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The validity of the MEGX test in correlation with histology after orthotopic rat liver transplantation

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Abstract Lidocaine metabolism (MEGX test) as an indicator for liver function in the assessment of different degrees of liver disease and as a predictor for liver outcome after transplantation is well established. Since reduced liver function is associated with an alteration in parenchymal and non-parenchymal cells, we evaluated whether MEGX values correlate with histology in an in vivo model of orthotopic rat liver transplantation (ORLT) to assess histological damage without taking biopsy specimens. Livers from syngeneic Lewis rats were transplanted with rearterialization after 15–30 h of cold storage in UW solution and rinsing with Carolina Rinse Solution prior to implantation. Forty-eight hours after transplantation, the MEGX test was performed and metabolites were measured with a commercial kit as described elsewhere. Biopsy specimens were taken and graded three degrees of damage (mild, moderate, and severe) in a double blind fashion by a pathologist. MEGX values were assigned to the histological results. Statistical analyses were done with a Mann-Whitney test ($n = 58$) for mean values. The mean MEGX values attributed to histologies with a mild, moderate, severe degree of damage were 159.96, 78.46 and 44.42 ng/ml, respectively. When the histological groups were compared with the mean MEGX values, mild vs moderate, mild vs severe and moderate vs severe were significant ($P - 0.0001$). In conclusion, MEGX values correlate significantly with histological grading in a linear fashion after ORLT. The MEGX test may be of clinical value because it reflects the histological pattern of livers and may reduce the necessity to take biopsy specimens before and after transplantation.

Key words MEGX · Liver Transplantation · Histology

Introduction

The MEGX test has been shown to predict liver graft viability and initial graft function after orthotopic liver transplantation [1–3]. The principle of the liver function test is the selective metabolism of lidocaine by the microsomal cytochrome P450 IIIa of the liver. The formation of the metabolite monoethylglycine xylidide (MEGX) is measured 15 and 30 min after administration of lidocaine intravenously and is quantitatively determined by either the fluorescence polarization immunoassay (FPIA) or high-performance liquid chroma-

tography [4–7]. Although some transplant centers perform the MEGX test routinely during donor hepatectomies as an additional criterion to asses liver graft quality for further transplantation, the MEGX test has not fully been accepted as a standard parameter to assess liver graft function [8]. While the MEGX test is usually performed in donors, measurement of lidocaine metabolism in patients after liver transplantation may reflect the metabolic capacity of stored and reperfused and meanwhile regenerating liver grafts.

In a model of orthotopic rat liver transplantation with physiological cuff rearterialization of the donor celiac to recipient common hepatic artery [9, 10], we investigated the correlation of MEGX values with the degree of liver damage after cold storage and reperfusion assessed by histology 2 and 6 days after transplantation. Liver damage was achieved by prolonged cold storage of UW/preserved liver grafts. Clinical chemistry served as an additional criterion to assess liver graft damage.

Materials and methods

Animals

Male LEW (RT1[1]) rats, 10–16 weeks of age, were purchased from Charles River (Zentralinstitut für Tierversuchszucht, Hannover, Germany) and were used as donors and recipients for syngenic LEW → LEW transplants. All animals had free access to standard rat chow and tap water pre- and postoperatively. Surgery was performed using a clean, non-sterile technique. Ether was used for the anesthesia. All animal procedures had been approved by the local ethics committee, and the animal procedures were carried out according to the usual guidelines for the care of animals.

Surgery

Livers were transplanted using an arterialized model essentially as described by Steffen et al. [8]. Donor livers were flushed via the portal vein and hepatic artery with chilled UW solution, excised, and then stored in UW solution surrounded by an ice-water bath. Prior to implantation grafts were rinsed with Carolina Rinse Solution [10]. Implantation surgery required 60 min whereas anhepatic time was less than 15 min.

Experimental groups

Liver recipients received grafts that were preserved in UW solution at 1–4 °C. Groups with different storage times (< 1 h, 15 h and 30 h) were chosen to induce time-related preservation damage. Preservation damage was assessed by histology and clinical chemistry; therefore, liver recipients were sacrificed at post-operative day 2 or post-operative day 6. Each group comprised 10–12 animals.

Clinical chemistry

ASAT, ALAT, GLDH, GGT, AP, direct and indirect bilirubin, cholinesterase, and albumin were analyzed using standard methods.

Histology

Specimens of liver grafts were collected from interindividual constant anatomical sites after performance of the MEGX test and terminal exsanguination. Blocks for histology were fixed in 5% formaldehyde, embedded in paraffin, cut into 5-µm-thick pieces, and were stained with H&E for light microscopy. The degree of preservation damage was semiquantitatively assessed by a pathologist in a double-blind fashion. The following grading system was used:

Degree 1: normal liver or mild preservation damage (single hepatocyte necrosis, vacuolization)

Degree 2: preservation damage limited to pericentral regions of liver lobules

Degree 3: severe preservation damage in all zones of liver lobules, resulting in necrosis.

Five microscopic fields at × 100 magnification were analyzed per grafted liver.

MEGX test

At either post-operative day 2 or post-operative day 6 animals were anesthesized and lidocaine (1 mg/kg bodyweight) was injected intravenously through the inferior vena cava with a butterfly cannula; 0, 15 and 30 min after lidocaine injection blood was drawn, centrifuged at 150 g, and stored at −70 °C. The metabolite monoethylglycine xylidine (MEGX) was measured with a commercial test kit (Abbott TDx system, Abbott Laboratories) according to the method described by Oellerich in 1987 [11].

Statistics

The Mann-Whitney test was used for statistical analyses. MEGX values were compared with the different degrees of histological grading.

Results

Survival

Fifty-eight of the 68 rats included in the study survived after ORLT. Seven of the 10 rats that died received grafts that were stored for 30 h and developed multiorgan failure due to preservation injury; 3 rats died due to biliary complications.

Clinical chemistry

Serum transaminases ranged between 12 and 8460 U/l.

At post-operative day 2 very high levels of serum transaminases were observed in recipients that received liver grafts stored for 30 h. Elevated levels were also observed when grafts were preserved for 15 h, while livers

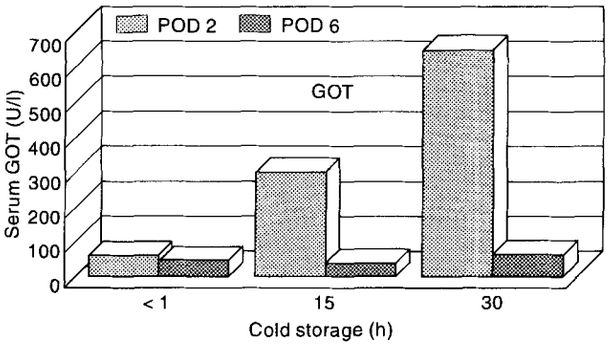

Fig. 1 Serum transaminase after different times of cold storage and orthotopic rat liver transplantation: POD 2 and POD 6 (*POD* post-operative day)

which were transplanted immediately after harvesting released transaminases with nearly baseline levels. At post-operative day 6, serum transaminase in the liver grafted rats returned to the normal range (Fig. 1).

While serum transaminase showed significant differences among the experimental groups, bilirubin, serum cholinesterase levels and albumin did not indicate the degree of preservation damage according to the length of cold storage.

Histology

At post-operative day 2 histology displayed a mild degree (degree 1) of preservation damage when grafts were stored for less than 1 h. The parenchyma and portal structures appeared normal. Only occasional mononuclear cells were detected in portal areas. Bile ducts displayed no abnormalities (Fig. 2). At post-operative day 6 histology revealed no significant differences.

Livers stored for 15 h in UW solution showed moderate damage (degree 2) with single foci of ischemic necrosis scattered throughout the lobules, predominantly in pericentral regions. At post-operative day 6, the damage was pronounced with inflammatory cell infiltrates (Fig. 2). Grafts stored for 30 h (degree 3) were severely damaged with extensive ischemic and hemorrhagic necrosis of hepatic parenchyma (Fig. 2). At post-operative day 6 severe cellular infiltrates were discernible throughout the lobules.

MEGX test

Determination of MEGX values correlated significantly ($P < 0.001$) with different histological degrees of preservation damage. The liver grafts that showed a mild degree

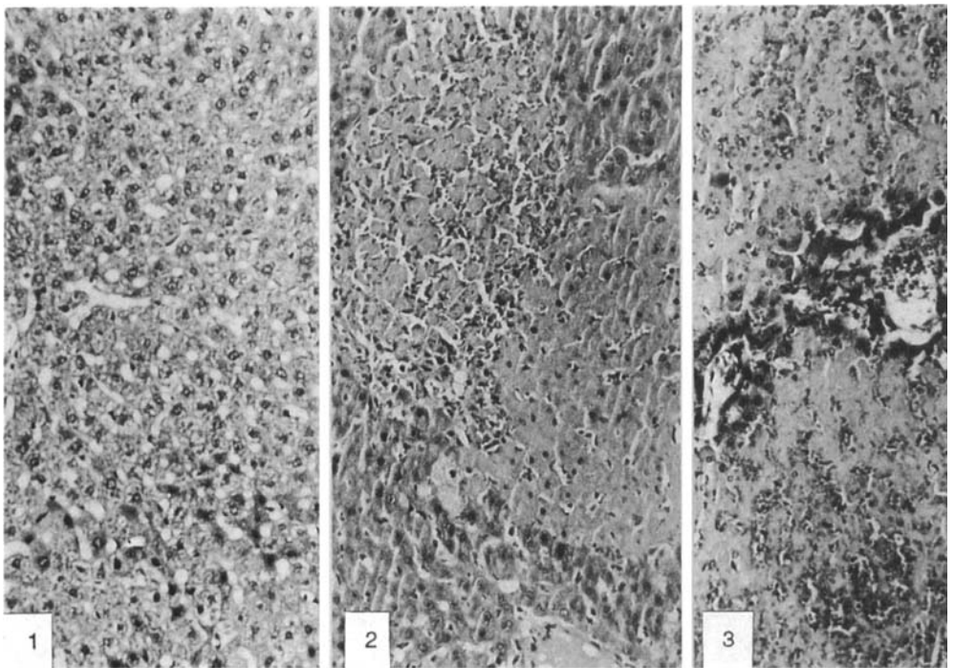

Fig. 2. Degrees of histology (1, 2, 3) after < 1, 15, and 30 h of cold storage and orthotopic rat liver transplantation

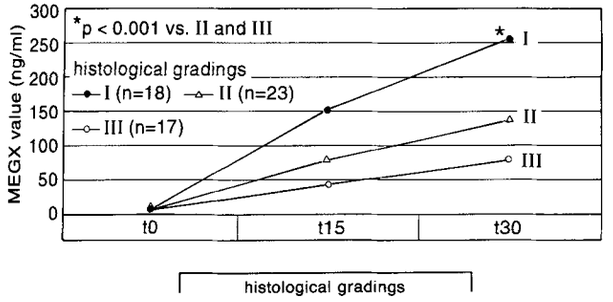

Fig. 3 Correlation of MEGX values and histology

Table 1 MEGX values compared with histological damage. MEGX values: Mean ± SD [ng/ml]

Histological grading	I	II	III
n	18	23	17
t0	4.9 ± 3.4	5.7 ± 5.2	6.0 ± 1.8
t15	152.6 ± 65.5	79.4 ± 45.2	43.6 ± 17.3
t30	257.6 ± 104.1 [a]	137.1 ± 80.0	79.2 ± 36.2

[a] $P < 0.001$ vs II and III

(degree 1) of damage achieved the highest MEGX formation rate 15 min after lidocaine injection. The grafts that showed severe damage (degree 3) achieved the lowest rate of MEGX formation (Fig. 3). Thirty minutes after lidocaine injection the relationship between histological degrees 1 and 2 was essentially the same. Statistical analysis showed high significance (Table 1).

Discussion

Livers stored for transplantation surgery are subject to graft dysfunction of variable degree due to donor-related conditions and diseases, storage injury, and reperfusion injury [12]. Liver damage after cold storage and reperfusion is usually assessed by the highest peak of serum transaminase at post-operative day 2. While our data clearly show a difference between serum transaminase levels of liver grafts stored for either < 1 h at either 15 h or 30 h, serum transaminase levels in humans after liver transplantation do not correlate well with the final outcome in terms of survival. Conventional liver function tests, including serum transaminases, total bilirubin, and PTT of patients during the early postoperative period, have been shown to be unhelpful in predicting the outcome of allografts. ICG and the MEGX test, however, displayed good correlation with outcome after liver transplantation [13]. Since both tests reflect hepatic blood flow, deterioration of graft function is obviously accompanied by reduced graft perfusion.

The MEGX test may therefore serve as an additional criterion to diagnose graft dysfunction and initial non-function post-operatively. While the MEGX test has been established at many centers as part of donor management, its status in recipient follow-up must still be determined. The usefulness of the MEGX test in liver recipients was shown by Schroeder et al. who demonstrated low MEGX formation rates in the presence of rejection or ischemic injury [14]. A decline in MEGX values preceded alterations in conventional liver function tests.

Clinical studies of MEGX tests determined the time point to measure the concentration of MEGX 15 min after bolus injection of lidocaine intravenously. This interval was derived from measurement of MEGX in healthy subjects who showed the highest levels of the metabolite 15 min after injection and maintained high levels for up to 2 h [2]. In contrast, patients with liver cirrhosis developed a slow increase of MEGX after 15 min and rose continously to a peek at 240 min. In our experiments we observed rising MEGX values from 15 to 30 min after injection, comparable to the slow rise of MEGX in cirrhotic patients. The normal half-life of lidocaine in rats is much shorter than in humans (30 vs 90 min) [15], indicating an accelerated metabolic rate in rats. Biochemical studies, however, demonstrated closely related properties of the human and rat cytochrome P-450 enzymes in hepatic microsomes [16]. Interestingly, genetic deficiencies in the metabolism of lidocaine have been reported in Dark Agouti (DA) rats in comparison to Wistar rats. In addition, male and female DA rats showed differences in metabolic activity [17]. Lewis rats were the only inbred strain used here and all animals were male, so it was possible to rule out any possible interference with intersexual differences.

Since the survival of liver graft recipients that received grafts with different times of cold storage was not a parameter in this study, no correlation between MEGX values and survival is identifiable. The most remarkable finding in this study was that MEGX formation is significantly dependent on the structural integrity of liver grafts. Severely damaged liver grafts due to prolonged preservation may result in fewer intact hepatocytes with an overall reduced amount of cytochrome P-450. An enzyme induction occurring in the remaining viable hepatocytes is unlikely since graft recipients were not treated with any medication. In our studies MEGX formation rates reflect the degree of histological damage

and also correlate with the amount of serum transaminase. However, in models of partial hepatectomy the MEGX formation rate did not correlate well with the removed liver mass and seemed to be more indicative of the traumatic removal per se than the amount of tissue removed [18].

Our findings are consistent with other reports that consider the MEGX test to be a global indicator for functional integrity of harvested and transplanted livers. Therefore, the MEGX test may be used in the decisive process of accepting liver grafts for transplantation. The MEGX test is also a valuable tool during the first few days after liver transplantation to assess damage due to preservation and reperfusion injury, initial non-function, and may even predict and foresee the short-term clinical course of the recipients.

We conclude that the MEGX test is a rapid dynamic test that can also able predict the potential function of liver grafts after transplantation and that the correlation of MEGX values and histological grading after liver transplantation may be of great interest in terms of reducing the amount of biopsies needed in the early postoperative period.

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