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Intervertebral Disc Degeneration, İnflammation, and Bioactive Lipids

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ABSTRACT

Intervertebral disc (IVD) degeneration is a common condition that is associated with significant morbidity and is considered an inflammatory condition. There is currently no specific treatment available for IVD except for surgical intervention. IVD may result from an imbalance between pro- and anti-inflammatory eicosanoids derived from arachidonic acid (AA) and other polyunsaturated fatty acids, such as eicosapentaenoic and docosahexaenoic acids (EPA and DHA, respectively). We propose that IVD can be prevented and managed by local administration of lipoxin A4 (LXA4), a potent anti-inflammatory, cytoprotective and anti-osteoporotic metabolite formed from arachidonic acid (AA).

Keywords: Intervertebral Disc, Degeneration, Lipoxin A4, Arachidonic Acid, Inflammation.

Introduction

Intervertebral disc (IVD) degeneration is caused by the deterioration or breakdown of one or more of the discs between the vertebrae of the spinal column. The outer part of the disc, the annulus fibrosus, is tough and fibrous, whereas the inner nucleus pulposus is soft and gelatinous and serves as the shock absorber and distributes hydraulic pressure in all directions within each IVD (see Figure 1). The nucleus pulposus cells included large vacuolated notochord cells, small chondrocyte-like cells, collagen fibrils, and aggrecan, which contain glycosaminoglycan (GAG). The shift of the extracellular fluid from the outside to the inside of the nucleus pulposus is necessary to prevent IVD, the reduction of which results in IVD degeneration (Das, 2019). Thus, integrity and healthy annulus fibrosis are needed to prevent IVD degeneration.

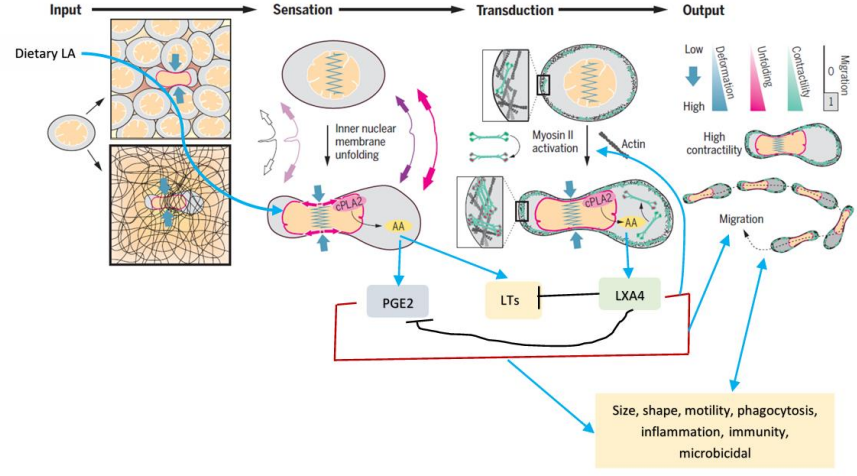

Figure 1A. The nucleus acts as an elastic mechanotransducer of cellular shape and controls dynamic behavior. In response to pressure, the cell shape changes, leading to inner nuclear membrane unfolding, which results in activation of the cPLA2-AA pathway. AA is the precursor of various eicosanoids that have several physiological and pathological actions. The unfolding of the inner nuclear membrane transduces myosin II to the cell cortex, where it regulates actin cytoskeleton contractility, which results in cell motility as needed.

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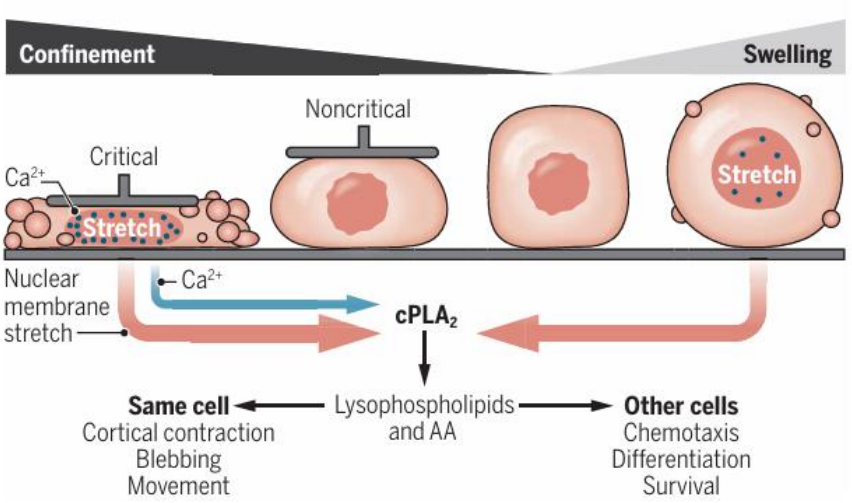

Figure 1B. Nuclear membrane transduction. In response to pressure or stretch stimuli, stretch in the nuclear membrane occurs in conjunction with calcium, which activates cPLA2 release and the release of AA. Eicosanoids formed from AA mediate cell autonomous and paracrine effects.

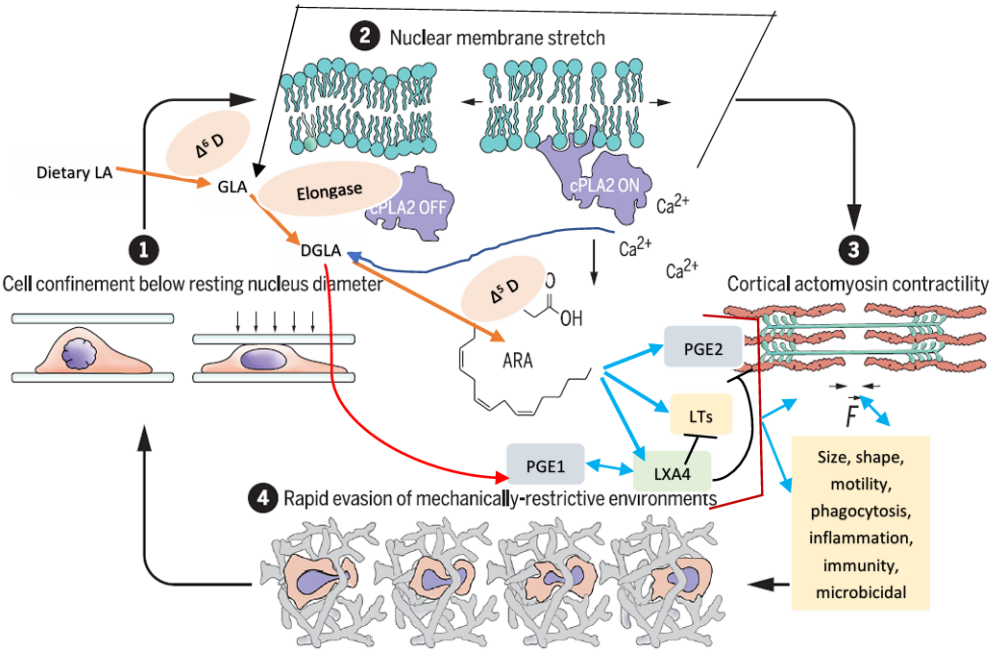

Figure 1C. In response to physical pressure, cell nuclear deformation and unfolding and stretching of the nuclear envelope (2) trigger calcium release, cPLA2 activation and AA release, the precursors of several eicosanoids. These events lead to actomyosin force generation (3) and increased cell migratory capacity (4) (Figures 1A, B and C were created and modified from references (Lomakin et al., 2020; Shen & Niethammer, 2020; Venturini et al., 2020).

IVD is an inflammatory condition

Studies showed that anulus fibrosis cell apoptosis is dependent on the JNK and p38 mitogen-activated protein kinase (MAPK) pathway. Loading conditions produce a significant increase in the expression of matrix metalloproteinases (MMP1, MMP2, MMP3, and MMP13), IL-1β and TNF-α and an increase in TUNEL positive cells in the intervertebral cells, suggesting that intervertebral disc degeneration is an inflammatory condition (Das, 2019).

Cell membrane

Cell membrane integrity is essential for cellular homeostasis. The cell membrane responds to a multitude of stressors in the extracellular and intracellular environments. The integrity of the cell membrane is essential for the optimal response of the cell to various external and internal stimuli. This is understandable since all stimuli must be conveyed to the genome through the cell membrane. In a similar fashion, all the responses elicited by

the cell genome are also conveyed to the cell external milieu through the cell membrane. Thus, the cell membrane structure and consequently its functions are crucial for receiving and sending signals.

The cell membrane is mainly composed of lipids and proteins (and their associated carbohydrate molecules). Proteins are like bricks on the wall and are inflexible. In contrast, lipids are flexible and are capable of influencing cell membrane fluidity. The presence of higher amounts of unsaturated fatty acids renders the membrane more fluid, whereas higher contents of saturated fatty acids and cholesterol make the membrane more rigid. Alterations in cell membrane fluidity influence the expression of receptors and their affinity for their respective molecules. This implies that the constitution of the cell membrane and its lipid content are critical to cell function.

How cells sense space and pressure

During both health and disease, cells need to travel short and long distances in response to chemical and physical stimuli to heal wounds, replace cells that have undergone apoptosis and, in the case of cancer, metastasize to distant organs. To perform these functions, embryonic, immune and cancer cells need to gauge space around them and respond as the situation demands. These cells do so by deformation of their nucleus, especially when physical pressure is applied to their surface. This results in stretching in the nuclear membrane, which activates the cytosolic phospholipase A2 (PLA2) enzyme, resulting in the release of arachidonic acid (AA) from the cell membrane lipid pool. AA is the precursor of several eicosanoids that have both pro- and anti-inflammatory effects and several other functions. These eicosanoids help cells crawl within or out of narrow spaces to perform various functions expected of them.

Stretching of the nuclear membrane activates the enzyme cytosolic phospholipase A2 (cPLA2), which initiates cell blebbing and movements that may help cells crawl within or out of narrow spaces (Lomakin et al., 2020; Shen & Niethammer, 2020; Venturini et al., 2020; Martino et al., 2018) (See Figures 1--2). These studies suggest that the nucleus, in addition to its genetic functions, directly senses the physical environment of the cell and responds accordingly, in which there is a critical role for both the cell and nuclear membranes.

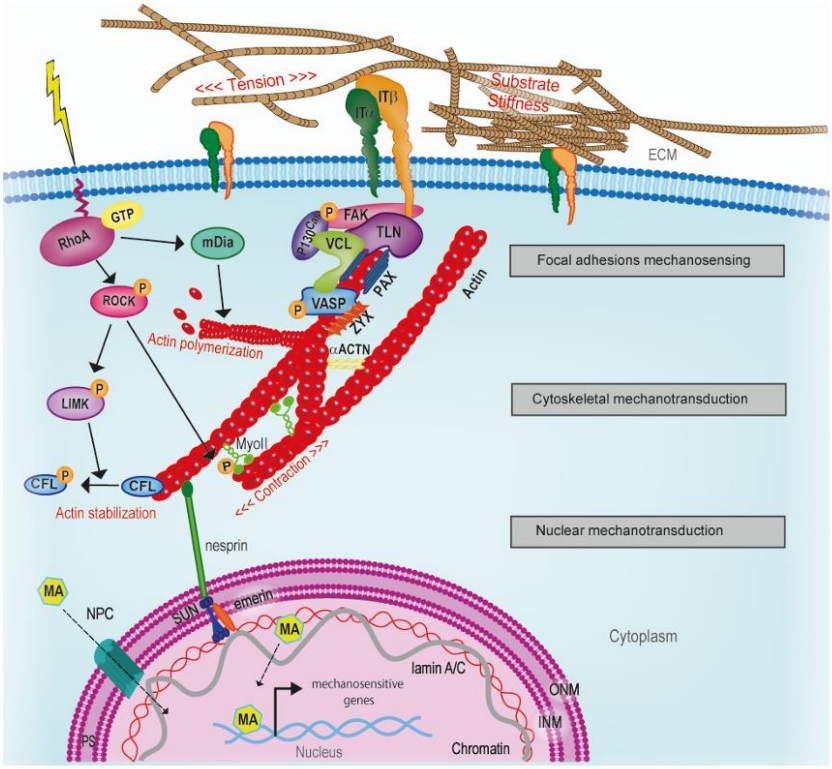

Figure 2. Scheme showing the cellular mechanotransduction process**.**

Extracellular physical stimuli are perceived by the cell membrane, and the signals are propagated by the cytoskeleton and transferred to the nucleus, where mechanosensitive genes are activated. This figure is taken from reference 5.

ACTN = actinin; CFL= cofilin; FA= focal adhesion kinase; INM = inner nuclear membrane; IT = integrin;

LIMK = LIM kinase; mDia = diaphanous-relatedformin-1; MyoII = myosin II; NPC = nuclear pore complex;

ONM = outer nuclear membrane; PAX = paxillin; PS = perinuclearspace; ROCK = Rho-associated protein kinase; TLN = talin; VASP = vasodilator-stimulated phosphoprotein; ZYX = zyxin.

(intervertebral cells) response to mechanosensory stimuli and the role of bioactive lipids in IVD

Intervertebral cells (IV cells) of the nucleus pulposus are under constant mechanical stress. Nucleus pulposus (NP) cells are derived from the embryonic notochord and are responsible for the synthesis and maintenance of the extracellular matrix of the intervertebral disc. A decrease in their cell number, loss of developmental phenotype, and infiltration of alternate cell types lead to alterations in mechanical function associated with intervertebral disc degeneration and suggest the onset of IVD (Sakai et al., 2012) (see Figure 3). A population of progenitor cells (progenitors of nucleus pulposus cells) that are Tie2 positive (Tie2+) and disialoganglioside 2 positive (GD2+) in the nucleus pulposus and express type II collagen and aggrecan have been identified. They are clonally multipotent, differentiate into mesenchymal lineages and are capable of inducing reorganization of nucleus pulposus tissue when transplanted into experimental animals. The frequency of Tie2+ cells markedly decreases with age and in those with degeneration of the intervertebral disc. These findings suggest that the capacity of Tie2+ cells to regenerate nucleus pulposus cells is decreased or exhausted. However, progenitor cells (Tie2+GD2+) can be induced from their precursor cells (Tie2+GD2-) in vitro (Bridgen et al., 2017). Angiopoietin-1, a ligand of Tie2, is crucial for the survival of nucleus pulposus cells. Notably, LXA4, a potent anti-inflammatory compound derived from AA, enhances the formation of angiopoietins.

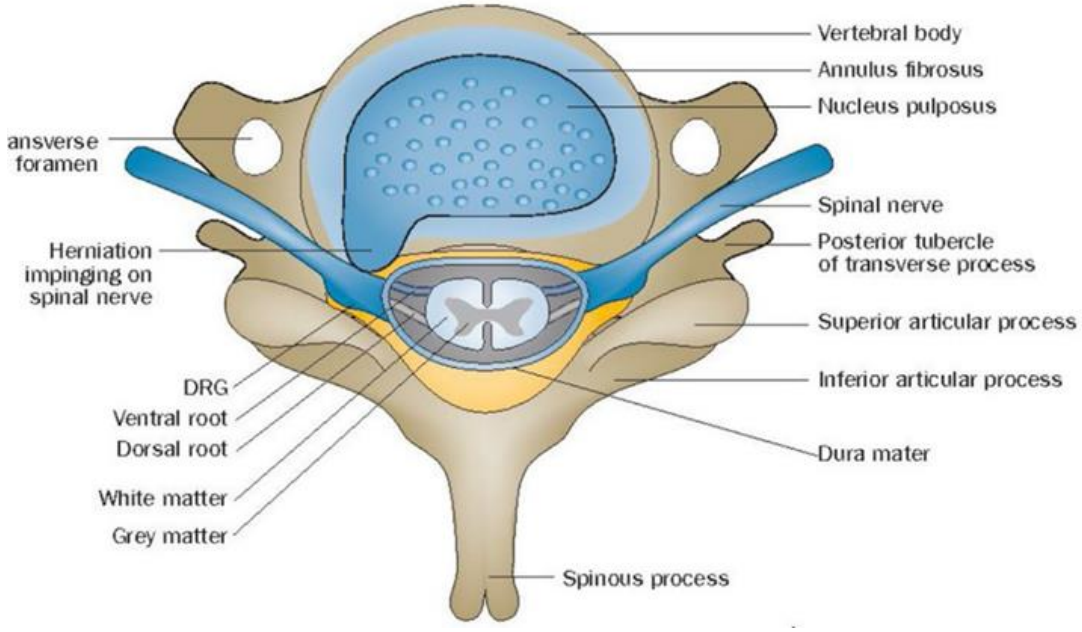

Figure 3. Cross section of vertebra with intervertebral disc

An in vitro study using cells derived from the nucleus pulposus and annulus fibrosus cultured with cyclic mechanical stress (CMS) revealed increased expression of COX-2 (cyclo-oxygenase-2) and prostaglandin E2 (PGE2), a proinflammatory molecule derived from AA (see Figure 4 for the metabolism of essential fatty acids, including AA). Cultured herniated human IVD specimens spontaneously release increased amounts of nitric oxide (NO), IL-6, and PGE2. NO inhibits IL-6 production, and its (NO) suppression increases proteoglycan synthesis in IVD samples in a dose-dependent manner. LXA4, which is also derived from AA, benefits lumbar disc herniation by inhibiting ERK, JNK and NF-kB/p65; suppresses proinflammatory IL-1β and TNF-α; and upregulates the expression of anti-inflammatory TGF-β and IL-10 (Miao et al., 2015; Wang et al., 2017). 15-EET (epoxyeicosatetraenoic acid, also derived from AA) protects rat nucleus pulposus cells against death induced by TNF-α in vitro by inhibiting the NF-κB pathway. Local administration of 14,15-EET prevents IVD degeneration (Li et al., 2017). These studies (Miao et al., 2015; Wang et al., 2017; Li et al., 2017) suggest that the balance between proinflammatory PGE2 and anti-inflammatory LXA4 (and possibly resolvins derived from eicosapentaenoic acid and docosahexaenoic acid and protectins and maresins derived from docosahexaenoic acid) and EETs (see Figure 4) is important for preventing IVD degeneration.

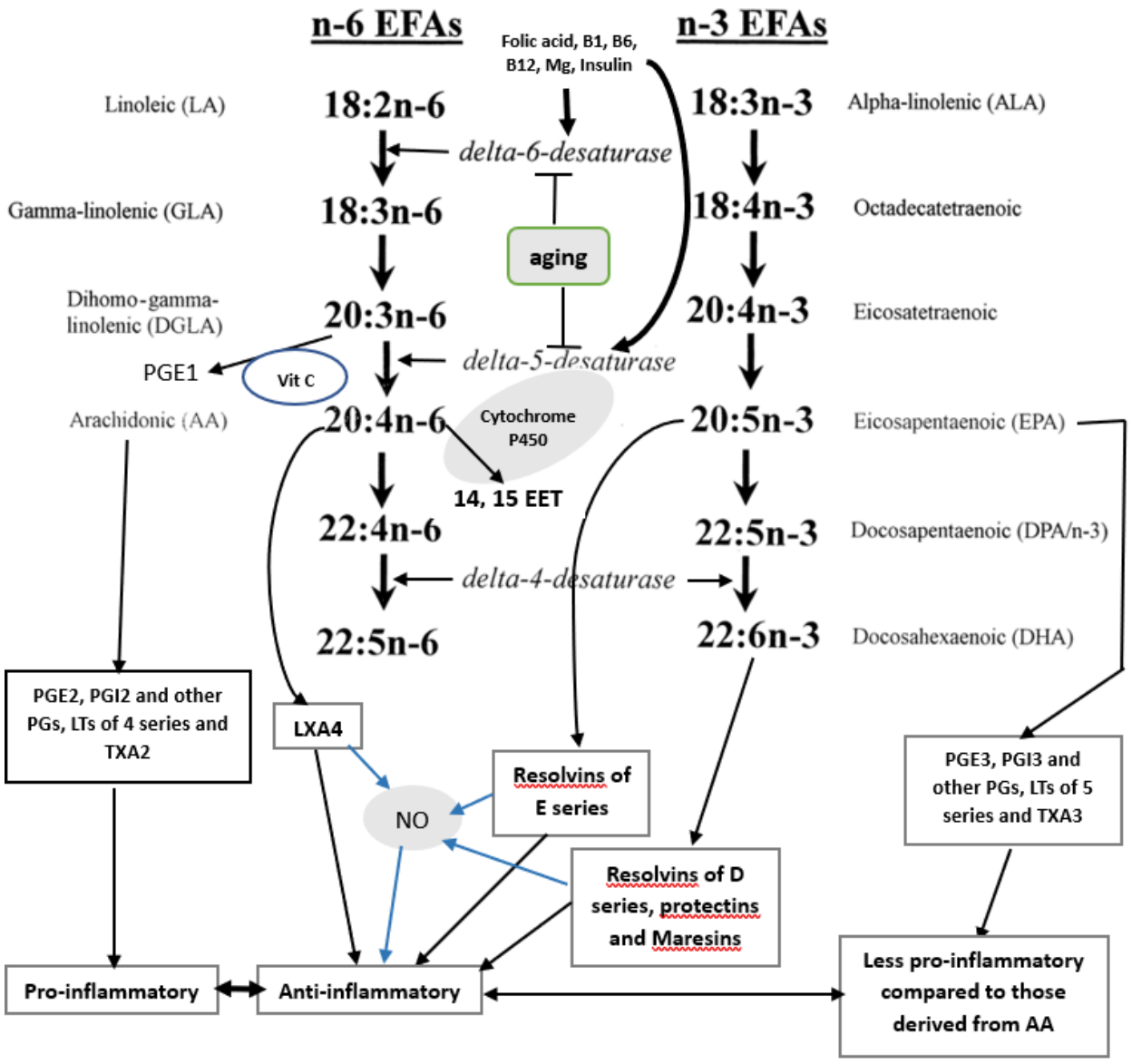

Figure 4. Metabolism of essential fatty acids (EFAs).

In this context, it is interesting to note that with advancing age, a decrease in the activities of desaturases, which are needed for the metabolism of dietary essential fatty acids, linoleic acid and alpha-linolenic acid, to their respective long-chain metabolites, AA (from LA) and EPA and DHA (from ALA), occurs (Das, 2021; Das, 2018). This results in a decrease in the plasma and tissue concentrations of AA, EPA and DHA. Thus, in those with IVDs, the cells derived from the nucleus pulposus and annulus fibrosus are likely deficient in AA, EPA and DHA. Because of this decrease in the levels of AA, EPA and DHA, the formation of their anti-inflammatory metabolites, LXA4 (from AA), resolvins and protectins (from EPA and DHA), decreases. This is due to precursor deficiency (see Figure 5) with a concomitant increase in the production of pro-inflammatory PGE2 (Arnardottir et al., 2014). It is paradoxical that a decrease in AA results in an increase in the production of proinflammatory PGE2. In contrast, supplementation with AA does not result in increased production of PGE2 and, in fact, may lead to an increase in or no change in LXA4 production (Tateishi et al., 2015; Tateishi et al., 2014). These results suggest that the availability of physiological (optimal) levels of AA, EPA and DHA results in the synthesis of adequate concentrations of LXA4, resolvins, protectins and maresins and a decrease in the formation of proinflammatory PGE2 and possibly thromboxanes and leukotrienes. In view of this, it is safe to administer AA.

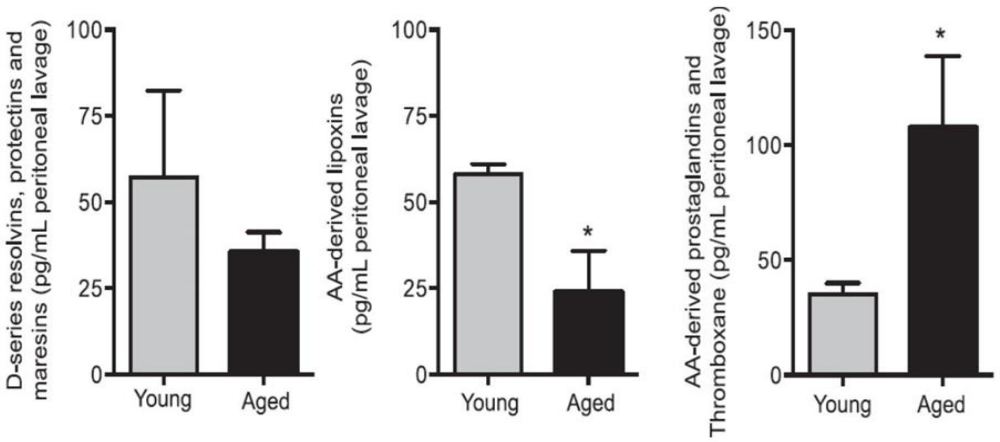

Figure 5. Aged mice presented reduced levels of resolvins, protectins, maresins and LXA4 in the peritoneal lavage fluid of zymosan-challenged animals. * $p < 0.05$ compared with young mice. These data are taken from Arnardottir, H.H.; Dalli, J.; Colas, R.A.; Shinohara, M.; Serhan, C.N. Aging Delays Resolution of Acute Inflammation in Mice: Reprogramming the Host Response with Novel Nano-Proresolving Medicines. J. Immunol. 2014, 193, 4235–4244.

A recent study (Zeng et al., 2023) revealed that amygdalin, a suppressor of COX-2 and iNOS, delays cartilage endplate degeneration and improves intervertebral disc degeneration by inhibiting NF-κB and other inflammatory events, suggesting that IVD degeneration is an inflammatory condition. Amygdalin is a potentially toxic or lethal compound (Milazzo & Horneber, 2015). LXA4 and EETs are anti-inflammatory in nature and beneficial in treating IVD degeneration (Das, 2019; Miao et al., 2015;Wang et al., 2017; Li et al., 2017). Hence, the local administration of LXA4 and EETs needs to be considered to prevent and manage IVD prolapse or degeneration.

Conclusions and therapeutic implications

It is evident from the preceding discussion that IVD is an inflammatory condition in which there is a critical role for proinflammatory PGE2 and anti-inflammatory LXA4, both of which are derived from AA. This finding implies that a delicate balance between PGE2 and LXA4 needs to be maintained to prevent IVDs. To withstand cyclic mechanical stress (CMS) and suppress inappropriate expression of COX-2 and production of PGE2, the cells of the nucleus pulposus and annulus fibrosus need a constant supply of AA to form adequate amounts of LXA4 to inhibit ERK, JNK, NF-kB/p65, IL-1β and TNF-α and upregulate the expression of anti-inflammatory TGF-β and IL-10; and 14, 15-EET. Hence, I propose that local administration of AA, LXA4 and EETs can be employed to prevent and manage IVD prolapse or degeneration. Despite the concern that AA administration might enhance the formation of proinflammatory PGE2, previous studies have suggested that this is unlikely (Tateishi et al., 2015; Tateishi et al., 2014). Hence, the local administration of AA/LXA4 to IVDs is safe.

The intrathecal administration of drugs is not uncommon. In clinical practice, the intrathecal administration of drugs is generally considered safe. For example, the FDA approved the intrathecal administration of morphine, ziconotide, baclofen and other opioids (Simpson & Jones, 2008; Prager et al., 2014). Hence, it is safe to administer AA/LXA4 locally to the intervertebral disc region. Furthermore, it is anticipated that the administration of AA/LXA4/EET needs to be given only once or not more than 2-3 ties in the lifetime of a subject with IVDs since LXA4 is known to stimulate its synthesis in an autocrine fashion. This is evident from our previous studies in which the intraperitoneal administration of LXA4 for 5 days resulted in a sustained increase in the plasma levels of LXA4 to near-normal levels for almost one month (Gundala et al., 2017a; Gundala et al., 2017b). Despite the fact that stem cell therapy is an attractive option in the treatment of IVD, we previously showed that even stem cells exert their beneficial effects by elaborating LXA4/resolvins, protectins and maresins (Das, 2020).

Despite the evidence and arguments presented here, more preclinical studies are needed to establish the safety, tolerability, and efficacy of AA/LXA4/EET before its clinical use in the treatment of IVD. Exploring the possibility of combining AA/LXA4/EET with existing therapeutic measures for treating IVD is worthwhile. It will be interesting to explore the potential of developing a biomaterial-based delivery system of AA/LXA4/EET that can release active material over long periods to obtain sustained relief from IVD. Another potential area that needs investigation is the development of methods for monitoring plasma and CSF (cerebrospinal fluid) concentrations of AA/LXA4/EET as markers of the progress and therapeutic response of IVD.

Statement & Declarations

Since the article's author is also the journal's chief editor, a conflict of interest exists. To prevent this conflict of interest, a guest editor has been invited for this article.

Ethics Committee Approval: Since this article is a review study, ethical approval is not required.
Informed Consent: Since this study is a review article, participant consent is not required.
Peer-review: Externally peer-reviewed.
Author Contributions: Concept – UD, AH; Design- UD, AH; Materials – UD, AH; Data Collection and/or Processing– UD, AH; Analysis and/or Interpretation – UD, AH; Literature Search –UD, AH; Writing Manuscript– UD, AH; Critical Review – UD, AH; Other–UD, AH.
Conflict of Interest: The authors have no conflicts of interest to declare.
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