (mice, rats and hamsters). As early as 2003, Euler *et al.*,[15] therefore emphasized the need for studies in large animals–like the study presented here–in order to obtain a more reliable dose planning for the use of ECT in humans.

Four electrodes can be connected to each of the three treatment channels of an ECU 300 direct current generator, which was used in this study. In the past, a number of authors investigated different combinations of electrode positions. Since we used tumor-free tissue from a slaughterhouse for ablation, we followed the example of Hinz *et al.*,[26] and von Euler *et al.*,[15] and positioned the electrodes at the corners of a square when we performed the experiments with four electrodes. We were able to show that the resultant volume of necrosis was significantly larger than the volume created with only two electrodes. When two additional electrodes were used, the area of ablation was approximately twice as large in the perfusion model.

Previous studies were performed with different electrode spacings. Svane and Nordenstroem,[27] who treated breast cancer patients with ECT, placed the anode centrally into the tumor and the cathode approximately 10 cm away from the tumor margin in an axillary direction. Xin *et al.*,[28] who used ECT in the treatment of lung cancer, found that the necrotic area had a diameter of approximately 2-3 cm and recommended an electrode spacing of less than 2 cm in lung cancer patients. Song *et al.*,[29] advocated an electrode spacing of 1-2 cm in the treatment of malignant tumors on the body surface. By contrast, Matsushima *et al.*,[30] used an electrode spacing of 1-2 cm in animal experiments and 3-4 cm in clinical studies. These authors, however, did not explain the reason for the different spacings. When we used two electrodes in our perfusion model, we found a clear relationship between electrode spacing and necrosis. When we separated the electrodes by a larger distance, the volume of necrosis increased as well. When this distance exceeded a certain limit, however, we observed an area of potentially viable tumor tissue between the anode and the cathode when a defined electric charge was applied. In our isolated liver perfusion model, we found that the areas of necrosis reliably came into contact at an electrode spacing of no more than 2 cm and an electric charge of 200 C. At lower electric charges or at an electrode spacing of more than 2 cm, we detected morphologically intact cells between the electrodes.

In contrast to our experiments with two electrodes, larger electrode spacing did not significantly influence the size of the resulting areas of necrosis when we inserted four electrodes. Our findings suggest that, when four electrodes are used, direct current flows not only in one direction from Electrode A to Electrode B but also from A to B and C, etc. This potential interaction between different directions of ion transfer may alternate the effect of ECT.

The literature also reveals controversy regarding the evaluation of the forms of necrosis at the cathode and the anode. Whereas Miklavcic *et al.*,[31] reported a larger effect at the cathode, a number of authors documented variable responses to anodic and cathodic fields.[32] In 1998 and 2002, Robertson[33] and Wemyss-Holden[34] reported larger ablation areas after anodic treatment compared with cathodic treatment. There is, however, broad consensus about the existence of a shift of extracellular water from the anode to the cathode during ECT with a local swelling at the cathode and dehydration at the anode.[21] According to von Euler *et al.*,[15] this process leads to a larger area of necrosis at the cathode. Our findings confirm the results of von Euler *et al.*

As expected, we also observed the release of gas at the electrodes. We did not, however, determine the composition of the gas bubbles that we detected around the electrodes. According to the literature, hydrogen is produced at the cathode and chlorine gas and oxygen at the anode.[13]

Four days after intrahepatic ECT, Finch *et al.*,[10] found histological evidence of necrotic tissue and an eosinophilic zone, which we too detected, in the region between the anode and cathode. In addition, Finch *et al.*, described a sharp demarcation between necrotic tissue and a surrounding zone with fibroblast and biliary proliferation and a mixed picture of white-cell infiltration. Predominantly neutrophils were found in their study. Such results cannot be obtained with a perfusion model that is used to investigate an isolated organ and does not address systemic interactions.

Using an *in vivo* dog model, Euler *et al.*,[15] harvested dog livers immediately after ECT and examined the specimens both macroscopically and microscopically. They observed a sharp border between the necrotic area and the surrounding healthy liver tissue. Using our *ex vivo* perfusion model, we were able to confirm this finding both macroscopically and microscopically.

Authors who used different experimental designs (e.g. the ECT of subcutaneous tumors in a mouse model) reported a linear relationship between the dose of coulombs and the resultant area of necrosis.[13] Griffin *et al.*, too, described a linear dose-response relationship between the electric charge delivered and the necrotic zone created in mice that were inoculated with cells from a mammary carcinoma.[14] Likewise, Samuelsson *et al.*, who investigated liver and lung tissue in a rabbit, detected a linear dose-response relationship.[35] These three research groups, however, applied low electric charges ranging between 2 and 40 coulombs. Griffin *et al.*,[14] and Robertson *et al.*,[33] detected a linear relationship between low electric charges of no more than 30 coulombs and necrosis and found in their experiments on small animals that the level of electric current did not influence the resultant necrosis with the electric charge remaining unchanged. By contrast, Euler *et al.*,[15] reported a logarithmic dose-response relationship when they delivered 5, 10 or 90 coulombs to rat or dog livers. Our data suggested a linear dose-response relationship in the liver when low and medium electric charges (up to 300 or 400 coulombs) were applied. When doses of more than 500 C were delivered, however, the effect of treatment no longer increased linearly but logarithmically.

Hinz *et al.*,[26] postulated an exponential increase in ECT time with an increase in tumor size. In our opinion, this is only partially right. Our results suggest that, when constant levels of electric current and voltage were used, there was initially a linear relationship between the electric charge (in coulombs) and the time (in minutes) required to deliver the preset dose to the target organ. At low doses of up to 80 C, there was a linear relationship between the dose (in coulombs) and the response in terms of volume of necrosis. At higher doses between 300 C and 600 C, however, there was an exponential relationship between electric charge and necrotic tissue since it is impossible to increase the necrotic area infinitely using this treatment method.

CONCLUSIONS

Using a perfusion model, we established an experimental design that allowed us to simulate the effects of ECT in the liver of large animals. An electrode template helped us improve the standardized analysis of dose-response relationships. ECT created reproducible and sharply demarcated areas of necrosis, the size of which depended on the charge delivered as well as on the number and spacing of electrodes. As a result of the logarithmic relationship between the electric charge delivered and the volume of necrosis induced, which we were able to demonstrate using isolated livers, it is not possible to infinitely increase the volume of necrosis by increasing the dose of coulombs. For this reason, the ablation of larger foci requires the insertion of additional electrodes.

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