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# Deleterious effects of indomethacin in the mid-gestation human intestine

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ABSTRACT

The use of the anti-inflammatory drug indomethacin (INDO) in preterm infants has been associated with an increased risk of developing enteropathies. In this study, we have investigated the direct impact of INDO on the human mid-gestation intestinal transcriptome using serum-free organ culture. After determining the optimal dose of 1 μM of INDO (90% inhibition of intestinal prostaglandin E2 production and range of circulating levels in treated preterm babies), global gene expression profiles were determined using Illumina bead chip microarrays in both small and large intestines after 48 h of INDO treatment. Using Ingenuity Pathway Analysis software, we identified critical metabolic pathways that were significantly altered by INDO in both intestinal segments including inflammation and also glycolysis, oxidative phosphorylation and free radical scavenging/oxidoreductase activity, which were confirmed by qPCR at the level of individual genes. Taken together, these data revealed that INDO directly exerts multiple detrimental effects on the immature human intestine.



## 1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin (INDO) have been widely used in the neonatal period for prevention and closure of persistent patent ductus arteriosus (PDA) [1–3] as well as a tocolytic agent [4]. PDA remains one of the most frequent complications in the clinical management of preterm infants. A persistent PDA in preterm neonates causes pulmonary congestion [5] and decreased blood flow to vital organs [6] leading to various additional complications. PDA has an incidence of 40–80% in premature infants with low birth weight [1,7] but can be treated effectively with NSAID administration leading to permanent ductal closure in 60–80% of infants [2,3]. INDO has also been efficiently used since the 1970s to delay delivery in cases of preterm labor [4]. However, the use of NSAIDs in the neonate is associated with a broad spectrum of life-threatening adverse effects on various systems, most notably on the gastrointestinal tract [4,8] where INDO treatment was associated with an alteration in blood flow and increased risk of developing necrotizing enterocolitis (NEC) with intestinal perforation [9–13].

In the adult, NSAIDs are known to cause severe gastrointestinal injuries ranging from frequent asymptomatic inflammation, erosions, ulcers and altered mucosal permeability [14–16]. These side effects of NSAIDs are a result of their ability to inhibit the COX enzymes, COX1 and COX2, which are involved in the conversion of arachidonic acid to prostaglandin. While the decrease of prostaglandin synthesis is widely acknowledged as the basis of the gastrointestinal toxicity caused by these drugs [14–16], the inhibition of COX alone may not completely explain all the gastrointestinal damage caused by these drugs. For instance, it has been demonstrated that disruption of the COX genes alone did not provoke gastrointestinal injury in rodents in the absence of injurious stimuli [17] suggesting that complementary mechanisms may be involved in causing digestive mucosal damage [18,19]. Additional studies on experimental animal models suggest that the development of NSAID-mediated enteropathy requires uncoupling of intestinal mitochondrial oxidative phosphorylation and/or inhibition of the respiratory chain [20]. Oxidative stress is another putative mechanism proposed to be associated with the adverse effects caused by NSAIDs. Indeed, previous studies have reported that INDO can induce apoptosis in intestinal epithelial cell lines [21] by generating the production of reactive oxygen free radicals and could be one of the mechanisms by which INDO damages intestinal mucosa [22]. In a recent study, INDO was shown to favor bacterial translocation across the epithelial

*Abbreviations:* COX, cyclo-oxygenase; INDO, indomethacin; IPA, Ingenuity Pathway Analysis software; NEC, necrotizing enterocolitis; NSAIDs, nonsteroidal anti-inflammatory drugs; PDA, patent ductus arteriosus; $PGE_2$, prostaglandin $E_2$.

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monolayer in a reactive oxygen species-dependent mechanism [23] further emphasizing INDO-induced barrier dysfunction. Nitric oxide (NO) could also be involved in the side effects of NSAIDs. NO is a scavenger of free radicals [24] playing an important role in maintaining mucosal integrity [25]. Evidence suggests that NSAIDs may induce gastrointestinal injuries by interfering with both mucosal synthesis and availability of NO [26]. Taken together, data generated in experimental animal models, although still fragmentary, suggest that NSAIDs may affect intestinal mucosa integrity via both COX-dependent and independent mechanisms.

Despite increasing scientific progress in establishing the pathways involved in NSAID-induced damage, understanding the pathogenesis of gastrointestinal injury by NSAIDs in neonates relies on a limited number of studies carried out in animal models. In the present study, we exploited our experience in maintaining mid-gestation human intestine in serum-free organ culture [27,28] with global gene expression analysis [29–31] to investigate the specific intestinal effects of INDO on the overall physiology of both small and large intestines at mid-gestation. Our results established that INDO negatively impacts several crucial metabolic and physiological pathways, such as glycolysis, oxidative phosphorylation, and free radical scavenging in the immature intestinal mucosa and begins to define the molecular elements directly affected in the mid-gestation intestine under defined conditions by this anti-inflammatory drug.

## 2. Results

### 2.1. Determination of INDO concentration for inhibition of intestinal $PGE_2$

We first performed a dose–response curve analysis to determine the appropriate working concentration of INDO to use in organ cultures of mid-term intestinal tissue. In preterm infants, previous in vivo studies showed that optimum PDA closure rates with intravenous INDO could be achieved with plasma levels varying between 0.5 and 5 μM [32,33]. Levels of $PGE_2$ were measured and assumed to reflect COX activity, the target of INDO inhibition. As shown in Fig. 1, increasing concentrations of INDO markedly decreased the production and release of $PGE_2$ by both small and large intestinal segments compared to controls. INDO significantly inhibited $PGE_2$ production by over 90% at concentrations of 100 nM INDO or more, confirming that intestinal organ cultures were responsive to INDO. In the context that previous studies indicating that INDO was active on intestinal epithelial cell lines in ranges between 1 and 1000 μM [21,23], we therefore decided to perform our studies with 1 μM INDO which is also representative of the minimum necessary dose required to close the PDA and which successfully inhibits its target in both segments in organ culture.

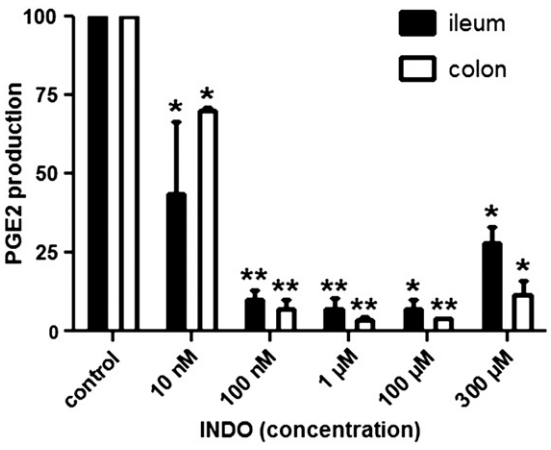


**Fig. 1.** Inhibition of $PGE_2$ production by INDO in the human small intestine and colon at mid-gestation. Changes in intestinal $PGE_2$ levels after 48 h of culture in the presence of increasing concentrations of INDO. Data are expressed as percentage of inhibition of $PGE_2$ compared to corresponding untreated control segments. Values shown are the mean of 3 independent biological samples. *$p<0.05$ and **$p<0.001$ vs untreated control segments.

### 2.2. Global gene expression analysis of the effect of INDO on the mid-gestation human small and large intestines

To investigate the modulatory influence of INDO on gene expression profiles in the mid-gestation intestine, matched control and INDO-treated (1 μM) explants prepared from 4 ileums and 4 colons were cultured in serum-free medium for 48 h. Gene expression profiles were then determined using Illumina whole genome expression beadchip microarrays providing coverage of more than 47,000 transcripts for each of the 16 samples. Statistical analyses revealed that 295 and 138 genes were significantly and differentially expressed by INDO in the ileum and the colon, respectively, in comparison to controls (Supplementary Tables 1 and 2 for gene lists). We noted that only 35 of these genes were common between the two segments. Of the 295 differentially expressed genes in the ileum, 153 were up-regulated and 142 were down-regulated, while only 41 were up-regulated and 97 were-down-regulated of the 138 found in the colon (Fig. 2). The original data have been deposited in the National Center for Biotechnology Information's Gene Expression Omnibus and are accessible through GEO Series accession number GSE38406.

### 2.3. Common pathways modulated by INDO in the mid-gestation human small and large intestines

All significantly and differentially expressed genes in response to INDO treatment in the ileum and colon were independently subjected to IPA software which groups genes by molecular and cellular functions. IPA analysis identified several enriched functional networks modulated by INDO in the immature intestine. Table 1 shows three of the most significant and representative networks for each segment (see Supplementary Tables 3 and 4 for complete network and gene lists). It is noteworthy that several network biological functions identified in both segments were related to inflammatory functions such as “cellular movement”, “cell death”, “DNA replication and cellular growth”, “inflammatory response”, and “gastrointestinal diseases”, consistent with the expected anti-inflammatory effect of INDO in the immature intestine.

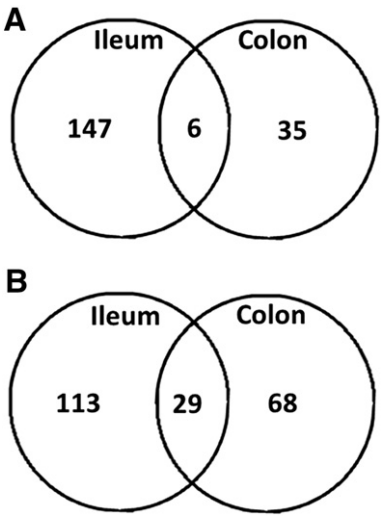


**Fig. 2.** Genes modulated by INDO in the human small intestine and colon at mid-gestation. A) Intersection between up-regulated genes in the small intestine (ileum) and colon. Numbers represent differentially up-regulated genes by INDO in each tissue. B) Intersection between down-regulated genes in the ileum and colon. Numbers represent differentially down-regulated genes by INDO in each tissue.