THROMBOPOIETIN GENE EXPRESSION IN HEALTHY DOG TISSUE

A.S. FIGUEIREDO¹, S.C. BULLA¹, K.V. LUNSFDOR², C. BULLA¹.
¹Department of Pathobiology and Population Medicine and ²Department of Clinical Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS, USA

Abstract

Thrombopoietin (THPO) is the major cytokine that regulates megakaryopoiesis and platelet production. Several human and mice studies have demonstrated that THPO is primarily synthesized in the liver, but the kidney, spleen and bone marrow are also implicated for its expression. There is no information concerning THPO mRNA expression levels in dogs. To gain a better understanding of the production of THPO in dogs we assessed the THPO mRNA expression in different tissues by quantitative reverse transcription-qPCR (RT-qPCR). Tissue samples such as bone marrow (n=5), liver (n=10), lung (n=10), kidney cortex (n=10), kidney medulla (n=5) and spleen (n=10) were obtained from 10 healthy, hound cross research dogs, between 6-8 months of age. mRNA extraction was performed by phenol-chloroform followed by quality and concentration verification by spectrophotometer analysis. RT-qPCR was performed by one-step reactions with primer and labeled-probe designed specifically for the canine THPO mRNA. For normalization purposes, a labeled-probe based detection (FAM-BHQ1) of the canine GAPDH was also designed. To avoid amplification of genomic DNA the reverse primer of both THPO and GAPDH assays were designed to span exon junctions. All samples were run in triplicates. The 2-ΔΔCT method was employed for relative quantification. The highest THPO mRNA levels were found in the liver and the lowest levels were found in the kidney (p < 0.0001) with a 13.66 ± 8.87-fold higher in liver than kidney (p > 0.0001). The lung and spleen had higher levels than kidney, which showed the lowest expression; in contrast to what was found in mice and adult human. The bone marrow showed the second highest level even in the absence of thrombocytopenia. The results reported here indicate that the liver is the major site of THPO expression.

Introduction

Thrombopoietin (THPO) is the major cytokine that regulates megakaryocyte production. It stimulates megakaryocytes to increase in cell size and ploidy, and to form proplatelet processes that then fragment into single platelets¹.

The liver is the main site of megakaryopoiesis during human fetal development, and then it is taken over by the bone marrow postnatally². The bone marrow becomes the major site of megakaryopoiesis in the adult, but liver is the major site of THPO mRNA production throughout life³. THPO mRNA is also found in several human, mouse and rat tissues in addition to the liver such as the kidney, bone marrow, lung, spleen, and central nervous system⁴,⁵. Although other regulatory mechanisms have been proposed⁶, THPO is considered to be constitutively produced by the liver and kidney, but is inducible by inflammatory processes⁷ and increased in the bone marrow by thrombocytopenia⁸.

To the best of our knowledge, there is no information concerning THPO expression levels in canine tissues. A clearer understanding of the primary production sites and the regulatory mechanisms involved in THPO expression in the dog is the first step toward the development of therapies with THPO mimetics for thrombocytopenic conditions in this species and toward developing potential comparative models. To gain a better understanding of the expression of THPO and its distribution, we quantified the THPO mRNA in healthy dog tissues.

Materials and Methods

Tissue fragments collected / RNA extracted

↓

Canine specific THPO and GAPDH hydrolysis probes

↓

THPO mRNA quantified by reverse transcription real-time PCR

Discussion and Conclusion

The liver showed the highest levels of THPO mRNA, with 13-fold and 2-fold more levels than kidney and bone marrow, respectively. Interestingly, the lung and spleen had higher levels than kidney, which showed the lowest expression; in contrast to what was found in mice and adult human.³,⁴ The bone marrow showed the second highest level even in the absence of thrombocytopenia.

The results reported here indicate that the liver is the major site of THPO expression in the dog.

Results

Figure 3. Fold difference of THPO mRNA relative to renal cortex (±SD). *Statistical significance among tissues, P < 0.05.

References


Figure 1. Ten-fold dilution series of GAPDH. Efficiency: 95.2% and R²: 1.

Figure 2. Ten-fold dilution series of THPO. Efficiency: 102.5% and R²: 0.99.