Title

"Ferrotoxicity and its amelioration by endogenous vitamin D in experimental acute kidney injury"

Description

"Supplemental material for Ferrotoxicity and its amelioration by endogenous vitamin D in experimental acute kidney injury by Chandashekar A, Rajesh NG, Pragasam V in Experimental Biology and Medicine"

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Running title: CATALYTIC IRON AND VITAMIN D IN ACUTE KIDNEY INJURY

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Supplementary material 1

Quantitative real-time PCR (qRT–PCR) analysis of iron-regulatory and 1α-hydroxylase genes

Freshly harvested kidneys from control and experimental rats were frozen using liquid nitrogen. Total RNA was extracted using TRIzol reagent (Sigma Aldrich, St. Louis, MO). 2 mg RNA was reverse transcribed to cDNA utilizing the higher capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA) and the mRNA expression was amplified by Quantitect SYBR® PCR kit (QIAGEN, Valencia, CA). Gene-specific primers were designed manually using NCBI/primer-BLAST tool software (supplementary table 1) and were purchased from Sigma Aldrich. qRT-PCR was performed to quantify transferrin receptors 1 and 2, ferritin, hepcidin, ferroportin and 1 α-hydroxylase (CYP27B1) genes. Transcription levels were assessed by the step one real-time thermal cycler with SYBR Green PCR master mix as per the manufacturer's instructions (Applied Biosystems, Foster City, CA). Amplification was carried out in the following cycling conditions: 94°C for 15 seconds, 60°C annealing for 30 seconds and 72°C extension for 30 seconds. The fold change in gene expression levels of the target genes was determined with normalization to β-actin values using the 2-ΔΔCt Comparative cycle threshold method. Each gene analysis was performed in triplicate.

Supplementary Table 1 Primer sequences used for qRT-PCR analysis of mRNA

Gene	Primer sequences
Transferrin receptor 1	Forward 5’ TTCAGGGAAGCTCTGTCGTT 3’ Reverse 5’ CTTTCTAAAGCCCGCAAGTG 3’
Transferrin receptor 2	Forward 5’ GACCCTGCAGTGGGTGTACT 3’ Reverse 5’ AGCATCCGTAGGTGTTCCAC 3’
Ferritin	Forward 5’ ATGATGTGGCCCTGAAGAAC 3’ Reverse 5’ GTGCACACTCCATTGCATTC 3’
Hepcidin	Forward 5’ ACAGAAGGCAAGATGGCACT 3’ Reverse 5’ GAAGTTGGTGTCTCGCTTCC 3’
Ferroportin	Forward 5’ CCCTGCTCTGGCTGTAAAAG 3’ Reverse 5’ GAAGTTGGTGTCTCGCTTCC 3’
1α-hydroxylase (CYP27B1)	Forward 5’ ACACCTAGCTTCCTGGCTGA 3’ Reverse 5’ AGCGCTCTGGACAATGACTT 3’