Development of a Real-Time Polymerase Chain Reaction Detection Method for the Quantitation of Myeloperoxidase Transcripts in Porcine Tissues

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Summary and Implications

Myeloperoxidase (MPO) is a hemoprotein present in azurophilic granules of polymorphonuclear (PMN) leukocytes and monocytes. It catalyzes the oxidation of halide ions to their respective hypohalous acids, which are used for microbial killing by phagocytic cells. Measurement of MPO activity is often used as a marker of neutrophil infiltration into tissues. We have designed a quantitative reverse transcription-polymerase chain reaction detection method for porcine MPO transcripts by using TaqMan real-time PCR technology. Total RNA was isolated from lung and spleen tissue collected 7 days post-intranasal inoculation with *Salmonella choleraesuis* (n=4) or saline (n=4). The lung and spleen samples were pooled before RNA isolation to create uninfected control and infected RNAs for each tissue. Expression of MPO mRNA was highest in infected spleen (12.5 ± 2.85 relative units (RU)), followed by the uninfected spleen sample (2.9 ± 1.50 RU). There was no difference in MPO expression between uninfected and infected lung samples. In conclusion, levels of MPO mRNA expression in porcine spleen and lung indicate a differential response to infection between the two tissues. This difference may be associated with bacterial-host adaptation of *S. choleraesuis*. The TaqMan assay for MPO also can be used to discover tissue-specific responses between individuals or groups of pigs exhibiting distinct phenotypic responses to infection.

Introduction

Early host-defense against infiltrating microorganisms involves the deployment of several devices by activated phagocytes, and an important one of these defenses is the release of MPO. In phagolysosomes, this enzyme works together with other oxidases and proteases to cause the destruction of ingested organisms (1). Low MPO levels are the most common neutrophilic lysosomal deficiency, but usually occur without a noticeable increase susceptibility to infection or reduced immune response (1,2). MPO detection also has been used as a marker of neutrophil infiltration into tissues (3,4). The purpose of our experiment was to develop a real-time PCR detection method that could measure relative levels of expression of MPO in several different tissues, and in normal verses infected states in the pig.

Materials and Methods

Forward and reverse primers for PCR were designed across an exon/exon splice junction for MPO and for a house-keeping gene ß-actin, so that only expressed RNA transcripts and not genomic DNA would be detected in the real-time PCR reaction. Fluorescent probes were then designed to sit down within the amplicon (Figure 1).

Total RNA was isolated from pooled samples of four pigs infected with *S. choleraesuis* (7 days after infection), and four uninfected pigs. Spleen and lung tissue from these animals were tested for the relative expression levels of MPO.

Results and Discussion

After normalization for ß-actin expression, the infected spleen tissue sample had the highest level of MPO expression, 12.5 ± 2.85 RU, by at least a 4-fold difference over that of the uninfected spleen sample, 2.9 ± 1.50 RU. (Figures 2 and 3). There was no difference in MPO expression between uninfected (1.0 ± 0.15 RU) and infected (0.7 ± 0.44) lung samples.

Therefore, after 7 days of infection there is a significant increase in MPO expression in spleen tissue of pigs. This contrasts with the MPO expression pattern seen in infected lung verses uninfected lung tissue. This difference may be associated with bacterial-host adaptation of *S. choleraesuis*, but a more thorough investigation of MPO expression in spleen and lung over a time-course of infection needs to be completed. The TaqMan assay for MPO can be used to discover tissue-specific responses between individuals or groups of pigs exhibiting distinct phenotypic responses to infection.

References

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Figure 1. Detection of transcripts during the TaqMan assay. Note, amplification initially starts with a cDNA template after first-strand synthesis from total RNA.

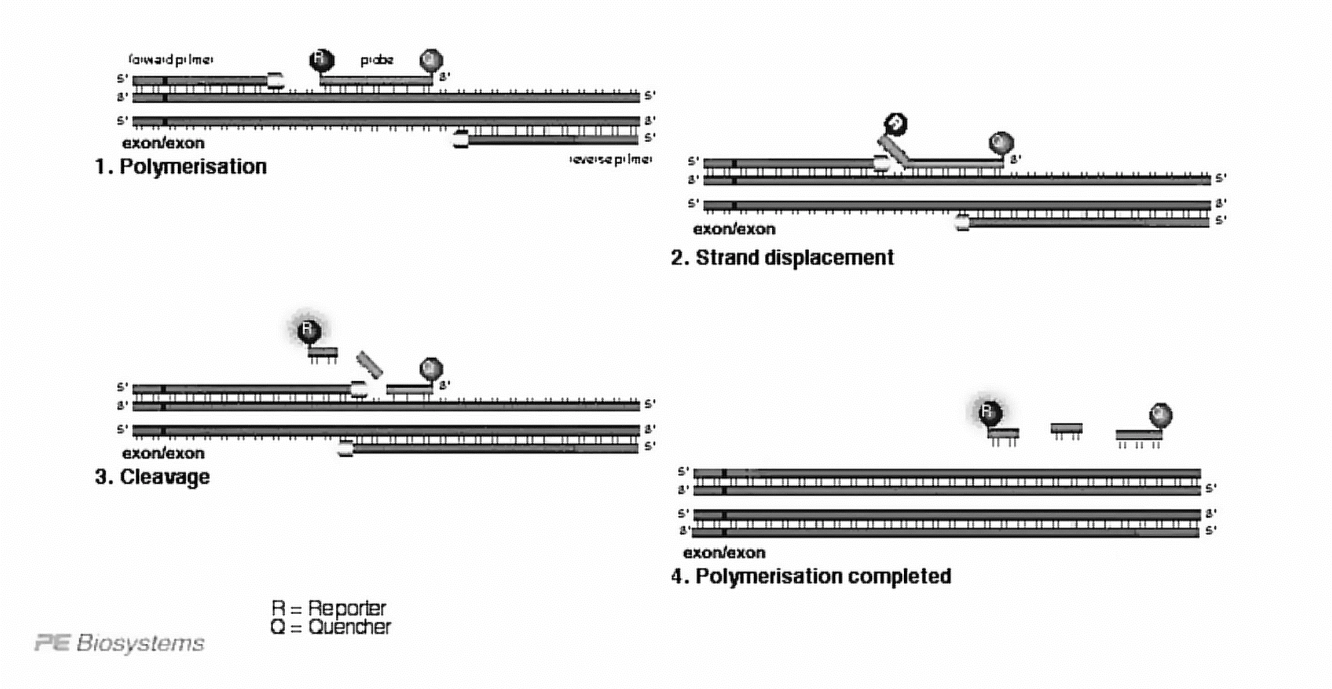

Figure 2. The relative measure of MPO expression is determined during initial cycles of exponential amplification. When a significant increase in fluorescence above background levels is detected, the cycle threshold (number) is compared with the cycle thresholds of other RNA sample.

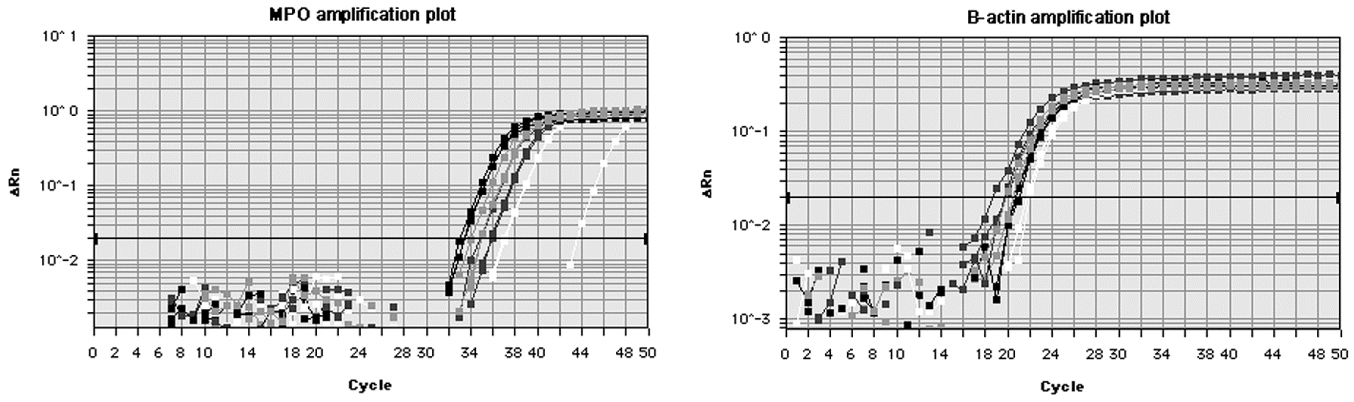

Figure 3. Relative expression of MPO compared with RNA isolated from control lung tissue (C-L, control lung; D-L, diseased lung; C-S, control spleen; D-S, diseased spleen).