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#### **Research Article**

Prediction of Graft Function after Liver Transplantation in Rats Based on Mitochondrial Permeability — a New Prognosis Scoring System to Identify Marginal Organs after Ischemia-Reperfusion Induced Graft Injury

Haiges Daniela<sup>1\*</sup>, Babaryka Gregor<sup>2</sup>, Otto Angela<sup>3</sup> and Matevossian Edouard<sup>1</sup>

<sup>1</sup>Department of Surgery, Technische Universität München, Germany <sup>2</sup>Department of Pathology, TU Munich, Germany <sup>3</sup>IMETUM, TU Munich, Germany

#### Abstract

Aim: Transplantation of expanded donor criteria organs due to prolonged cold ischemia is one of the major options to increase the use of such organs and therefore reduces mortality. However this may lead to dysfunction of the organ. Therefore it's necessary to develop a predictive analytic method in order to identify such expanded donor organs and exclude them from transplantation.

Method: The government of Bavaria, Munich, approved the animal studies.

We used special LEWIS-(RT)-rats which were divided into three groups (n=4), depending on cold ischemia time and loss of ATP production after 2, 4 and 8 hours

(post explantationem). The evaluation of the ATP production and histopathology was limited to such animals where an hepatic phase was <20 minutes und the transplantation time <60 min. For statistical analysis we used a program called software R, Version 2.12.2 (Development Core Team). The significance was 5 %.

**Result:** There was an inverse correlation between the ATP/protein date and the histopathology of the ischemia-reperfusion-injury (p=0,003).

**Discussion:** We evaluated the degree of the ischemia-reperfusion injury by comparing the mitochondrial permeability and the histopathology. By having a prolonged ischemia of 8 hours, the evaluation of the ATP/protein production was more sensitive than the histopathology. Thus, this fact could be used in order to obtain a score that could identify extended donor organs.

#### **ABBREVIATIONS**

LTx: Liver Transplantation; ATP: Adenosintriphosphate; IR: Ischemia-Reperfusion injury

## INTRODUCTION

At the present time, liver transplantation is the only chance

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#### \*Corresponding author

Daniela Haiges, Department of Surgery, Klinikum rechts der Isar der Technische Universität München, Ismaninger Straße 22, 81675 Munich, Germany, Tel: 0049-151-7033-4520; Email: daniela\_haiges@web.de

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#### **Keywords**

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to ensure survival when suffering from a chronic liver failure and all other conservative therapies had been exhausted [1]. A liver transplantation is performed when a chronic liver disease, a metabolic disease, genetic disease, vascular diseases, malignant diseases or trauma occurs in the liver [2]. Though the promising outcome and the prolongation of life there must not be forgotten the fact that there is an extremely lack of compatible organs

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which minimizes the transplantation rates. In Germany 1491 people needed a liver and only 779 where transplanted from deceased donors [3]. In order to solve the dilemma with a lack of organs in regards of the need, organs with "expanded criteria" were used for the potential donor pool [4].

Using those organs raises a lot of questions, which are still unsettled. At the present time there is no precisely definitions of the characteristics of expanded criteria organs. In literature the use of those organs is sometimes associated with a transplant dysfunction afterwards [5].

Therefore it is necessary to develop a experimental method which is able to identify such organs which would be qualified for a transplantation and would not be associated with a transplant dysfunction afterwards [6].

Due to former experiments and results in our working group, we decided to focus on the Adenosintriphosphate (ATP) production in mitochondria in order to access the vitality of the expanded criteria organs. As a consequence of the ischemia-reperfusion injury (I/R) the mitochondrial permeability increases which therefore leads to a decrease in production of ATP.

We focused on following questions:

Is the level of ATP in correlation with the histopathology a tool to evaluate the vitality of these organs and could therefore ATP be used as a measurement of these organs?

# **MATERIALS AND METHODS**

ATP measurement: We used male syngeneic Lewis-CD-rats with a weight between 220-240gram.

The explantation of the rat livers was performed at the Klinikumrechts der Isar, Munich. Isofluran 5% was used for anaesthesia. The steps of the explantation are described later. After explantation the rat livers were carried in a culture medium to the research centre IMETUM, Garching, Germany. The medium consisted of Dulbecco's Modified Eagle's Medium (DMEM base) + 0,35 g/l NaHCO3 + 2mM L-Glutamin + 5% FCS +20mM HEPES + 25 mM Glucose with a pH around 7,25.

Regarding the ischemia, the rat livers were assigned to different ischemia time: 2h, 2,25h, 2,5h, 3h, 3,5h, 4h, 4,5h, 5h, 5,5h, 6h, 7h, 8h, 9h, 10h. Each group consisted of 4 rat livers.

The ATP measurement was based on an ATP lite Luminescence ATP Detection Assay System (Perkin Elmer). The mechanism is based on a light emission, which is proportional to the ATP concentration.

After adding 400µl Mammalian Cell Lysis Solution of the KIT to the explanted livers, which marked ischemia time. Then the livers were crushed using a ultrasonic wand. After centrifuging the samples with 2500 rpm, 50 µl of each sample were transferred to 96 well plate in which 100 µl PBS had been put. Then 50 µl of a new substrate solution were put into the wells. By adding the lyophilized substrate Solution, a low ph was created, which catalysed the reaction. The exact details are seen on Figure 1.

To exclude different weights of the liver and different masses, we did a protein measurement, using the BRADFORD-test.

Based on the ATP measurement we divided the rats in groups



Figure 1 Function of the ATP KIT http://www.perkinelmer.com/ Catalog/Product/ID/6016947.

for the liver transplantation. This lead to 3 groups with an ischemia time of 2, 4 and 8 hours. Each group consisted of 4 rats.

We performed a liver transplant surgery after the technique of Kamada et al [7]. Instead of using splints, every vessel was sutured. We ligated the liver vessels and the ductus choledochus distant from the rat livers for explantation. We inserted a plastic splint (22 G, Vasculon<sup>®</sup> Plus) [8] into the ductuscholeochus and fixed it with a suture in order to simplify suturing the ductus choledochus when transplantation the liver into a new rat recipient (Figure 2). The aorta abdominalis was clamped and perfused with CUSTODIOL through inserting a small catheter (14 G, Venflon<sup>®</sup>) into the aorta. Subsequent the ligation of left Vena phrenica, infrahepatic Vena cava inferior, Vena renalis, V. suprarenalis and the A. hepatica communis followed. At last we ligated the infrahepatic Vena cava.

When explanting the liver of recipient rat, we ligated the vessels close to the liver.

After the ischemia time of 2, 4 and 8 hours we transplanted the livers. Therefore we sutured the vessels of the Venacava inferiors first (Figure 3,4). Before anatomising we used isotonic saline solution to flush the lumen of the vessels to prevent air bubbles and as a consequence to prevent a lung embolism [9]. We also sutured the potal vein and the Ductus choledochus. After finishing the transplantation, we used the production of bile for the success of the transplantation. Then after 5 minutes of a sufficient blood flow, the rats were euthanatized and the livers were sent to pathology. Due to literature, donation after circulatory death provides a similar outcome as donation if brain-death donors do [10].

The histology was analysed at the Department of Pathology using Elastica-van-Gieson and hematoxylin-eosin stain.

## **RESULTS AND DISCUSSION**

During prolonged ischemia due to the lack of oxygen, the

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Figure 2 The anastomosis of the portal veins.



**Figure 3** The donor liver in situ of the recipient.

levels of ATP production decrease slowly resulting in cell death.

The lack of oxygen inhibits the respiratory chain leading in decreased production of ATP [11]. Furthermore the ATP synthase starts then hydrolyzing remaining ATP to ADP which further aggravates the loss of ATP.

Various intracellular ion channels and enzymes are based on the availability of ATP. Therefore a lack of ATP leads to an accumulation of lactate [12] and other metabolic products. This leads further on to morphologic changes of the cells: swelling, necrosis and apoptosis. The time how long ischemia is tolerated in certain organs is still for some organs unclear and prolonged ischemia might even increase cell death rather than being used as a precondition to induce tolerance [13,14].

We noticed a significant decline in the ATP production from

a ischemia time from 2-10 hours with an average of  $0,0067\mu g/ml$  (95% confidence interval: [0,0019; 0,0115], p=0,010). A steady state was reached after 7 hours with an ATP production of  $0,01\mu g/ml$  (Figure 5).

The results of the histopathologies were divided into 3 groups. The first group included all livers with singular cell necrosis <3 and no group necrosis, no karyorhexis and beginning oedema. The second group was characterised by small group necrosis (< 10 hepatocytes), no karyorhexis, sinusoidal dilatation, and multiple cell oedema. Group necrosis >10 and karyorhexis were found in group 3 as long a strong sinusoidal dilatation and cell oedemas.

In the light of all results, it showed that there was an inverse correlation between the ATP measurement and the histopathology. Although the ATP measurement showed clearly that there was basically no production of ATP at 8 hours anymore,



Figure 4 Anastomosis of the Vena cava through suturing.



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the histopathology only illustrated group 2, which means small group necrosis (< 10 hepatocytes), no karyorhexis, sinusoidal dilatation, multiple cell oedema. There was no bigger group necrosis found (Figure 6).

# **CONCLUSION**

The ATP measurement is more sensitive than the histopathology. Previous studies showed that the histopathology indicating cell death showed no difference between the time periods. Even if cell swelling and necrosis indicate the death of the cells, the time of death remained unclear [12]. On top of it was shown, that low ATP levels and low adenine nucleotide pool lead to cell death [13]. Cell death will occur when the adenine nucleotide pool is down to 25% to 30% [12]. Cell swelling and necrosis are the beginning of cell death due to various cellular mechanisms. The loss of ATP marks the irreversibility of beginning cell death.

Given that fact, it should be considered whether the ATP measurement should be the standard to test the function of eligible livers for transplantation. This could be a suitable tool in order to identify extended criteria organs. Measuring ATP could be used in future time to establish a quick test for selecting vital organs which won't be associated with a transplant dysfunction afterwards. Up to now ATP measurements are still time-consuming giving no advantage to a pathologist analysing samples from eligible donor organs. But with future progress in the technical realization of producing an ATP measuring device less time consuming than doing a sample. This would speed up the explantation process and also guarantee a better survival of the patient receiving an organ and might be less associated with

transplant dysfunction afterwards.

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# REFERENCES

- Al-Chalabi A, Matevossian E, V Thaden AK, Luppa P, Neiss A, Schuster T, et al. Evaluation of the Hepa Wash® treatment in pigs with acute liver failure. BMC Gastroenterol. 2013; 13: 83.
- Farkas S, Hackl C, Schlitt HJ. Overview of the indications and contra indications for liver transplantation. Cold Spring Harb Perspect Med. 2014; 4. pii: a015602.
- 3. Hartert M, Senbaklavacin O, Gohrbandt B, Fischer BM, Buhl R, Vahld CF. Lung transplantation: a treatment option in end-stage lung disease. Dtsch Arztebl Int. 2014; 111: 107-116.
- 4. New York State Department of Health Workgroup. Workgroup on expanded criteria organs for liver transplantation. Liver Transpl. 2005; 11: 1184-1192.
- 5. Attia M, Silva MA, Mirza DF. The marginal liver donor--an update. Transpl Int. 2008; 21: 713-724.
- 6. Sass DA, Reich DJ. Liver transplantation in the 21st century: expanding the donor options. Gastroenterol Clin North Am. 2011; 40: 641-658.
- 7. Kamada N, Calne RY. Orthotopic liver transplantation in the rat. Technique using cuff for portal vein anastomosis and biliary drainage. Transplantation.1979; 28:47-50.
- 8. Matevossian E, Doll D, Hüser N, Brauer R, Sinicina I, Nährig J, et al. Liver transplantation in the rat: single-center experience with technique, long-term survival, and functional and histologic findings. Transplant Proc. 2009; 41: 2631-2636.
- 9. Kern H, Bald C, Brill T, Fend F, Von Weihern CH, Kriner M, et al. The influence of retrograde reperfusion on the ischaemia-/reperfusion injury after liver transplantation in the rat. Int J Exp Pathol. 2008; 89: 433-437.
- 10. Di Lisa F, Canton M, Menabò R, Kaludercic N, Bernardi P. Mitochondria and cardio protection. Heart Fail Rev. 2007; 12: 249-260.
- 11. Kalogeris T, Baines CP, Krenz M, Korthuis RJ. Cell biology of ischemia/ reperfusion injury. Int Rev Cell Mol Biol. 2012; 298: 229-317.
- 12. Jennings RB. Historical perspective on the pathology of myocardial ischemia/reperfusion injury. Circ Res. 2013; 113: 428-438.
- 13. Jennings RB, Murry CE, Steenbergen C Jr, Reimer KA. Development of cell injury in sustained acute ischemia. Circulation. 1990; 82: II2-12.
- 14.Summers DM, Watson CJ, Pettigrew GJ, Johnson RJ, Collett D, Neuberger JM, et al. Kidney donation after circulatory death (DCD): state of the art. Kidney Int. 2015.

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