Journal of Clinical and Basic Cardiology

An Independent International Scientific Journal

Journal of Clinical and Basic Cardiology 2004; 7 (1-4), 26-27

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Indexed in
Chemical Abstracts
EMBASE/Excerpta Medica

Krause & Pachernegg GmbH · VERLAG für MEDIZIN und WIRTSCHAFT · A-3003 Gablitz/Austria

Real Time PCR (Light Cycler) and Quantification of Gene Expression Levels of the Insulin-Dependent Glucose Transport Molecule GLUT4 in Human Myocardium

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Key words: glucose transport, gene expression, real time PCR, hypertension

Transmembrane glucose transport and its disturbances in metabolism play a crucial role in pathological processes resulting in hypertension and myocardial ischemic disease [1]. The glucose transport molecule GLUT4 is responsible for the facilitative, insulin inducible glucose transport into cardiac muscle cells [2]. Quantitative analysis of GLUT4 expression levels by Real Time PCR (light cycler) was established as a method to investigate differences between groups of hypertensive and normotensive patients attending the Universitätsklinik Graz, Austria.

Materials and Methods

Myocardial tissue samples were collected from patients undergoing cardiac surgery. Samples were dissected in three parts and adipose tissue was removed. Dissected samples were snap frozen separately in liquid nitrogen and stored at –70 °C until RNA isolation.

Prior to RNA isolation the frozen myocardial tissue was grinded with a disposable pestle in the reaction tube. Tissue powder was resuspended in 400 µl lysis buffer and homogenized by passing this lysate 5–10 times through a 20-gauge needle fitted to a syringe. RNA isolation was carried out following the instructions of the Roche High Pure RNA Isolation Kit. RNA was eluted with 100 µl elution buffer, divided into four aliquots and immediately frozen in liquid nitrogen. RNA was stored at –70 °C until Real Time PCR.

GLUT4 Real Time PCR was carried out based on the works of Razeghi et al [3] and Depre et al [4]. Primers and probe see in Table 1. We carried out a single step method for reverse transcription from RNA to cDNA and subsequent quantification without opening of the reaction tube using a Roche Light Cycler apparatus by following program: once 30 minutes at 61 °C, 50 cycles of 20 s at 95 °C, 30 s at 57 °C, 30 s at 72 °C and a final cooling step to 40 °C. A concentration standard was generated by conventional PCR of myocardial RNA without TaqMan probe and subsequent dilution after photometric measurement. 5 µl RNA were used for experimental reaction.

Table 1. Primers and probe were delivered by TIB Molbiol, Germany. Primer sequence according to [3].

Primer	Conc. nM	Sequence
Forward	5	5'-GCTACCTCTACATCATCCAGAATCTC-3'
Reverse	5	5'-CCAGAAACATCGGCCCA-3'
TaqMan	0,4	5'-FAM-CTGCCAGAAAGAGTCTGAAGCGCCT-TAMRA-3'

Results

Three samples, one negative control and additionally two standards were amplified by Real Time PCR. All reactions except the negative control showed a result listed in Table 2. No mean value was calculated because no patient data was available from these samples and therefore not comparable. Data analysis on the computer can be conducted in two different ways. The Second Derivative Maximum Analysis is automatically done by the computer and suitable if more than 1000 copies of sample are amplified. It can be used as an accurate predictor of concentration. The second method (Fit Points) needs a manual baseline correction of the background and unspecific fluorescence signal and is used for less than 1000 copies.

In our case the Second Derivative Maximum Analysis was appropriate. The Second Derivative Maximum is the point at which the rate of change of fluorescence is fastest. It usually occurs in the cycle where the sample fluorescence can first be distinguished from background fluorescence (crossing point). Data analysis is shown in Figure 1.

Discussion

The RNA was isolated from the right auricle of human myocardium and used for subsequent Real Time PCR. The quantitative analysis of human GLUT4 gene expression was established successfully. For more precise quantification

Table 2. Results of the GLUT4 Real Time PCR. Amplification of two standards allow the analysis of sample concentration. No mean value was calculated because no patient data was available.

Nr.	Name	Calculated concentration	Crossing point
1	Glut4/sample 1	4.832E + 04	29.02
2	Glut4/sample 2	3.521E + 04	29.43
3	Glut4/sample 3	5.817E + 04	28.77
4	Glut4/NC		
5	Glut Std. 10E3	1.000E + 03	34.08
6	Glut Std. 10E6	1.000E + 06	25.06

Received and accepted: August 6th, 2004.
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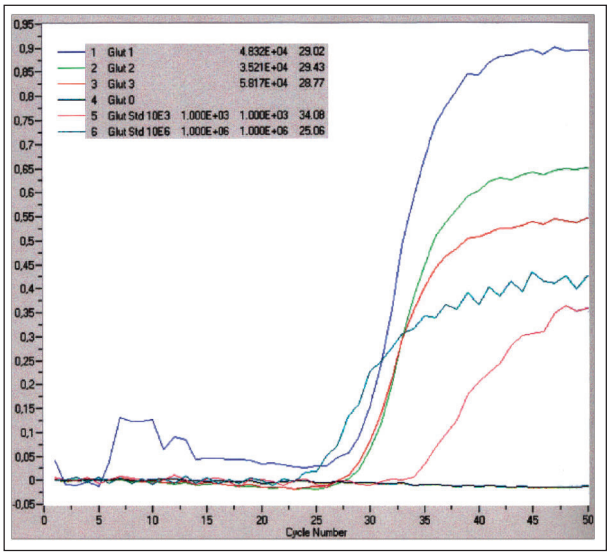

Figure 1. Analysis of the GLUT4 real time PCR by second derivative maximum algorithm computed automatically. RNA concentrations can be seen in the left upper corner and in Table 2.

house-keeping gene reaction could be additionally established.

This method will open up new opportunities to examine the association between GLUT4 and therefore facilitative, transmembrane glucose transport and metabolic syndrome, diabetes and hypertension.

Acknowledgement

This project (OENB Nr. 10829) has been sponsored by a research grant of the Austrian National Bank.

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