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Value of the in vitro or in vivo monoethylglycinexylidide test for predicting liver graft function

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Abstract

**Background** An adequate function test for donor livers is still lacking. The monoethylglycinexylidide (MEGX) test, performed in vivo in the donor to measure the metabolic rate of lidocaine conversion to MEGX, has been proposed as a function test for donor livers to predict post-operative organ function.

**Methods** In the present study, we investigated whether the MEGX formation rate measured in needle biopsy specimens in vitro correlates with the rate of MEGX formation in vivo. The in vivo MEGX test was performed in the donors and in the recipients on day 1 and 2. The in vivo and in vitro MEGX tests were compared with posttransplant liver function in the recipients in order to investigate their possible relevance as predictor for graft function.

**Results** The MEGX formation rate in needle biopsy specimens in vitro showed a significant correlation with the MEGX serum concentration found in the donor. A low rate of MEGX formation in the biopsy specimens tended to predict initial poor function of the grafts. In the donor, the MEGX test did not correlate with general liver function after transplantation. Only the MEGX serum concentration in the recipients on day 2 gave an indication of graft function.

**Conclusions** MEGX formation in liver biopsy specimens in vitro properly reflects metabolic function of the particular liver. Therefore, liver biopsies may be a valuable tool to help predict liver function in vivo. However, the MEGX test alone is not sufficient to provide the gold standard to determine liver function in donor and transplantation livers.

**Introduction**

The proper selection of donor livers is a persistent problem in liver transplantation. No consensus on the acceptance criteria for potential donor livers has been achieved. Donor parameters, such as age and length of stay on an intensive care unit have been reported to be related with post-operative organ function. The metabolic rate of lidocaine de-ethylation to monoethylglycinexylidide (MEGX),
mediated by cytochrome P450 3A4, has been suggested as a function test for donor livers and was presented as the MEGX test. The MEGX test was proposed to be performed in donors before procurement in order to predict post-operative organ function. In the study of Oellerich et al., the probability of graft survival over 120 days was significantly higher for livers from donors with MEGX test values above 90 µg/l than for livers from donors with MEGX serum values of 90 µg/l or lower. However, Tesi et al. showed that liver donors with MEGX serum concentrations values of 50-90 µg/l can be transplanted safely. Also, in other reports, the unreliability of the MEGX test in predicting postoperative organ function was described. One of the possible causes for these controversial findings may be that lidocaine is a drug with a high first-pass extraction. Therefore, an alteration in hepatic perfusion will largely influence lidocaine clearance. Moreover, many liver donors receive vasoactive drugs, which may influence liver blood flow and, consequently, lidocaine clearance. Another reason may be that after performance of the MEGX test, the donor liver is subject to other events that may influence graft performance, such as procurement and reperfusion.

Our study addresses several questions. First, we wanted to assess the possibility of performing the MEGX test in vitro on needle biopsy specimens in donor livers in order to exclude the influence of hepatic blood flow. Therefore, we investigated the metabolic rate of lidocaine in biopsy specimens and correlated it with the value obtained in the donor in vivo. Secondly, we wanted to investigate whether such an in vitro MEGX test was a better predictor of postoperative graft function than a MEGX test performed in the donor in vivo before procurement. Moreover, because the biopsies were performed after cold storage of the organ, possible initial preservation damage might be evaluated. Finally, we investigated whether the MEGX test in the recipients might be an early marker of the liver function after transplantation and whether it could be used to predict initial poor function of the liver graft.

Materials and Methods

Materials

Lidocaine for the in vitro studies was obtained from Centrachemie (Etten-leur, The Netherlands). Lidocaine used in vivo was Xylocaine.
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Monoethylglycinexylidide (MEGX) was a kind gift of ASTRA (Sodertalje, Sweden). University of Wisconsin organ preservation solution (UW) was obtained from Du Pont Critical Care (Waukegan, Illinois, USA). All other chemicals were of analytical grade and were obtained from commercial sources.

**MEGX test in vivo**

Consent from the legal authorities and from the families concerned was obtained for the explantation of organs for transplantation purposes. The research protocols were approved by the medical ethical committee of our institution.

Lidocaine metabolism was assessed before removal of the liver by administering 1 mg/kg as a single intravenous bolus. Before dosing and 30 minutes after the bolus was given, a venous blood sample was drawn. Thereafter, the organ procurement was continued according to standard procedures. Just before UW perfusion, an additional venous blood sample was taken for routine analysis of donor blood. Donors, who received dopamine to stabilize the blood pressure, were categorized in two groups: those with a high dose of dopamine infused > 4 µg/kg/min and those with a low dose 0-4 µg/kg/min.

The donor livers were preserved in cold UW on melting ice (0-4°C). Blood samples were stored on ice. Analysis of the samples was performed when the organ reached the transplantation hospital.

To assess the function of the donor liver in the recipient, the patients received 1 mg/kg lidocaine 12 hours (day 1) and 36 hours (day 2) after transplantation in a single bolus. A venous blood sample was drawn before and 30 min after the bolus was given and the lidocaine metabolite MEGX was determined in serum.

**MEGX test in vitro**

During the transplant operation, just before the donor liver was taken from the cold UW solution for implantation, a needle biopsy was taken from the right lobe with a Hepafix® 1.6 mm biopsy needle. The needle biopsies (20-200 mg) were immediately immersed in UW solution (at 0-4 °C) transported and were kept in ice-cold UW, until the experiment was performed (not exceeding 12 hours).

The biopsy specimens were cut into fragments of 1-3 mm³, and incubated in 3 ml of Krebs-Henseleit buffer with 1 % BSA in a shaking water bath at 37 °C under
carbogen (5% CO₂/95% O₂) gassing. After preincubation for 30 minutes, lidocaine was added to a final concentration of 5 mM. After 30 and 60 minutes medium samples (0.8 ml) were taken, HClO₄ was added to a final concentration of 6% (v/v), and the samples were quickly frozen in liquid N₂ and stored at -80 °C until analysis by high-performance liquid chromatography, as previously described.

**Graft function**

The recipients were routinely evaluated on day 1, 3, 5 and 7 after the transplantation. On these days a venous blood sample was drawn, to determine the standard blood tests for recipients.

The recipients were grouped according to graft function with initial poor function (IPF) or immediate function of the graft using the classification described by Ploeg et al. Ploeg et al. defined IPF as a form of primary dysfunction with on posttransplant days 2-7: serum glutamate-oxal-acetate-transaminase (GOT) > 2000 U/l, prothrombin time (PTT) >16 sec and ammonia levels >50 µmol/l.

In a second evaluation, patients were grouped according to graft function: good, moderate and severe dysfunction as previously proposed by Gonzalez et al.

The moderate graft function was defined as serum glutamatepyruvate-transaminase (GPT) 1000-2500 U/l, the bile output 40-100 ml/day and prothrombin activity >60% (<20 sec) (with fresh-frozen plasma). Severe dysfunction of the graft was assessed if the serum GPT >2500 U/l, the bile output <40 ml/day and the prothrombin activity <60% (>20 sec) (despite fresh-frozen plasma).

**Statistics**

Normal distribution was tested by Kolmogorov-Smirnov goodness of fit test. If non-normal distribution was assessed, a log transformation was performed and distribution was tested again by Kolmogorov-Smirnov goodness of fit test. Linear regression analysis was used to detect possible significance of correlation. The two groups formed based on the classification of Ploeg et al. (IPF or immediate graft function) were compared with respect to the experimental data by the independent two-tailed t-test. The three groups formed according to the classification of Gonzalez et al. (good, moderate or severe dysfunction) were compared with respect to the experimental data by using analysis of variance. A p-value < 0.05 was considered statistically significant. SPSS version 6.0 statistical software (SPSS, Inc. Chicago, IL) was used.
Results

In total, 47 liver transplantations were included in this study. The livers were stored in ice-cold (0 °C) UW for 11 ± 3 hours. The in vitro MEGX test was studied in 41 needle biopsy specimens taken from donor livers. The in vivo MEGX test was performed in 22 donors (including 16 of the donors where also a biopsy was taken), in 21 recipients on day 1 and in 15 recipients on day 2 (Figure 1).

After 41 liver transplantations had been evaluated (6 liver transplantations could not be evaluated due to organizational reasons), 6 grafts with IPF and 35 grafts with good graft function were observed according to the classification of Ploeg et al. 1. According to the classification of Gonzalez et al. 16, 22 recipients had good graft function, 11 moderate functions and 8 severe dysfunction. The MEGX formation in vitro in biopsy specimens was linear up to 60 minutes. The distribution of the MEGX formation rate measured in the needle biopsies is depicted in Figure 2.

Figure 1  The number of samples of MEGX formation in Needle biopsies, in the donors (Donor) and in the recipients on day 1 and 2 (Day 1 and Day 2). n is the total number of samples. The numbers between parentheses are the number of samples that were compared.

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The in vitro MEGX formation data suggested a bimodal distribution where two groups could be identified, those with a metabolic rate higher than 20 nmol/g liver, min (fast metabolizers) and those with a low rate < 20 nmol/g liver, min (slow metabolizers). Analysis of the distribution with the Kolmogorov-Smirnov goodness of fit test revealed that MEGX formation in vitro was distributed normally with borderline significance (p=0.05). After log transformation of the MEGX formation the in vitro values were normally distributed (p=0.69). Therefore, the correlation of the in vitro data with all other parameters was also tested with log-transformed data. However, no differences were observed between linear data and the log-transformed data with respect to the significance of the correlations between the in vitro and in vivo data.

In vivo - in vitro correlation
The in vivo MEGX formation was normally distributed (data not shown). Linear regression was performed with the in vitro MEGX data and the in vivo MEGX formation. When the rate of lidocaine metabolism assessed in needle biopsy specimens from donor livers was compared with the values obtained in vivo in the donor a significant linear correlation ($r^2 = 0.30$, $p<0.05$) was found (Figure 3).
When the in vitro MEGX data were compared with the MEGX concentrations from the recipient on day 1 ($r^2=0.05$, $p=0.43$) and day 2 ($r^2=0.20$, $p=0.17$), no significant linear correlation (NS) was observed (Figure 4).

**Figure 3** Donor MEGX in µg/L serum versus MEGX in nmol/g liver, min in needle biopsies from donor livers. (n=16)

**Figure 4** A. MEGX in µg/L serum in recipient on day 1 versus MEGX in nmol/g liver/min in needle biopsies from the donor livers. (n=16; NS) B. MEGX in µg/L serum in recipient on day 2 versus MEGX in nmol/g liver/min in vitro in needle biopsies from the donor livers. (n=11; NS)
Patients who received a donor liver with a low (<20 nmol/g liver, min) in vitro metabolic activity exhibited a significantly lower MEGX on day 2 (44.6 ± 18.2 µg MEGX/l serum), than those (100.5 ± 55.8 µg MEGX/l serum) who received a donor liver with high in vitro activity, as tested with an independent t-test. No such correlation was found using the day 1 MEGX test data.

In vivo correlation

The MEGX serum concentration in the donors showed a highly significant correlation ($r^2 = 0.95$, $p < 0.0005$) with the MEGX serum concentration found on day 1 in the recipients (Figure 5 A).

However, no correlation was found between the MEGX serum concentration in the donors and the MEGX serum concentration at day 2 in the recipients ($r^2 = 0.39$, $p = 0.37$) (Figure 5 B), but in this case only four samples could be compared. The day 1 MEGX concentration in the recipients did not correlate with the day 2 MEGX concentration in the recipients ($r^2 = 0.21$, $p = 0.1$) (Figure 6).
The mean values of the MEGX concentration formed in vivo in the donors and recipients were compared: the MEGX formation on day 1 in the recipient was significantly lower, as shown by independent t-test and paired t-test, than the MEGX observed in the donors and on day 2 in the recipients (Figure 7).

Figure 6  MEGX in recipients on day 2 in µg/L serum versus MEGX µg/L serum in recipients on day 1 (n=13; p=NS).

Figure 7  The MEGX in µg/L serum in donors and recipients on days 1 and 2. Data are expressed as mean ± SEM (n= number of experiments. *p<0.05).
The dose of dopamine administered to the donors apparently did not influence the results of the MEGX test in vivo: no differences were found in the high and low dose groups, when compared by independent t-test, with respect to the MEGX test in the donors, MEGX test in the recipients on day 1 and 2 and MEGX formed in vitro.

Comparison of the in vitro and in vivo MEGX tests with graft function

The MEGX formation rate observed in needle biopsy specimens showed an almost significant correlation with liver graft function as classified according to Ploeg et al. \(^1\) (p=0.054). Livers showing IPF had a lower MEGX in vitro than livers that did not show IPF. However, no significant difference in in vitro MEGX formation in the various groups was observed when the patients were classified according to the method of Gonzalez et al. \(^{16}\).

The MEGX serum concentration obtained in the donors did not correlate with liver function classified either according to Ploeg et al. or to Gonzalez et al. \(^{1,16}\). If the donor MEGX test was classified according to Oellerich et al. \(^5\), i.e., MEGX in the donor < 50 µg/l and MEGX between 50-90 µg/l and a group higher than 90 µg/l \(^5\), no difference was observed between these groups with respect to graft function.

The MEGX concentration found in the recipients on day 2 showed a significant correlation with liver function as classified according to Gonzalez (p=0.038). The group with severe graft dysfunction had a lower MEGX concentration on day 2 compared to the groups with moderate and good graft function. There were insufficient data to compare IPF of the grafts as classified by Ploeg et al. \(^1\) with respect to the data from the recipients’ MEGX test on day 2.

Comparing post operative liver function tests with MEGX formation rate in vitro and in vivo

When the MEGX formation rate in vitro was compared to the post operative liver function tests, no correlations were found with the parameters measured in serum. However, in the group of patients who received a fast-metabolizing liver, serum GOT on day 3 and day 5 were significantly lower (p<0.05, independent t-test) than levels in the group receiving a slow-metabolizing liver. A similar trend was observed for serum GPT and pro-thrombin time on day 5 (p=0.059). The donor MEGX concentrations significantly correlated with serum GPT on day 1.
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The MEGX in the recipient on day 2 showed a significant correlation with serum GOT on day 1, and with serum GPT on day 3, 5 and 7. None of the routinely tested clinical parameters in donor blood was related to graft function in the recipient.

Discussion

In vivo and in vitro correlation
As was shown by our results it was possible to measure MEGX formation in needle liver biopsy specimens. This was also reported by Meyer et al. 17. The amount of MEGX formed in vitro showed large interindividual differences, which were also found in vivo 5. In addition, livers could be classified into two groups according to their lidocaine metabolic rate, as was also reported for MEGX formation observed in vivo 5.
The values of MEGX found in the biopsy correlated significantly with the MEGX found in vivo, indicating that the needle biopsy might be used to predict the in vivo rate of lidocaine metabolism. The needle biopsies were performed after preservation of the graft in ice-cold UW. This suggests that lidocaine metabolism is either similarly altered in all stored donor livers or that cold storage of the donor liver in ice-cold UW does not influence MEGX formation. The latter hypothesis is supported by the conclusions obtained in a study with human liver slices showing well preserved metabolic rate of lidocaine metabolism in slices during 18 hours of storage in UW 8.
In a case study, Sallie et al. 19 observed MEGX formation after a lidocaine bolus (1 mg/kg) in an anhepatic patient. After 30 minutes, 17 µg/l MEGX was found, indicating extrahepatic metabolism. Nevertheless, in the present study we observed that the metabolic activity determined in the in vitro preparation from the liver correlated well with that found in vivo. This might indicate that biotransformation in the liver is the major site of metabolism of lidocaine, which is also indicated by the fact that MEGX concentration found in the donor are usually much higher than 17 µg/l (figure 3).

In vivo correlation
The MEGX serum concentration in the donors correlated significantly with the MEGX found in the recipients on day 1. However, the concentration of MEGX on
day 1 was significantly lower compared with the value obtained from the donors, indicating possible reperfusion damage. The increased MEGX formation on day 2 in the recipients indicates restoration of the metabolic function to pretransplantation levels. One of the possible causes of this reperfusion damage may be the cascade of interleukines produced and observed after reperfusion in liver transplantation. In studies with rats and pig hepatocytes, IL-6 was shown to initiate down-regulation of cytochrome P450 concentrations. Furthermore, in healthy subjects lidocaine is bound for about 50% to plasma proteins, predominately to α1-acid glycoprotein. After transplantation α1-acid glycoprotein increases, which may lead to lower concentrations of the unbound lidocaine, and subsequently to a lower amount in the liver and finally to a lower rate of MEGX formation. Experiments are currently underway to elucidate these hypotheses. The donors with a hemodynamic instability, which was corrected with relatively high doses of inotropic agents, showed no differences in the MEGX formation rates compared to hemodynamically stable donors. These results indicate that the temporary period of hemodynamic instability does not irreversibly influence lidocaine metabolic rate. Moreover, the livers obtained from donors who received high amounts of inotropica showed no differences in MEGX formation rate measured in vitro or after transplantation in the recipient. These results imply that inotropica administered to the donor did not influence the metabolic function of the graft.

**Comparing MEGX test in vitro and in vivo with graft function**

The group with good function of the graft tended to have a higher in vitro MEGX formation rate than the group with an initial poor function of the graft (according to Ploeg et al.). This trend suggests that needle biopsies may be used to assess the graft function. However, no differences in in vitro MEGX formation rate were found between the groups classified according to Gonzalez et al. The present results suggest that MEGX test in vivo in the donor has no predicting value for the graft outcome. The only MEGX value that correlated with liver function, classified according to Gonzalez et al., was the MEGX on day two in the recipient, indicating that MEGX test reflects graft function in the recipient.

**Comparison of post operative liver function tests with MEGX concentration**

Donor livers with low in vitro metabolism produced a high serum GOT level in
the recipients. This suggests that a lower MEGX concentration in vitro indicates a lower viability of the liver graft. A negative correlation was also found between serum GOT and GPT in the recipient and the MEGX formation rate on day 2. This again suggests that MEGX on day 2 is correlated to the viability of the graft. In conclusion, in the present study, the MEGX test in the donor in vivo appeared not to have predictive value for graft function. The MEGX test in the recipients on day 2 after transplantation seemed to be useful for indicating liver function. A MEGX test on day 1 in the recipient is not recommended for this purpose, because post-operative events, such as changes in protein binding and reperfusion damage, may probably influence the metabolic activity of the graft. The in vitro MEGX test, which showed a trend for predicting IPFs, may be a better alternative. However, the power of this test is limited and the in vitro MEGX test is unlikely to become the gold standard. However, testing several functional parameters at the same time in needle biopsy specimens might help to determine different aspects of donor liver function and might result in a more powerful test to predict liver graft function.
References

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19. Sallie RW, Tredger JM, Williams R. Extrahepatic production of the lignocaine metabolite mono-
20. Steininger R, Roth E, Fugger R, et al. Transhepatic metabolism of TNF-alpha, IL-6, and endotoxin in
the early hepatic reperfusion period after human liver transplantation. Transplantation 1994; 58
(2): 179.
22. Monshouwer M, Witkamp RF, Nijmeijer SM, Van Amsterdam JG, Van Miert ASJPAM. Suppression
of cytochrome P450- and UDP glucuronosyl transferase dependent enzyme activities by
pro-inflammatory cytokines and possible role of nitric oxide in primary cultures of pig hepatocytes.
23. Drayer DE, Lorenzo B, Werns S, Reidenberg MM. Plasma levels, protein binding, and elimination
data of lidocaine and active metabolites in cardiac patients of various ages. Clin Pharmacol
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