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Neurite Outgrowth of Dorsal Root Ganglia Is Delayed and Arrested by Aspirin

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In this study, the effect of increasing doses of aspirin on the neurite outgrowth of Dorsal Root Ganglia (DRG) was investigated. DRG were cultured in complete medium (DMEM+10% FCS +100ng/ml NGF +collagen Type*1*in substratum in 96 multiwell plate) in the presence of concentration of 1.25, 2.5, 5 and 10 mM aspirin. The neurite outgrowth of DRG was followed in comparison with controls that lack aspirin. 10 mM aspirin treated DRG showed delayed neurite outgrowth and after 7 days it reached the same DRG neurite outgrowth control wells after 18 hrs. This growth has delaye approximately one week and showed no further development and in such stage the cells became apoptos. However at concentrations of 1.25, 2.5, 5 mM of aspirin, outgrowth was observed after 18-24 hrs. Although the rate of growth was lower than control, it was not significant. In the other experiment, when DRG cultured for one week in complete medium then treated with aspirin, at 10 mM, DRG neurite outgrowth was stopped,while it was continued in the control. It seem that the aspirin affected DRG became apoptosis.

Key Words: **neurite outgrowth; Dorsal Root Ganglia; culture; delay; aspirin.**

Aspirin as an old drug, fall into the category of the non-steroidal anti-inflammatory drugs (NSAIDs) (1) and is extensively used for its analgesic, anti-pyretic and anti-rhumatic properties. Retrospective and prospective epidemiological studies concluded that regular uses of aspirin reduces the risk of heart attack and stroke (1). In the early 1970s NSAIDs were found to prevent the production of prostaglandins (PGs) by inhibiting the enzyme cyclooxygenases (COXs), suggesting a biochemical mechanism function for these drugs. PGs are produced by most cells and tissues, and have a diverse array of biological functions (4) including stimulation of cell proliferation and suppression of immune reaction, which both are linked to tumor progression stage. Recently, the structural basis of the aspirin activity inferred from the crystal structure of the inactivated prostaglandin H2 synthetase was reported (1). On the other hand, all of NSAIDs, cause untoward side effects in a significant number of treated patient, and this frequently limits therapy. The most common side effects associated with NSAIDs therapy are gasterointestinal, with hemorrhage and frank ulceration seem in some patients and such these lesions apparently can lead to increased morbidity in long term NSAIDs users (4). Renal and CNS effects were also observed (5) in addition teratogenicity of aspirin was observed (6). In this paper, the effect of aspirin on neurite outgrowth of DRG was studied. Recent studies have considered the DRG outgowth as a neural toxicity test for various substances and such protocol has been applied as a routine test (7).

MATERIALS AND METHODS

Preparation of collagen solution. Collagen solution was extracted and prepared from rat tail as previously reported (8). Briefly, 1g of rat tail collagen fibers was sterilized in alcohol for overnight peroid, then dissolved in 300 ml of 1:1000 acetic acid in steril distilled water and stirred at 4 ° C for 48 hr. Subsequently the solution was left to stemd without agitation for 24 hrs, allowing the undissolved fibers to sediment and the clear solution was gently poured off into a sterile container. Preparation of gel collagen: 8/2ratio of collagen and DMEM medium was used. This solution is yellow and acidic, 2-3 drop of sterile 1N NaOH was added until solution became reddish as an indication of neutral medium (9).

Extraction of 7s NGF. 7s NGF (nerve growth factor) was extracted from submaxillary glands of 100 mature male mice as according to Varon, and *et al.* (10). Assay of 7s NGF protein: 7s NGF content was estimated by Lowery assay (11), and Bovine Serum Albumine, at concentrations of 0, 20, 40, 60, 80 μg was used for standard curve determination. The extracted NGF showed a concentration of 5.7 mg/ml. Bioassy of NGF was done using E8 chicken DRG and optimized concentration of 100 ng/ml (unpublished data).

Effect of aspirin on DRG chicken embryo (E8). DRG were isolated from E8 chick in complete Hank's buffer under stereomicroscopic conditions. For constructing collagen gel, 96 multiwell plates (Nunc) were applied. 50 μl of collagen solution was loaded in each well and incubated at 37° C, 5% CO2 and 95% air for 30 min. to gel collagen, then 200 μl complete medium (DMEM+ 10%FCS+ 100 ng/ml NGF) in duplicate condition was poured then one ganglion was added to each well. To investigate the effect of aspirin on DRG outgrowth,

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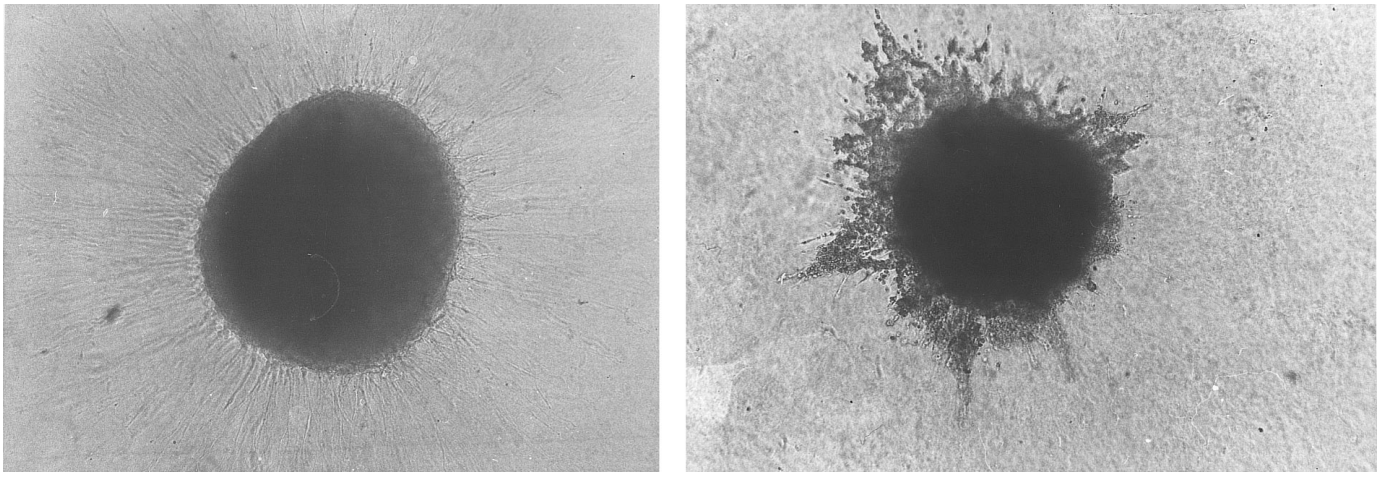

FIG. 1. Neurite outgrowth of DRG as control (left) and 10mM Aspirin treated DRG after 8 days (right).

complete medium was used for preparation of increasing concentration of aspirin as follow: 1.25, 2.5, 5 and 10 mM. For each concentration, two wells were considered. DRG outgrowth after 18 hrs was studied. Each experiment was done 3 times.

RESULTS

Outgrowth was observed by the use of phase contrast inverted microscope after 18 hrs. Outgrowth was seen in all control wells in the presence of 100 ng/ml NGF. Outgrowth was observed in wells contained 1.25, 2.5, and 5mM aspirin showed lower effect than the control and no differences was observed during the next 2 days. 10 nM aspirin treated DRG, caused no growth after 18 hrs but after 7day, DRG outgrowth was similar to 18 hrs DRG outgrowth control wells. The delayed outgrowth remained at the same stage and seemed to reach apoptosis. When, DRG cultured one week and then treated with 10 mM aspirin, DRG outgrowth remained at the same stage and did not develope more, while, in the control wells DRG outgrowth was continued (Fig.1).

DISCUSSION

Aspirin, has been thought as a dangerous drug by few people. Yet, chronic use of aspirin and other so called non-steroidal anti-inflammatory drugs (NSAIDs) can cause stomatch bleeding, kidney failure or worse. Traditional aspirin and other NSAIDs, block the activity of so called cyclooxygenase (COX) enzymes. COX-1 produces the "good" prostaglandins that act in the stomatch and other tissues but another COX enzyme (COX-2) responsible for the troublesome prostaglandins made in response to injury (12). Aspirin and related anti-inflammatory drugs caused irreversible inhibition of cyclooxygenase by acetylating the active site of enzyme (13). In our study aspirin delayed and arrested neurite outgrowth of DRG and finally caused apoptosis. It has been reported that some of PGs synthetase were recovered in the cytosol during chicken embryonic DRG development (14,15). Prostaglandins are a class of naturally occurring cycle 20-carbon fatty acid with poten-

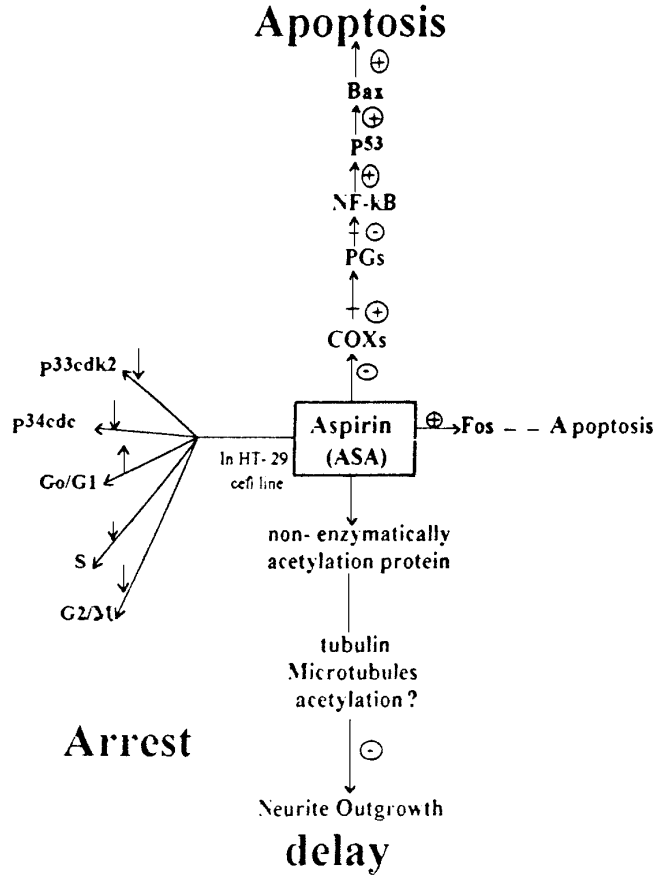

FIG. 2. See Discussion.

tial biological properties. In eukaryotic cell, they are synthesized from arachidonic acid (AA) and other polyunsaturated fatty acid precursors derived from the phospholipid stimuli (16), and act as intracellular signal mediators in the regulation of physiological and patholgical processes, including inflammation immune responses, febrile responses, cytoprotection (16) and cell proliferation and differentiation. It has also reported that PGs cause protection of microtubules network. PGs ,especially, PG A1(16) and PG E2 (17),can inhibit nuclear factor kappa B (NF-kB). NF-kB is an inducer of apoptosis (18,19,20,21). NF-kB is most probably not the only transcription factor that plays a role in the ceramide-dependent apoptotic signaling pathway in mesencephalic neurons in culture, but it is clearly associated with the death process also by activating c-MYC (22) and p53 proteins and subsequently the induction of BAX gene by p53 (19). Aspirin can inhibit the production of PGs. So NF-kB may not cause inhibition and induction of apoptosis. Another point which should be mentioned is that, acetylation is one of the tubulin modifier (23,24). Aspirin can unenzymatically (25) acetylate some of the protein. One suggestion is that aspirin acetylate tubulins unregulary and affected tubulin polymerization and subsequently delayed neurite outgrowth. NGF could induce production of PGs in PC12 cells and therefore differentiation of PC12 to nerve cell (26). So, PGs could be one of the important factor for neurite outgrowth that is inhibited by aspirin. On the other hand, it has reported that aspirin can induce *fos* protein which is an inducer of apoptosis (27). It has also been reported that in some cell line such as HT-29 colon adenocarcinoma cell in vitro, aspirin and other NSAIDs altered the cell cycle phase distribution of these cells. They increased the proportion of cells in G0/G1 phase and reduced the proportion in the S phase of the cell cycle. Aspirin also reduced the percentage of cells in the G2/M phase. Parallel to their effect on cell cycle, aspirin also reduced the levels of p34cdc2 and p33cdk2, two cyclin-dependent kinases that are important for cell cycle progression (28). Therefore, aspirin could be a toxic agent and especially women should not use it during proganency due to its teratogenic effect, and in some of disease that the congenitally inhibited peripheral nerves was interpreted as a result of aspirin administration, should be reevaluated. The pathways of aspirin involvement is summerized in (Fig.2).

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