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SCIENCE DIRECT
Toxicology 216 (2005) 204–223
TOXICOLOGY
www.elsevier.com/locate/toxicol

Skeletal developmental effects of selective and nonselective cyclooxygenase-2 inhibitors administered through organogenesis and fetogenesis in Wistar CRL:(WI)WUBR rats☆

Franciszek Burdan[a,*], Justyna Szumilo[b], Barbara Marzec[c], Robert Klepacz[b], Jaroslaw Dudka[b]

[a] *Experimental Teratology Unit of the Human Anatomy Department; Medical University of Lublin, 4-6 Jaczewskiego Street, PL-20090 Lublin, Poland*
[b] *Clinical Pathomorphology Department; Medical University of Lublin, PL-20950 Lublin, Poland*
[c] *Medical Genetic Department; Medical University of Lublin, PL-20950 Lublin, Poland*

Received 12 July 2005; received in revised form 10 August 2005; accepted 10 August 2005
Available online 22 September 2005

Abstract

Cyclooxygenase (COX) inhibitors are the most commonly ingested drugs. The aim of the study was to evaluate the prenatal skeletal effect of selective (DFU) and nonselective (tolmetin, ibuprofen, piroxicam) COX-2 inhibitors. All the tested compounds were administered intragastrically to pregnant Wistar rats from 7 to 21 gestation day. The initial dose was set at 8.5 mg/kg/dose for tolmetin and ibuprofen, 0.3 and 0.2 mg/kg/dose for piroxicam and DFU. The middle dose was increased 10-times. The highest dose, except for ibuprofen, was elevated 100-times. The highest dose for ibuprofen was set at 200 mg/kg/dose. Tolmetin and ibuprofen were administered three times a day. Piroxicam and DFU were dosed once daily. After routine teratological examinations, extremities of randomly selected 21-day-old fetuses were taken for histological, immunohistochemical and molecular studies. The proximal femoral epiphyses were separated and their ultrastructure evaluated. The expression of genes coding cytokines (IL-1α, IL-1β, IL-6, TNF-α, TNF-β) and proteins (COX-1, COX-2, cathepsin K, collagen types I, II and X; osteocalcin, osteopontin) was evaluated in femoral epiphyses by RNase Protection Assay and/or immunohistochemically. The articulate development was checked histologically and found undisturbed in any of the experimental groups. The epiphysis of the 21-day-old fetuses, presented physiological expression of COX-1 and COX-2, as well as cathepsin K, collagen types I, II and X; osteopontin, osteocalcin and TNF-α. Increased developmental skeletal variation was noted in groups exposed to the highest dose of nonselective drugs. Unlike the increased number of skeletal variations observed in fetuses exposed to highest doses of nonselective compounds, both groups of COX inhibitors did not disturb joint formation and morphology of femoral epiphyses when administered even in high maternal toxic doses.

Keywords: Bone development; COX-1; COX-2; Cyclooxygenase; Fetus; NSAID; Pregnancy; Skeleton

1. Introduction

Number of animal and human studies showed that cyclooxygenase (COX) inhibitors in spite of their beneficial effect on blocking inflammatory reaction may

☆ Developmental skeletal effects of COX-inhibitors.
* Corresponding author. Tel.: +48 603 767649; fax: +48 81 5328903.
E-mail address: fb3@wp.pl (F. Burdan).

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doi:10.1016/j.tox.2005.08.009

disturb bone and cartilage physiology. They inhibit bone healing and could decrease synthesis of cartilage matrix (Seidenberg and An, 2004; Burdan, 2005b). According to Dingle (1999) non-selective COX inhibitors, also known as aspirin-like or non-steroidal anti-inflammatory drugs (NSAID), could be divided into three categories with respect to their action on the extracellular matrix of cartilage. The first group contains drugs which can stimulate glycosaminoglycan, hyaluronic acid and collagen synthesis, e.g., aceclofenac, tenidap and tolmetin. The second group contains drugs which appear to be without significant effect on matrix synthesis, e.g., aspirin, tiaprofenic acid, piroxicam. The third group contains drugs that decrease matrix synthesis, e.g., ibuprofen, indomathacin and naproxen. No adequate data for selective COX-2 inhibitors were found.

The cartilage injury can appear only in the presence of the tumor necrosis factor-α (TNF-α) and interleukin 1β (IL-1β), which increase degenerative changes and are induced by various xenobiotics including COX inhibitors (Dingle, 1999; Blanco et al., 1999). It is also known that TNF-α is indispensable in the IL-1β synthesis that is the strongest stimulant factor in cartilage collagen fiber and glycosaminoglycan degeneration, and inhibits bone restoring (Klimiuk and Sierakowski, 2001; Nedelec et al., 2001). It also stimulates synthesis of IL-6 which inhibits chondrocytes proliferation and synthesis of extracellular matrix in cartilage. IL-6 simultaneously inhibits TNF-α expression but such feedback control was not revealed between IL-6 and IL-1 (Klimiuk and Sierakowski, 2001). Dingle and Parker (1997) and Gonzalez et al. (1994) showed inhibition of the proinflammatory cytokines in cartilage of patients with osteoarthritis and rheumatoid arthritis during therapy with some COX inhibitors, e.g., aceclofenac and tolmetin. Such therapy led to increased expression of insulin-like growth factor (IGF), which stimulated collagen and glycosaminoglycan synthesis (Gonzalez et al., 1994; Dingle, 1999; Blanco et al., 1999). The study done with adult tissue showed that TNF-α and IL-1 stimulate COX-2 expression (Riendeau et al., 1997; Blanco et al., 1999). Secondary are synthesized prostaglandin E_2 (PGE_2) which stimulates cartilage matrix degeneration, as well as prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostacycline whose effect on cartilage morphology and metabolism is unclear (Morisset et al., 1998). On the other hand it was also proved that prostaglandins, especially PGE_2, stimulate the expression of COX-2 on positive feedback mechanism. Such changes accelerate the pathophysiological cascade of cartilage destruction (Pilbeam et al., 1993; Morisset et al., 1998). It is postulated that prostacycline and $PGF_{2\alpha}$ may exert positive effects on cartilage, increasing the level of glucocorticoid receptors in chondrocytes (DiBattista et al., 1991), influencing chondrocytes differentiation (O'Keefe et al., 1992) and proliferation (Capehart and Biddulph, 1991), and mediating the effects of vitamin D (Schwartz, 1992).

On the other hand experimental data suggest that COX-inhibitors protect against bone demineralization (Lane et al., 1990; Jungkeit and Chole, 1991). The mechanism of such action is secondary to blockade of PGE_2 synthesis which in very low doses increases collagen synthesis but in higher doses is one of the strongest mediators for osteoclasts (Kawaguchi et al., 1995). Such animal observations were partially confirmed in human studies (Bauer et al., 1996; Morton et al., 1998).

Unlike the relatively large number of adult experimental and clinical studies, prenatal skeletal effect of COX inhibitors, especially selective ones, were not extensively evaluated although most of the drugs easily cross placental barrier and reach fetal blood concentration close to maternal one, e.g., ibuprofen (Adams et al., 1969; Briggs et al., 1998). The bone and/or cartilage examination performed on single or double-stained specimens, which is one of the obligatory step in prenatal toxicological studies (Christian, 2001), showed that in utero exposure to COX inhibitors may disturb skeletal formation (Ostensen and Skomsvoll, 2004). Higher incidence of bone developmental variations was seen in fetuses whose mothers were treated with high toxic doses of non-selective COX inhibitors (Burdan, 2004, 2005a). Similar developmental effect was found for selective COX-2 inhibitor—DuP-697 in dose over the COX-2 selective level (Burdan et al., 2003). Such observations indicate that COX-1 may play a principal role in bone formation since its expression was revealed in fetal bone and cartilage, while COX-2 expression was detected only at the end of fetal period (Stanfield et al., 2003). Unlike adult studies, chondrotoxicity was not evaluated on the molecular level during pregnancy but some epidemiological data showed higher risk of joint deformity in populations exposed to the COX inhibitors during gestation (Briggs et al., 1998).

The current article presents results from an ongoing scientific project taken to evaluate prenatal toxicity of selective and non-selective COX-2 inhibitors (Fig. 1). The aim of this paper is to show the cartilage effect. Tolmetin, ibuprofen and piroxicam were selected from among the nonselective inhibitors (Brideau et al., 1996; Riendeau et al., 1997). In earlier adult toxicological studies, they were shown to stimulate, to inhibit or