THE CYCLOOXYGENASE-2 INHIBITOR CELECOXIB INHIBITED CELL PROLIFERATION AND INDUCED CELL APOPTOSIS IN HUMAN OSTEOSCLAST-LIKE CELL LINES.

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Introduction: Cyclo-oxygenases (Coxs) are the key enzymes that mediate the production of prostaglandins from arachidonic acid. Cox-2, an inducible isozyme, of the Coxs family, can be dramatically up-regulated during pathological conditions such as inflammation and cancers. The Cox-2 gene is rapidly induced in response to tumor promoters, cytokines and growth factors. Cox-2 expression is regulated by oncogenes and p53 (1). Evidence suggests that the increase of its tumorigenic potential is associated with resistance to apoptosis. Selective inhibitors of Cox-2 may protect against the formation or reversion of cancers through induction of apoptosis and suppression the cell proliferation. Cox-2 inhibitor has been becoming the most frequently used analgesic in clinic. It was recently found that the specific Cox-2 inhibitor can suppresses cell growth and inhibits the formation of collagen type I expression on the cells.

Methods: Saos-2, an osteoblast like cells, was used in this study (ATCC). Cells (2x10⁴) were cultured in 6 well plates with 0 nM, 25 nM, 50 nM and 100 nM of celecoxib (Pfizer Inc), 100 nM Rofecoxib (Merck & CO Inc) , a specific Cox-2 inhibitor, for 24, 48 and 72 hours. Apoptosis cells were determined by flow cytometry with Annexin V/propidium iodide fluorescein stain kit (Santa Cruz Biotechnology, Inc.). Alterations of collagen type I protein levels were determined by Western blot analysis. Cell lysates were loaded and separated in 7.5% SDS/PAGE gels. Then, the protein was immuno-blotted using anti-human collagen I monoclonal antibody (Accurate Chem & Sci. CO.).

ALP activity was measured with alkaline phosphatase kit (Sigma diagnostics). The activity of caspase-1, caspase-3 and caspase-8 were measured by using Ac-YVAD—AMC, Ac-DEVD-AMC and Ac-IETD-AMC (BD Pharmingen) as substrates. The results were determined by spectrophotometer reading in excitation wavelength of 380 nm and an emission wavelength of 460 nm. Cell proliferation was measured by flow cytometry with 5-6-darboxyfluorescein diacetate succinimidyl ester (CFSE) stain Kit (Molecular probes). Alkaline phosphatase (ALP) activity was measured with alkaline phosphatase kit (Sigma diagnostics). The activity of caspase-1, caspase-3 and caspase-8 were measured by using Ac-YVAD—AMC, Ac-DEVD-AMC and Ac-IETD-AMC (BD Pharmingen) as substrates. The results were determined by spectrophotometer reading in excitation wavelength of 380 nm and an emission wavelength of 460 nm. Cell proliferation was measured by flow cytometry with 5-6-darboxyfluorescein diacetate succinimidyl ester (CFSE) stain Kit (Molecular probes). Alkaline phosphatase (ALP) activity was measured with alkaline phosphatase kit (Sigma diagnostics).

Results: After 24 hours incubation, the cells in 50 nM and 100 nM Celecoxib showed shrunken, round and detached from the dish (Fig.1), but it did not found in other groups. Morphology and flow cytometry analysis demonstrated that the Celecoxib induced apoptosis in Saos-2 cells, the incidence of the cell death was associated with the concentration of Celecoxib (Fig.2). Both Caspase-3 activity and cytochrome c were elevated in Saos-2 cells after incubated with 50 nM and 100 nM Celecoxib, the increase of cytosol cytochrome c was close correlated with increase of caspase-3 activity, ADP:ATP ratio and percentage of cell apoptosis. However, no significant alteration on caspase-1 and caspase-8 activity was found in the cells. In other hand, Rofecoxib treated cells did not show a notable increase on apoptosis and caspase-3 activity. Cellular ALP activity was also decreased after incubation with Celecoxib (Fig.3). However, the decrease can be attenuated partially by BMP-2. By means of western blot we showed that collagen type I expression were down-regulated after 24 and 48 hours incubation with Celecoxib (fig 4). Measured by CFSE staining method, the cell cycle was arrested with a reduction in cell proliferation number (Fig.5). The decrease of alkaline phosphates activity and cell proliferation number induced by Celecoxib could not be attenuated by prostaglandin E2. Moreover, 100 nM Rofecoxib did not show marked effect on Saos-2 cell proliferation and ALP activity.

Discussion: A large number of studies have showed that selective Cox-2 inhibitor, especially Celecoxib, could attenuate cell growth and induce apoptosis in different kinds of cell. Though Cox-2 inhibitor is widely used in clinic recently, its effect on osteoblast remains elusive. The discovery that Cox-2 inhibitor reduce the fracture healing suggested that this agent may influence cell function and clinic outcome. In this study we revealed that celecoxib can induce apoptosis in osteoblast-like cell. Moreover, our founding also suggested that Cox-2 might halt osteoblast growth and reduce fracture healing by inhibiting cellular proliferation and viability. Collagen I is an extracellular matrix protein, which play an important role in bone hearing and rebuilding after trauma. Suppression of collagen I synthesis, as we found here, will disturb the recovery process. The relationship between apoptosis and suppression of cell proliferation/function is not clear. In this study we found Celecoxib can inhibit Saos-2 cell proliferation in 25 nM without notable apoptosis, which means that the effect of proliferation arrest is not dependent on apoptosis. On other hand, our data showed Rofecoxib did not induce apoptosis and suppress cell proliferation. Meanwhile, PGE2 could not attenuate the cellular alteration caused by Celecoxib. These results hint that Celecoxib does not induce the cell changes by block Cox-2. In summary, Celecoxib could induce apoptosis in Saos-2 and suppress the cell proliferation function. The effect may be not performed by inhibit Cox-2.

Figure 1: Morphologic change of Saos-2 cells (24h)
Figure 2: Percentage of apoptosis
Figure 3: ALP activity of Saos-2 cells
Figure 4: Celecoxib inhibited Collagen I expression on the cells
Figure 5: Proliferation of Saos-2 cell

Reference:
3. Aaron Daluiski, Keri Pearson, Yuexian Shi et al. (Unpublished)