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Experimental Teratology Unit of the Human Anatomy Department
Department of Clinical Pathomorphology, Medical University of Lublin

FRANCISZEK BURDAN, JUSTYNA SZUMIŁO, JAROSŁAW DUDKA, MONIKA CENDROWSKA-PINKOSZ, BARBARA MADEJ, ROBERT KLEPACZ, MAŁGORZATA FRĄCZEK, ELŻBIETA KOROBOWICZ, ZBIGNIEW WÓJTOWICZ

Immunoexpression of the constitutive and inducible cyclooxygenase isoforms in maternal and fetal rat lungs

Both cyclooxygenase isoforms – constitutive (COX-1) and inducible (COX-2) – are coded by different genes, which are regulated by various factors. COX-1 (22 kB) is localized on chromosome 9 and has only few binding sites for inducible transcription factors. Its expression is constant and normally observed in most tissues. Unlike COX-1, COX-2 is coded by gene (8.3 kB) located on chromosome 1, that is stimulated by various signaling molecules, including growth factors (e.g., FGF, IGF, PDGF), inflammatory mediators (e.g., IL-1, IL-6, TNF-α), lipopolysaccharide (LPS) and others. For this reason COX-1 belongs to housekeeping genes, whose expression regulates physiological processes, whereas COX-2 is typical of immediate-early gene activated mostly during different pathological processes. However, COX-1 was also detected in rheumatoid and atherosclerotic lesions. Contrary, COX-2 expression was physiologically detected in various organs (10). In spite of well known expression and function of COX-1 and COX-2 in the central nervous system, gastric mucosa, kidney, endothelial cells in adult and fetal tissues (10, 13, 14), data on localization of both isoforms in lungs are still unclear.

The aim of the present study is to evaluate immunoexpression of the both COX isoforms in maternal and fetal lungs at the end of pregnancy in untreated rats.

MATERIAL AND METHODS

The study was conducted on sexually mature albino rats of Wistar CRL: (WI) WUBR strain and approved by the Local Board for Supervising Ethics in Medical Experiments (guidelines #372/2002). All the animals were obtained from an accredited breeder (Warsaw-Rembertów, Poland), housed and maintained in an animal care facility. On mating days, females (weight 200–250 g) were placed in cages with males (5:2) for approximately 14 h. The following morning, a vaginal smear was done to determine if copulation had occurred. The day when sperm was found was designated gestation day 1 (GD1). Sperm positive females were randomly taken for the examined group (n=6). No xenobiotics were administered during the study.

On gestation day 21, dames were sacrificed, fetuses delivered by caesarean section and routinely examined (2). Both maternal and fetal lungs were removed in-block with other thoracic viscera and sectioned. The lung samples were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 μm and then stained routinely with hematoxylin and eosin (H&E).

Immunohistochemical reaction for COX-1 and COX-2 was performed on the 4 μm slides obtained from the paraffin blocks used previously for histological examination. After dewaxing and rehydration the slides were placed for three cycles of heating in a microwave oven (750W) for 5 min in citrate buffer (0.01 M, pH 6.0) for antigen retrieval. Then endogenous peroxidase activity

was blocked with 3% hydrogen peroxide for 5 min, and the slides were incubated for 60 min with the primary monoclonal mouse anti-human antibodies (Novocastra; Newcastle, UK) against COX-1 (clone 12E12, dilution 1:20) and COX-2 (clone 4H12, dilution 1:200). The next step was the incubation with DakoEnvision^{+TM}/HRP, Mouse kit (DakoCytomation; Glostrup, Denmark) according to manufacturer directions. The specific immune reaction was visualized using 3',3-diamino-benzidine tetrahydrochloride (DAB) (DakoCytomation; Glostrup, Denmark) and finally the sections were counterstained with Mayer's hematoxylin. TBS buffer rinsing was used after each step. The whole procedure was performed at the room temperature. In all cases the appropriate positive and negative controls were performed. The sections treated in the same way, but with mouse pre-immune serum except for examined primary antibodies were used as negative controls. For the positive COX-1 and COX-2 controls the human colonic mucosa and osteochondroma were applied, respectively. Before starting the proper immunohistochemical study the cross-reactivity with rat tissues was verified. All slides were evaluated under light microscope (Olympus BX45; Tokyo, Japan).

RESULTS

In maternal lungs, immunoexpression of COX-1 was revealed in variety of cells. The strongest staining was found in alveolar macrophages. Less intensive immunoreactivity was observed in endothelial cells, epithelial cells of bronchi and bronchioli (Fig. 1A, B). Strong COX-2 immunoreactivity was found in smooth muscle cells of bronchial tree and blood vessels, alveolar macrophages, as well as type II pneumocytes (Fig. 1D, E). Contrary, bronchial epithelial cells and type I pneumocytes exhibited a weak staining focally.

The examined lungs of 21-day-old fetuses corresponded to physiological succular phase of the organ development. Strong COX-1 expression was observed in both bronchial and alveolar epithelial cells (Fig. 1C). Similar localization but with lower intensity was revealed for COX-2 isoform (Fig. 1F).

Immunohistochemical reaction for COX-1 was found exclusively in the cytoplasm of cells while reaction for COX-2 was observed in both cytoplasm and nuclei.

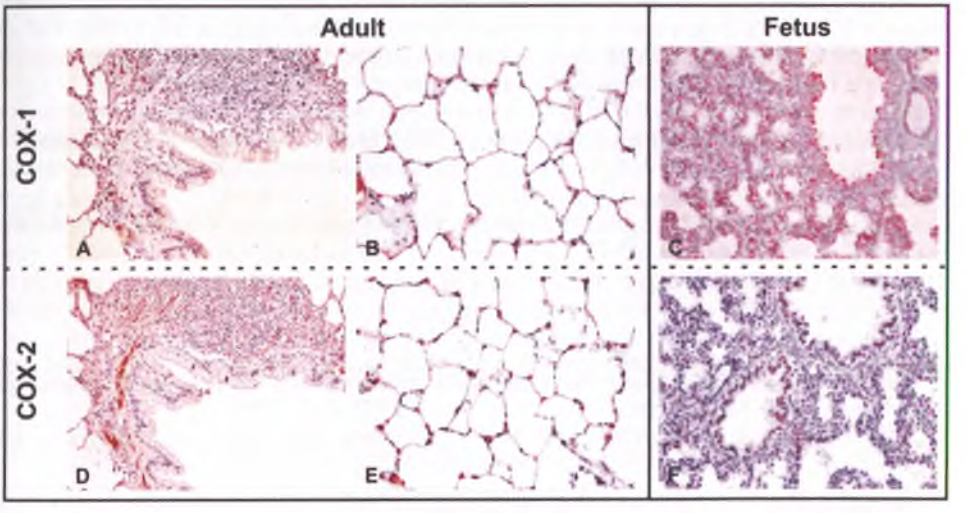

Fig. 1. Immunoreactivity of COX-1 (A, B, C) and COX-2 (D, E, F) in maternal (A, B, D, E) and fetal (C, F) lungs (DakoEnvision^{+TM}/HRP; A, D magn. x100; B, C, E, F magn. x200)

DISCUSSION

Similar to our results COX-1 expression was previously detected in most of the lung structures including epithelial and stromal cells in both animal (3, 4, 13, 14) and human (5, 7, 8) samples. It was also revealed in chondrocytes of bronchial cartilages as well as in interstitial cells of alveolar septum and bronchial adventitia. In rats, strong expression of COX-1 was seen predominantly in bronchial epithelial cells and smooth muscle cells of large hilum veins (5). Lower expression was found in alveolar macrophages and endothelial cells of pulmonary artery. Chida and Voelkel (3) reported physiological expression of COX-2 in unstimulated adult rat lungs. According to Ermert et al. (4) in rats the most intense COX-2 staining was noted in macrophage- and mast cell-like cells, detected in the close vicinity of the bronchial epithelium and in the connective tissue surrounding the vessels. Strong expression was also found in smooth muscle cells of muscular vessels and large pulmonary veins. Bronchial epithelial cells displayed weak COX-2 immunoreactivity. Occasionally the staining was also observed in alveolar macrophages and alveolar septal cells. Unlike in animals, a weak COX-2 expression was seen exclusively in mature lung epithelium in humans (8, 11). Moreover, higher COX-2 that COX-1 expression is characteristic of cultured pulmonary epithelial cells (1, 16).

It should be noted that the expression of COX-2 isoforms decreased during lung maturation (7). The observation in human showed the highest expression in non-malformed fetuses of gestational age of 16–32 weeks. The enzyme was detected in the ciliated epithelial bronchial cells, as well as in cuboid cells and cells resembling type II pneumocytes. Similar localization but with less intensive staining was found in infants (38–42 gestational week).

The increase in COX-2 expression, detected using both genetic and immohistochemical methods was observed in hypoxia, inflammations and neoplasms. Tomlinson et al. (15) found the increase in this isoenzyme expression in pulmonary epithelial and endothelial cells, as well as in bronchial smooth muscle cells and alveolar macrophages during LPS administration. It should be noticed that the mRNA COX-1 level was not altered. Similar results were reported for various types of lung neoplasms, including adenocarcinoma which is characterized by the greatest COX-2 expression (6). Lower expression was detected in squamous cell carcinoma, while small cell carcinoma did not reveal the expression of both constitutive and inducible isoform (17, 18). Furthermore, Sousa et al. (12) reported the increase in COX-2 expression in bronchial epithelial cells in samples from patients with bronchial asthma, while Demoly et al. (5) observed no effect on the isoform expression. However, according to recently published results (9) the obtained differences may be due to corticosteroid intake, that decreases COX-2 expression.

In conclusion, it was found that both constitutive and inducible cyclooxygenase isoforms are physiologically expressed in fetal lungs. Unlike COX-1 immunoexpression, COX-2 expression decreases in postnatal period, however in adult pregnant rat females it is still detected in various cells.

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SUMMARY

Immunoexpression of constitutive (COX-1) and inducible (COX-2) cyclooxygenase isoenzymes was evaluated in maternal and fetal rat lung on day 21 of gestation. In maternal lungs, immunoexpression of COX-1 was revealed in alveolar macrophages, epithelial cells of bronchi and bronchioli, as well as endothelial cells. COX-2 immunoreactivity was found in smooth muscle cells of bronchial tree and blood vessels, alveolar macrophages, as well as type II pneumocytes. A week staining was also occasionally found focally in bronchial epithelial cells and type I pneumocytes. COX-1 expression was observed in both bronchial and alveolar epithelial cells in fetal lung. Similar localization but with lower intensity was revealed for COX-2. It could be concluded, that both constitutive and inducible cyclooxygenase isoforms are physiologically expressed in maternal and fetal lungs.

Lokalizacja izoenzymów cyklooksygenazy w płucach matek i płodów w końcowym okresie ciąży

Immunoekspresję izoformy konstytutywnej (COX-1) i indukowanej (COX-2) cyklooksygenazy oceniano w płucach matek i płodów u szczurów w 21 dniu ciąży. W płucach matek immunoekspresja COX-1 została wykazana w makrofagach pęcherzykowych, komórkach nabłonkowych

oskrzeli i oskrzelików oraz komórkach śródbłonka. Obecność COX-2 stwierdzono w komórkach mięśniowych gładkich drzewa oskrzelowego i naczyń krwionośnych, makrofagach pęcherzykowych, jak również pneumocytach typu II. Słaby odczyn sporadycznie obserwowano w komórkach nabłonka oskrzelowego i pneumocytach typu I. W płucach płodu immunoekspresja COX-1 była obecna w komórkach nabłonka oskrzeli i pęcherzyków płucnych. Analogiczną lokalizację odczynu, jednakże o mniejszej intensywności, wykazano dla COX-2. Należy stwierdzić, że płuca matek i płodów szczurzych wykazują ekspresję zarówno formy konstytutywnej, jak i indukowanej cyklooksygenazy.