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Parecoxib does not suppress thromboxane synthesis in newborn piglets with group B streptococcal sepsis☆

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ABSTRACT

Group B streptococci (GBS) cause fatal sepsis in newborns. Strong activation of thromboxane synthesis is assumed to correlate with severe pulmonary hypertension. In this study we compared the impact of indomethacin versus parecoxib on hemodynamics and outcome and investigated the pharmacological effects on thromboxane synthesis and EP-3 receptor gene expression. Whereas both parecoxib and indometacin reduced expression of thromboxane synthase and EP-3 receptor in infected lung tissue, parecoxib did not suppress urine levels of thromboxane like indometacin. We presume that COX-2 inhibition in GBS sepsis is associated with enhanced thrombogenicity.

1. Introduction

Group B streptococci (GBS) remain the leading cause of severe bacterial infections of the newborn. By early diagnosis and antibiotic treatment it is possible to prevent severe complications in most cases, however, a few neonates develop fatal pneumonia, meningitis or sepsis. A recent survey in Germany revealed a mortality of 4.3% [1]. The two most common strains isolated from newborns with early-onset-sepsis in Germany are Ia and III [2]. The pathogenesis of respiratory failure as well as hypotension and cardiac failure in the case of GBS sepsis have been the subject of investigation for more than 20 years. The most remarkable finding was a profound rise in thromboxane levels in the serum of miscellaneous animals after intravenous application of GBS [3,4]. This increase in thromboxane serum levels is triggered by phospholipids from the bacterial membrane [5] and correlates with pulmonary hypertension followed by systemic hypotension. Pulmonary hypertension as well as systemic hypotension were inhibited by indometacin in previous studies [6] supporting the idea that activated prostaglandins play an important role in the regulation of vascular tone in neonatal sepsis and may serve as a target for a new treatment option.

Thromboxane A2 (TXA2) is derived from platelets and causes vasoconstriction, bronchoconstriction and platelet aggregation. It is synthesized by thromboxane synthase (ECNumber 5.3.99.5), an enzyme that is suspected to be a downstream enzyme of COX-1 and may not be influenced by COX-2 inhibitors e.g. parecoxib. Systemic thromboxane synthesis is reflected by urinary excretion of TxB2 metabolites 2,3-dinor-TxB2 and 11-dehydro-TxB2. The increase of the index metabolites of thromboxane in urine has been shown to be specific for pulmonary hypertension caused by group B streptococci [7]. In the lung, prostaglandin E2 has been shown to suppress or to induce the proliferation of fibroblasts during acute lung injury in a concentration-dependent manner, via preferential signaling through EP2 or EP3 receptors, respectively [8].

In the present study we compared the effect of COX inhibition by indometacin and parecoxib, a specific COX-2 inhibitor, on outcome, hemodynamics and thromboxane metabolism as well as EP-3 receptor expression in a neonatal piglet model of GBS sepsis. We presumed that parecoxib would suppress the inflammatory response to GBS and stabilize the hemodynamic situation similarly to indometacin.

Abbreviations: CO, cardiac output; GBS, group B streptococci; IMV, intermittent mandatory ventilation; MAP, mean arterial pressure; MPAP, mean pulmonary arterial pressure; NSAID, non-steroidal anti-inflammatory drug; SVR, systemic vascular resistance; PVR, pulmonary vascular resistance.

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2. Methods

2.1. Animal experiment

Newborn piglets ($n = 25$) were anesthetized and prepared as described previously [9,10]. After tracheotomy IMV was performed with a neonatal respirator (Infant Star 950, Mallinckrodt, Hennef, Germany). An arterial catheter (20 gauge; Arrow, Erding, Germany) was placed in the femoral vein for continuous monitoring of blood pressure. A Swan-Ganz catheter was inserted into the pulmonary artery for continuous measuring of the pulmonary artery pressure and cardiac output via thermodilution. Group B streptococci strain Ia serotype 90 (1.75×10^6 cfu/kg), grown to a mid-logarithmic phase in tryptone-soya broth, were applied at a dose of 1.75×10^6 cfu/kg intravenously over a sheath placed in the right jugular vein for 30 min. The piglets were randomized to the following groups: control (no GBS, $n = 5$), GBS (application of group B streptococci, $n = 7$) and two intervention groups which both received GBS and additional treatment: either indometacin (5 mg/kg i.v., $n = 7$) or parecoxib (20 mg/kg i.v., $n = 6$) was administered 2 h after GBS application. Application of parecoxib was repeated after 8 h in surviving animals. The end of the observation period was determined by the death of the animal. Animals in the control group were killed after 12 h by application of methohexital (50 mg/kg) plus 20 ml of a 1 M potassium chloride solution.

All hemodynamic data were analyzed by two-way ANOVA.

2.2. Quantification of prostaglandin metabolites by mass spectrometry

Urine was obtained from a cystofix catheter prior to application of GBS and shortly before the death of the animal except for the case of anuria. Quantification of 6-keto-PGF1α, 2,3-dinor-6-keto-PGF1α thromboxane B2 (TxB2), 2,3-dinor-TxB2 and 11-dehydro-TxB2 was performed by mass spectrometry as described elsewhere [11].

2.3. Real-time RT-PCR analysis of lung tissue

Tissue samples were obtained from different lobes of the right lung. Total RNA was isolated using TRIZOL®. Quantitative RT-PCR was performed using the primers and TaqMan probes listed in Table 1. Gene expression was related to the housekeeping genes β-actin (A) and hypoxanthine guanine phosphoribosyl transferase (HPRT).

Table 1

Hypoxanthine guanine phosphoribosyl transferase (HPRT)
Forward: 5′-CGGCTCCGTTATGGCG-3′
Reverse: 5′-GGTCATAACCTGGTTCGTCATCA-3′
TaqMan probe: 5′-(FAM)-CGCAGCCCCAGCGTCGTGATTA-(TAMRA)39
IL-8
Forward: 5′-TTCTGCAGCTCTCTGTGAGGC-3′
Reverse: 5′-GGTGGAAAGGTGTGGAATGC-3′
TaqMan probe: 5′ (FAM)-TTCTGGCAAGAGTAAGTGCAGAACTTCGATG-(TAMRA) 3′
COX-2
Forward: 5′-GCCTGATGACTGCCCAACA-3′
Reverse: 5′-CCACAACTTCCTTTGAATCGG-3′
TaqMan probe: 5′ (FAM)-AAGCTCTTTCCTCCCTTTCACACCCATG-(TAMRA) 3′
Thromboxane synthase
Forward: 5′-CATAATGGCCACAGGATTGG-3′
Reverse: 5′-TCCTCCCATCTTTTGTCACGTA-3′
TaqMan probe: 5′ (FAM)-ATCCAAGCCGGTAGCTGACAGCATCC-(TAMRA) 3′
EP-3 receptor
Forward: 5′-TGTACACAGAAAAGCAGAACGAGTG-3′
Reverse: 5′-AGGGATCCAAGATCTGGTTCAG-3′
TaqMan probe: 5′ (FAM)-AACTTCTTCTTAATAGCCGTTCGCCTGGC-(TAMRA) 3′

3. Results

3.1. Hemodynamic parameters

Irrespective of the mode of treatment all animals died within 12–16 h. Two hours after intravenous infusion of GBS a slightly increased MAP was observed (not significant). Thereafter, MAP decreased continuously. At 2 h and 15 min, MAP in animals treated with indometacin reached a significantly higher level than that of untreated animals (Fig. 1A, $p < 0.01$). This effect was not evident anymore later after GBS infusion. Parecoxib did not increase MAP significantly. After application of GBS, MPAP was significantly elevated compared to the control group, reaching its first maximum at 15 min after the application of GBS (Fig. 1B). A second maximum was observed between the 2nd and 4th hour of the experiment. This GBS-induced rise of MPAP was prevented by parecoxib (Fig. 1A) at 3 h and 30 min, 5 h and 5 h and 15 min ($p < 0.05$). This is consistent with a decrease of the pulmonary vascular resistance (PVR, Fig. 1C). Later no difference between the groups was seen anymore. Cardiac output was lower after treatment with indometacin than following parecoxib administration, but this difference was not significant. Mean heart rate of the individual groups did not differ (data not shown). The systemic vascular resistance increased in the indometacin group compared to the parecoxib group in the 3rd and 6th hour (Fig. 1D, $p < 0.01$).

Pulmonary resistance was recorded in intervals of 15 min (Fig. 1E). In the parecoxib group, the resistance was significantly higher in the 10th and 11th hour after GBS application than that of the control and the GBS groups. Indometacin led to a slight but not significant increase of pulmonary resistance.

3.2. Inflammation in lung tissue is reduced by indometacin and parecoxib

IL-8 and COX-2 gene expression was quantified by TaqMan PCR. IL-8 mRNA levels increased 20-fold in the GBS group (Fig. 2A). Treatment with indometacin and parecoxib prevented the rise of IL-8 and COX-2 expression (Fig. 2A and 2B). Expression of thromboxane synthase rose 8-fold and could be suppressed by parecoxib and indometacin to the same extent. EP-3 receptor gene expression increased 4-fold after application of GBS (Fig. 2B).

3.3. Determination of index metabolites of prostacyclin and thromboxane in urine

Following application of GBS, urinary concentration of 6-keto-PGF1α and 2,3-dinor-6-keto-PGF1α rose, indicating a profound activation of prostacyclin. Furthermore, TxB2, 2,3-dinor-TxB2 and 11-dehydro-TxB2 concentration increased, demonstrating stimulation of thromboxane synthesis as well. Indometacin and parecoxib reduced 6-keto-PGF1α and 2,3-dinor-6-keto-PGF1α levels significantly to a comparable degree, while the effect on thromboxane metabolites varied: indometacin profoundly suppressed thromboxane metabolism, whereas parecoxib did not affect thromboxane concentration in urine (Fig. 3).

3.4. Quantification of prostaglandin E2 in urine

PGE_2 concentration in urine rose significantly in the GBS animals. COX inhibition led to decreased PGE_2 levels. There was no difference between the indometacin and the parecoxib group (Fig. 4).