Diagnosing acute liver graft rejection: experimental application of an implantable telemetric impedance device in native and transplanted porcine livers

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**Abstract**—Diagnosis of acute rejection is a complex and persistent problem in liver transplantation. Focused on the use of proprietary impedance technology a porcine liver model was designed to provide immediate information for differentiation of normal and acute rejecting tissue by an implantable telemetric device.

Electrical impedance was analyzed by electrodes implanted in the liver of pigs, where impedance is derived from measurements of voltage transients produced in response to programmed current pulses. Consequent electric recordings in porcine livers after transplantation and after mere laparotomy were evaluated in relation to biochemical parameters and histological results of liver biopsies.

Acute rejection was correctly predicted in all cases and correctly excluded in the remaining 32 biopsy related impedance recordings (p<0.004). Impedance measurements not only correlated with the diagnosis from liver biopsy specimen (r=0.84, p<0.0001) but also exemplified the severity of histological acute rejection.

I. INTRODUCTION

LARGE-scaled allograft monitoring to prevent postoperative liver transplant failure still merits a problem for health care. According to literature reports, over 50% of the liver grafts will have one or more episodes of acute rejection [1].

Because advanced stages of acute rejection are difficult to treat effectively, it is important to detect graft rejections at their earliest stages. It has been shown that neither the absolute level nor the change of biochemical parameters can distinguish acute rejection from other causes of graft dysfunction [2]. Radiologic imaging may represent an essential tool but is also not able to verify the diagnosis. Liver transplant histology is the most specific indicator for the detection of allograft rejection. However, liver core biopsy is invasive. Problems arise from the limited availability, the necessity for patient transfer, the timing of biopsy, biopsy sampling error, and the psychological burden to the patient [3]. The use of nonproprietary computer network solutions facilitates the ability to gain a stream of trended data in a way that was previously unrealizable [4]. Considering the basic research on hepatic impedance and the results from intramyocardial electrocardiogram monitoring of cardiac transplant recipients, a prototype of an implantable bioelectrical impedance analyzer has been evaluated in native porcine livers and transplanted pigs for the diagnosis of acute rejection [5].

II. MATERIALS AND METHODS

**Experimental setup** The experiments conducted were designed to test whether the impedance monitor can detect and differentiate acute allograft rejection from normal functioning grafts and native livers. As acute rejection of liver grafts in pigs is less frequent and severe, genetically different Pietrain pigs served as liver donors. Animals were handled in accordance to the principles of laboratory animal care (NIH publication No. 86-23, revised 1985). To enhance the probability of acute rejection, no immunosuppression with the exception of a single intraoperative steroid pulse (250 mg prednisolone, Urbason®, Aventis, Strasbourg, France) during graft reperfusion was administered. Biochemical measurements included blood counts with electrolytes, urea, creatinine, total bilirubine, serum glutamic-pyruvic transaminase (SGPT), serum glutamic-oxalacetic transaminase (SGOT) and serum glutamic-dehydrogenase transaminase (SGLDH), alkaline phosphatase (AP) and gamma-glutamyltranspeptidase (γ-GT).

**Impedance device** Arranged in parallel, the impedance electrodes were designed with two outer and inner voltage pins. The length and penetration depth of the four puncture electrodes was 4 mm. Protected by a silicone priming tube the matched coaxial cable were linked with the input of the battery and the impedance registration unit. The voltage electrodes were connected with a differential amplifier of...
high input impedance. The distance between the probes was 2–3 cm. The probes were prickled into the parenchyma of the right liver lobe. Dislocation was prevented by sealing with a fibrin-glue-coated adhesive sheet. Impedance recordings were obtained 6 times per hour in 16.0 ms intervals. Through the two outer electrodes a rectangle impulse current of 40 mA, with a frequency between 0.0625 and 140.4 kHz, outlasting 16 ms was applied. By means of the two inner electrodes the voltage drop was registered in 7.813 ms intervals up to a total of 62.5 ms. The size and design of the 54.4 g weighing telemetric chamber is comparable with a cardiac pacemaker. The telemetric chamber was implanted extraperitoneal from the inside of the abdominal cavity after creation of a pocket between the abdominal muscle layers. The main item of the telemetric chamber is an application specific integrated circuit component (ASIC). Following inductive connection of an extracorporal magnetic spool data were transferred via the long-wave band from the intra- to the extracorporal control unit. Using a modem as a server in the external control unit data were transferred via telephone to a central data collecting and analyzing registry. To avoid artificial interferences during impedance registrations all interrogations were performed with the animal asleep or resting quietly during recordings. To establish longitudinal daily analysis, data were imported to a computerized calculation program. Regardless of the experimental setting, the results from biopsy evaluation and blood chemistry, impedance data were analyzed by a qualified computer operator.

Liver transplantation group German landrace swines were used as transplant recipients. Non-related pure bred Pietrain pigs served as liver donors. In total 10 liver transplantations were performed. Liver donors and recipients were only matched for weight. Ex-vivo flush perfusion of the graft consisted of 2000 cm³ cold (4°C) histidine–tryptophan–ketoglutarate solution (Custodiol®, Köhler Chemie, Albach-Henlein, Germany) and 1000 cm³ of saline solution. Transplantation was performed orthotopic without the use of the veno-venous bypass[6]. Only survivors of more than 24 h were included in the study. Eight transplants met the prerequirements. Because of cardiac and neurological complications, four animals had to be excluded from evaluation. Proven acute rejection was found in all of the remaining 4 liver grafted pigs. Median survival of these pigs was 4 days (2–6 days). Altogether 11 liver biopsies were performed in the four animals that exhibited acute allograft rejection.

Native liver group 11 German landrace pigs were operated upon. Two animals were excluded from final evaluation because of a technical default. Median survival of the 9 animals meeting the prerequirements was 10 days (8–12 days). 25 liver biopsies were obtained in the native group.

Histologic monitoring Liver biopsy specimens were evaluated unaware of animal assignment to the control or transplantation group by a single pathologist. Samples were graded with the use of the Birmingham Score (BH-score) [7]. Based on quantitative scoring on a scale from 0-3 histologic features were added to produce a final score an classified as none, BH-score 0-2, borderline, BH-score 3, severe rejection, BH-score 4-5.

Data processing Preliminary in vitro testing of physical variables revealed that the offset of the impedance measurements was only influenced by external factors. In accordance to in vitro studies the most sensitive interval for postprocessing and quantitative analysis was located between 7.813 and 62.5 ms, frequency of 128 and 16 kHz of the 16 ms lasting cycle. The data input per day accounted a volume of 1.2 megabyte (MB). As a common postoperative phenomenon in native livers and in transplants first baseline measurement could be conducted 12–24 h postoperatively. During the subsequent period coherent measurements were possible. For this reason the offset of day 0 was set to 100%. The absolute of the mean of subsequent bisensoric (left and right impedance sensor) recordings for running analysis were referenced in percent to the offset of day 0 and defined as the absolute impedance gradient (IG). For quantitative description of daily discrepancies the relative IG was introduced as the percentile difference of between absolute IGs of consecutive days.

Statistical analysis Biochemical parameters and the postoperative course of relative IGs were expressed as means +/- SD. Differences between groups were assessed with the Kruskal–Wallis or Mann–Whitney test. Exact 95% confidence intervals were determined for proportions. All P-values are two sided, a P-value of less than 0.05 was considered to indicate statistical significance. Correlation between impedance recordings and the results of biochemical and histological data were evaluated with the Pearson correlation coefficient. As a measure of proximity between both variables we demanded that the correlation coefficient should be >0.7 (which for n=14 corresponds to a P< 0.001). Receiver operating characteristic analysis (ROC) was performed to illustrate how good impedance recordings discriminate rejecting from non-rejecting forms and to discriminate the optimal cut-off allowing a diagnosis of acute rejection. The analysis was performed using the software package SPSS V8.0.

III. RESULTS

Impedance analysis in native non-transplanted porcine livers The time-course of the relative IG discrepancies from native livers and liver transaminases and total bilirubine with a peak value on the day of surgery returned to normal until the second postoperative day. IG recordings required a duration of 1–2 days to stabilize. With postoperative recovery and prolonged survival approximation of interindividual relative IG discrepancies were assigned. Once reaching a postoperative plateau relative IGs during histologic quiescence revealed gradual stability over the median of 10 experimental days. Correlation of relative IG discrepancies with liver transaminases showed evident coherence with SGLDH (r=0.5, P<0.001) and SGPT (r=0.5,
Invasive examinations of the liver, such as biopsies, were generally associated with a uniform, non-significant and reversible positive increase of relative IG discrepancies. Compared with measurements preceding the day of biopsy the median increase of the relative IG accounted +5.3% (+2.4% to +10.5%). With a subsequent negative increase of IG recordings normalized after a mean duration of 10 h following core biopsy. In agreement with the findings of IG analysis of the 25 biopsies in the control group, consisting of 2.9 specimens each, were judged free of evidence of rejection. The median BH-score was 1 (range 0–3). The median of the biopsy related day-to-day variability of relative IGs was +6.4% (-2.2% to +11.4%). On the basis of ROC-analysis all recordings were correctly analyzed for the exclusion of acute rejection yielding a sensitivity and specificity of 96%. Units

**Impedance analysis in transplanted porcine livers**

Uncompromised function of the liver grafts characterized the initial course of the four animals meeting the objective of terminal acute allograft rejection. In conjunction with operative trauma and graft reperfusion a common positive increase of relative IGs and of liver transaminases with a maximum on the 2nd postoperative day was observed. A synchronous negative increase of relative IGs and liver transaminases characterized graft recovery. Corresponding to the observations of the native liver group relative IG discrepancies in transplants did not drop below the 100% offset during histologic quiescence. With a median BH-score of 2, six of the biopsies obtained during the initial postoperative course were assumed positive for the exclusion of acute rejection. The median of the biopsy related relative IG accounted -9.1% (+1% to -12.3%).

Histology of rejecting biopsy specimens in subsequent biopsies was characterized by infiltration of the portal tracts by lymphocytes, mononuclear cells and eosinophils. As rejection progressed endothelialization of the hepatic venules coincide with the infiltration and spread into the parenchyma with resulting necrosis of hepatocytes and disruption of the lobular architecture. The aggressive course of acute rejection correlated with the degree of negative increase of relative IGs in each specimen. Progression of acute rejection with a median BH-score 5 (3–6) at the end of the experimental period was joined with a median of -47% (+1.8 to -92.9%) negative increase of relative IG discrepancies.

Altogether statistical analysis emphasized significant interrelation between the negative increase of relative IG discrepancies and the assigned results of porcine liver biopsies (r=0.96, P<0.001). During borderline rejection the relative bisensoric IG was -6.5%. Unisensoric recordings were heterogeneous and yielded a decline of -27% versus an increase of +14%. The fact may find explanation in correspondence to the supposition of heterogeneous initialization and manifestation of onsetting acute rejection. Biochemical accounts did not predict the individual fate of the transplanted livers and revealed any statistical correlation with the results of biopsies or with the courses of impedance recordings during onset and/or progress of acute rejection.

Considering the small number of animals meeting acute rejection the reliability of impedance recordings further on was tested by inter-group analysis. All measurements were analyzed for the absence (n= 90, median relative IG +2.2%) or the presence of acute rejection (n=4, median relative IG 48.7%). Non-parametric two-tailed analysis revealed significant difference between both (P<0.004, Mann–Whitney Test). There was significant correlation between the scored results of liver biopsies and the changes of relative IG discrepancies.

This observation implied that the severity of a rejection episode can be described by the degree of negative percent increase of relative IGs (r=0.84, P< 0.0001). With regard to biochemical significant differences were only ascertained for bilirubin (P<0.002, Mann–Whitney). Of the biochemical parameters only bilirubin was correlated with the scored biopsy specimens (r=0.6, P<0.002).

**IV. Conclusion**

Acute rejection remains a threat to graft survival. Electrical impedance analysis to monitor organ damage during ischemia and acute rejection has been introduced in recent years in heart, liver and kidney [8]. Today, there is virtually no other implantable and automatized sensor technique that can be used for less invasive diagnosis of acute rejection in liver transplants. Electrical impedance of living tissues gives access to valuable tissue compartment parameters which are telemetrically sent within seconds using minimally invasive, simple metallic electrodes.

Liver transplantation in pigs however merits the problem that acute rejection is less often and that long-term survival can be achieved without evidence of acute rejection [9]. Furthermore, the detection and differentiation of viral hepatitis is similarly limited because of the lack of suitable porcine models. Nevertheless, the anatomic similarities of porcine and human livers render the pig model favorable for
establishment of the technical feasibility and general applicability of electrical impedance analysis for detection of rejection, particularly since genetic differences between pig races allow enhancement of rejection. Organ temperature, configuration of the liver capsula, regional blood flow and local pressure to the liver surface by the impedance electrodes all influence the individual offset and the course of impedance reactance of the liver [10]. To abolish systemic errors of impedance measurements in the given experimental setting the characteristics of the animals and the fixation technique of the sensoric leads to the liver were standardized. Considering the influencing effects consequently the independent method of gradient calculation was used for comparative quantification.

In-vitro pretesting studies used confirmed addiction of impedance recordings to organ temperature. During pathologic conditions, changes of passive hepatic electric conductance as attributed by Kehrer et al. are traced back on dynamic ionic shifts affecting the hepatocyte- and endothelial membrane complexes, the intra- and extracellular spaces and their interspheric gap junctions. Temporary and/or reversible changes of impedance recordings on the other hand seem to mirror both destructive and regenerative morphologic processes of the intra- and extracellular components of the hepatocyte and the sinusoidal lining cells [11]. In all transplanted animals a uniform and negative increase of relative IGs characterized acute rejection. This consistency and the stability of relative IGs during functional and morphologic quiescence in native and transplanted livers implies evidence of interrelation between histologic, functional and the passive electrophysiological changes. Proven by ROC-analyses diminution of relative IG discrepancies of -24% of the preceding day served as a diagnostic criterion for acute rejection in pigs. The correlation between the negative increase of relative IGs and the severity of acute rejection suggested that not only the onset, but also the progressive course of rejection can objectively be monitored by trended impedance data. A non-linear negative increase of unipolar leaded impedance recordings, exhibiting a borderline-lesion on microscopic examination that may indicate the supposition of inhomogeneous onset of graft rejection. Because of the disparate cellularity, the inhomogeneous distribution of necrosis and fibrosis it is not improbable that the heterogeneity of spatial tissue characteristics can alter regional differences of electrical conductance. It can be stated that continuous impedance recordings with the technical equipment used can be done without the loss of information. Theoretically, on-line liver graft monitoring can be conducted over an unlimited period. All data once taken can be obtained for medical reports and retrospective scientific evaluations. The technique of computerized clinical data processing is simple and can thus easily be applied in clinical routine. The next step for this technology, which has not been implemented yet is to establish its value in monitoring effects of anti-rejection treatment, to differentiate rejection from preservation injury and viral hepatitis.

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