Small molecule-based treatment approaches for intervertebral disc degeneration: Current options and future directions

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Abstract

Low back pain (LBP) is a major reason for disability, and symptomatic intervertebral disc (IVD) degeneration (IDD) contributes to roughly 40% of all LBP cases. Current treatment modalities for IDD include conservative and surgical strategies. Unfortunately, there is a significant number of patients in which conventional therapies fail with the result that these patients remain suffering from chronic pain and disability. Furthermore, none of the current therapies successfully address the underlying biological problem – the symptomatic degenerated disc. Both spinal fusion as well as total disc replacement devices reduce spinal motion and are associated with adjacent segment disease. Thus, there is an unmet need for novel and stage-adjusted therapies to combat IDD. Several new treatment options aiming to regenerate the IVD are currently under investigation. The most common approaches include tissue engineering, growth factor therapy, gene therapy, and cell-based treatments according to the stage of degeneration. Recently, the regenerative activity of small molecules (low molecular weight organic compounds with less than 900 daltons) on IDD was demonstrated. However, small molecule-based therapy in IDD is still in its infancy due to limited knowledge about the mechanisms that control different cell signaling pathways of IVD homeostasis. Small molecules can act as anti-inflammatory, anti-apoptotic, anti-oxidative, and anabolic agents, which can prevent further degeneration of disc cells and enhance their regeneration. This review pursues to give a comprehensive overview of small molecules, focusing on low molecular weight organic compounds, and their potential utilization in patients with IDD based on recent in vitro, in vivo, and pre-clinical studies.

Key words: small molecules, discogenic pain, intervertebral disc, degeneration, inflammation

Introduction

Discogenic back pain

Globally, chronic low back pain (CLBP) symptoms occur in ~60-80% of people during their lifetime, which has a significant socioeconomic impact via reduced quality of life and work efficacy [1-4]. CLBP is a multifactorial and complex clinical presentation, and symptomatic intervertebral disc (IVD) degeneration (IDD) is considered as the major cause of CLBP [5, 6]. The IVD is a fibrocartilaginous tissue that lies between two vertebrae and functions as a shock-absorber. It includes the jelly-like nucleus pulposus (NP), the surrounding fibrocartilaginous annulus fibrosus (AF), and the cartilaginous endplate (CEP) anchoring the IVD to the corpus vertebrae. IVDs are crucial structural components that form a fibrocartilage joint allowing for slight intervertebral
molecules may facilitate new treatment approaches. Furthermore, we will discuss how these small molecular weight organic compounds that have been investigated for their regenerative effects on IVDs. This review is focused on low molecular therapy have been used for the regeneration of the IVD. Even though these methods opened new possibilities, they also have their limitations [17, 18]. These therapeutic agents (or utilize well-known drugs which were proven effective in treating other diseases) aiming to relieve discogenic pain and regenerate damaged IVDs through restoration of tissue homeostasis. In this regard, several small molecules have shown promising results as alternative therapeutic agents in in vitro, in vivo, and clinical studies [19, 20]. These therapeutic agents demonstrate various phenomena to induce regeneration and prevent degeneration of the IVD, which include anti-oxidative, anti-inflammatory, anti-senescence, anti-apoptotic, anti-catabolic, and anabolic effects. This review is focused on low molecular weight organic compounds that have been investigated for their regenerative effects on IVDs. Furthermore, we will discuss how these small molecules may facilitate new treatment approaches for IVD regeneration.

Anatomy of the intervertebral disc

The IVD is the largest avascular structure in the human body that contains three main components; the soft mucoid NP core, the lamellar AF tissue that encloses the NP, and the CEPs which cover both top and bottom of the IVD (Figure 1A) [21]. Different cell populations produce a unique composition of ECM, forming a special microenvironment for the IVD, which plays an important role in its functionality and mechanical properties [22]. The high density of negatively charged proteoglycan (PG) molecules provides a capacity to absorb approximately three times their weight in water, giving the NP its mechanical resilience during compressive loading [23]. The NP is circumferentially surrounded by the AF, a fibrocartilaginous tissue consisting of highly organized collagen fibers that are arranged in concentric layers [24]. The AF is predominantly composed of both type I and II collagen and small quantities of PGs [25]. The outer layer of the AF is mainly made of collagen type I (95%); however, the amount of this collagen type is significantly decreasing in an almost a linear negative gradient when approaching the NP, where it constitutes less than 5% of collagen type I [26]. In contrast, an opposing pattern exists for collagen type II, decreasing in content towards the outer layers of the AF [26]. The endplate is an osteochondral structure that consists of two parts, including CEP and bony endplate (BEP) that physically limit the NP and AF to their anatomical partitions. Along with its mechanically supporting role, the CEP controls the fluid exchange, as well as an exchange of nutrients or metabolic waste, and acts as a semipermeable barrier between discs and vertebrae [27].

Damage, inflammation and IDD

The etiology of IDD is multifactorial and usually associated with genetic and environmental factors. [28]. IDD often occurs when the balance between catabolism and anabolism of the ECM is disturbed by decreased ECM production and enhanced ECM degradation [29]. The IVD (NP and AF) cells are responsible for keeping the balance between anabolic and catabolic processes including the synthesis, breakdown, and accumulation of ECM components [30]. The quality of ECM composition and the IVD mechanical properties are determined by these cellular processes that are high energy demanding and require glucose and oxygen consumption. During the IDD process, the expression of inflammatory cytokines (i.e., IL-1 and TNF) in disc cells is increased, which subsequently up-regulates matrix remodelling.
Through inflammatory matrix remodelling processes, the concentration of PGs and collagen type II is dramatically decreased, which is mainly mediated by two extracellular enzyme types: matrix metalloproteinases (MMP) and a disintegrin and metalloproteinase with thrombospondin motifs proteins (ADAMTS). Simultaneously, the amount of collagen type I is increased which altogether can change the ECM shear stresses [30]. Moreover, an in vitro study showed that aggrecan, the major PG of the IVD, can inhibit neural ingrowth, which is associated with the development of CLBP [30, 31]. Therefore, it is suggested that detrimental changes in the ECM are linked with discogenic pain.

Damage to the CEP can be another reason for IVD degeneration through both mechanical and nutritional factors. Damage to the CEP changes mechanical loading of the NP, stimulating metabolic disturbances in the disc [32]. With increasing age, calcification of the endplate occurs, which may disturb its permeability and transportation of nutrients and other metabolites, leading to hypoxia and an acidic pH. This impairs the normal activity of IVD cells in synthesizing and supporting the ECM [33].

Inflammation is another factor that is thought to play an important role in the development of IDD [34]. It is not known whether inflammation is the cause or consequence of disc degeneration and herniation. However, pro-inflammatory cytokines and chemokines, which are produced during both systemic and local inflammation, have been associated with IDD and lower back pain. Overproduction of chemokines and cytokines including interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), and interleukins (IL-1, 2, 4, 6, 8, and 17) by inflammatory cells present in the IVD can trigger the cascade of tissue degeneration. Moreover, several angiogenic and neurogenic factors (i.e., vascular endothelial growth factor, nerve growth factor) are also released during the IDD process, leading to blood vessel and nerve ingrowth [35]. It is hypothesized that endogenous factors, such as ECM breakdown products, can induce IVD inflammatory responses [36]. Fibronectin, collagen, elastin, laminins, and low molecular weight hyaluronan are produced in response to an imbalance of homeostasis in ECM proteins. These products, in turn, induce an inflammatory response in the IVD [37-40]. Finally, all these processes can lead to discogenic and/or radicular pain (Figure 1B).

Figure 1. The intervertebral disc structure and the hallmarks of IVD degeneration (IDD). (A) The structure of the IVD and its anatomical location in the vertebral column. (B) Compared to healthy IVD, inflammation, blood vessel and neuronal ingrowth escalated in the degenerated disc. Moreover, the disorganization of collagen fibers and increasing of the interlamellar distances between collagen bundles in the annulus fibrosus were usually observed during the degeneration process that often results in disc bulging. During IDD, the number of apoptotic disc cells dramatically increased as well as the cell-cluster formation of nucleus pulposus (NP) cells. The calcification of the cartilage endplate and osteophyte formation occur in advanced degeneration. IVD degeneration is diagnosed when the degenerated IVD in the spine becomes symptomatic and causes discogenic pain. Additionally, the degenerated IVD (protrusion, bulging etc.) presses on spinal nerves, often producing radicular pain.
Table 1. Mechanism of action of small molecules used for IVD regeneration

<table>
<thead>
<tr>
<th>Small molecule</th>
<th>Anti-apoptotic</th>
<th>Anti-inflammatory</th>
<th>Anti-oxidative</th>
<th>Anti-catabolic</th>
<th>Anabolic</th>
<th>Miscellaneous</th>
</tr>
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<td>[58, 127]</td>
<td>[58]</td>
<td>[58]</td>
<td>[50]</td>
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</tr>
<tr>
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<td>[50, 78]</td>
<td>[50, 78]</td>
<td>[50]</td>
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<td>[87, 128]</td>
<td>[63, 128]</td>
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<td>[63, 129]</td>
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<td>[130]</td>
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<td>[57]</td>
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<tr>
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</tr>
<tr>
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<td>[89, 131]</td>
<td>[65, 132, 133]</td>
<td>[63, 132, 133]</td>
<td>[132, 134]</td>
<td>[104]</td>
<td>[104] Senolytic; [102] mTOR inhibitor.</td>
</tr>
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<td>[135]</td>
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<td>[135]</td>
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<tr>
<td>o-Vanillin</td>
<td>[104]</td>
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<td>[104]</td>
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<tr>
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<td>[136]</td>
<td>[80, 136, 137]</td>
<td>[138]</td>
<td>[80]</td>
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<td>[82, 91, 140]</td>
<td>[82, 91, 139]</td>
<td>[82, 140]</td>
<td>[81, 82, 91]</td>
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<td>[52]</td>
<td>[105] BMP2 activator</td>
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<tr>
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<td>[86]</td>
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<td>[107, 142]</td>
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<td>APO866</td>
<td>[93]</td>
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</tr>
<tr>
<td>Dexmedetomidine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[74] Inhibit pyroptosis</td>
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<tr>
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<td></td>
<td></td>
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<td>[102] mTOR inhibitor</td>
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<td>[102]</td>
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</table>

Small molecules and IVD regeneration

In the context of this review, we focus on small molecules as low molecular weight (<900 daltons) compounds, including synthetic or natural products [41]. In the area of pharmaceuticals, small molecules are defined as compounds that bind to certain biological macromolecules and help regulating a particular biological process. The upper molecular weight limit for a small molecule, which requires rapid diffusion across the cell membrane and digestive system absorption, is 900 daltons. Basically, the molecules larger than 550 daltons face more challenges for absorption, while there are some up to 900 daltons that successfully cross barriers [42, 43]. Historically, they were provided as drugs (such as celecoxib) to modulate different cell processes. In the last few years, several small molecules that can selectively regulate cell fate and signaling pathways have been developed [44]. Indeed, small molecules have several advantages and only few limitations compared to large molecular compounds, as outlined in Table S1. Here we focus on recent advances in the use of small molecules that are effective in the regeneration of IVD cells through attenuation of inflammation, cell damage, and stimulation of anabolic processes. These molecules are listed in Table S2, and their mechanism of natural action is summarized in Table 1. In addition, we will discuss new strategies and approaches which were used in recent studies and the future direction for using these molecules to regenerate IVD cells. In vitro, in vivo, and clinical studies related to the discussed small molecules are listed in Table 2. The effective in vitro concentrations of different small molecules for regeneration of disc cells are listed in Table S3.

Table 2. Study setup of different investigations using small molecules for IVD regeneration

<table>
<thead>
<tr>
<th>Small molecule</th>
<th>In vitro (Cell culture)</th>
<th>Ex vivo (Organ culture)</th>
<th>In vivo</th>
<th>Clinical study</th>
</tr>
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<td>[38]</td>
<td>Rat [127]</td>
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<td>[118, 119]</td>
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<tr>
<td>Epigallocatechin 3-gallate</td>
<td>[50, 78]</td>
<td>Rat [50]</td>
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<td>Naringin</td>
<td>[63, 87, 128, 129]</td>
<td>Rat [63]</td>
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<td>Urolithin A</td>
<td>[143]</td>
<td>Rat [54]</td>
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<td>Estradiol</td>
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<td>Rat [121, 132]</td>
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<td>Curcumin</td>
<td>[144]</td>
<td>Rat [145]</td>
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<td>Icarin</td>
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<td>Rabbit, Rat [91, 117]</td>
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<td>Metformin</td>
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</table>
Anti-inflammatory effects of small molecules

Although detailed pathways and molecular interactions between discogenic pain, disc degeneration, and inflammation remain to be elucidated, some inflammatory cytokines and related pathways are known as potential targets for therapies in IDD [19]. Pro-inflammatory cytokines such as IL-1 and TNF-α are key cytokines, triggering ECM degeneration through activation of NF-κB and p38/MAPK pathways. One of the most important cell signaling pathways that seem to play a crucial role in IDD is MAPK signaling. Through this pathway, both matrix synthesis and degradation are modulated in the IVD by influencing PG degradation as well as by changing anabolic and catabolic gene expression levels [45]. Particularly, the PG metabolism is regulated by p38/MAPK/extracellular signal-regulated kinase (ERK) signaling pathways, as treatment with inhibitors of p38 or ERK considerably attenuated the cytokine-induced decrease in synthesis and release of PG. Moreover, ERK can activate the Wnt/β-catenin signaling pathway, which may contribute to the pathogenesis of IDD. Activation of p38 and ERK, which was shown to be higher in degenerated IVD cells can enhance apoptosis induced by experimental loading stress [45]. During IDD development, several growth promoting factors such as insulin-like growth factor 1 (IGF-I), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF) exert their beneficial effects (i.e., mitogenic action) via activating ERK by phosphorylation and subsequent DNA synthesis in degenerated disc cells, indicating that MAPKs may be involved in metabolic processes in the IVD [46]. TNF-α is a cytokine that has been closely related to IDD. This cytokine has two receptors (TNFRI and TNFR2) that bind the ligand with high affinity. Upon TNF binding to TNFR1, two distinct signaling complexes can be activated, 1) the anti-apoptotic complex I, and 2) the death inducing signaling complex (complex II). Signaling down-stream of anti-apoptotic complex I is mediated by NFκB/MAPK signaling pathways. IL-1 is another cytokine that has strongly been linked to IDD. Among 11 cytokines of the IL-1 family, IL-1α and IL-1β are the most studied cytokines regarding IDD. Like TNF, IL-1α and IL-1β can activate NFκB and MAPK signaling pathways [47]. As downstream effects, these cytokines activate MMPs and ADAMTS, which finally elevate ECM degradation.

In various in vitro studies, small molecules, including naringin, cannabidiol (CBD), epigallocatechin gallate (EGCG), curcumin, icariin, resveratrol, berberine, and tofacitinib showed an impact on the downregulation of IL-1 and TNF-α levels in IVD cells (Figure 2). According to previous literature, icariin, resveratrol, and EGCG can inhibit NF-kB and p38/MAPK signaling pathways, thereby modulating inflammatory responses and preventing the development of a degenerative cascade [48-51]. Gefitinib, kaempferol, and berberine are other small molecules that exclusively block the NF-kB signaling pathway [20, 52, 53]. On the other hand, intracellular p38/MAPK signals could be blocked by rhein and urolithin A in vitro [54, 55].

Lang et al. investigated the effects of tofacitinib in an inflammatory and degenerative bovine IVD organ culture model. Tofacitinib citrate (2.5 mg/mL) was added daily to the culture medium to simulate a systemic application of the drug. The results showed that tofacitinib could slow down the degenerative response and reduce inflammation in the organ culture model by selectively inhibiting the Janus kinase 3 (JAK3) pathway [56]. A recent in vitro study showed that luteoloside, a flavonoid glycoside, could suppress inflammatory factors, such as TNF-α and IL-6, in IL-1β-primed NP cells through inhibition of the NF-κB signaling cascade. They demonstrated that luteoloside promoted the nuclear factor erythroid 2-related factor 2 (Nrf2) translocation to the nuclei; Nrf2 can act through activation of the Nrf2/HO-1 (heme oxygenase-1) signaling in NP cells and mitigate inflammation by suppressing the NF-κB signaling cascade and by anti-apoptotic function [57].

Cyclooxygenase-2 (COX-2), which regulates prostaglandin E2 (PGE2) synthesis, is another candidate for modulation of inflammatory responses in IVDs. Chen et al. showed that pre-treatment with CBD suppressed the production of COX-2 and inflammatory cytokines (i.e. IL-6 and IL-1β) in NP cells [58]. Moreover, metformin, luteoloside, and icariin exhibited their anti-inflammatory impact on IL-1 primed NP cells via inhibition of COX-2 and inducible nitric oxide synthase (iNOS) expression, leading to a decreased synthesis of PGE2 [51, 57, 59]. Suzuki et al. used the JAK antagonist tofacitinib for pre-treatment of rat AF cells and then cells were incubated with inflammatory cytokines such as IL-6. Tofacitinib significantly decreased the expression level of COX-2 and, subsequently, the production of PGE2 [60]. Celecoxib is one of the most popular anti-inflammatory small molecules which has recently been used for regeneration of the IVD. Of the selective COX-2-inhibitors, celecoxib was the first on the market and has been in clinical use since 1999. In a preclinical canine study, local delivery and sustained release of this small molecule (as celecoxib-loaded microspheres) in the IVD showed promising results in the control of inflammation, attenuation of discogenic pain and inhibition of IDD development [61] (Figure 2).
Anti-apoptotic effect of small molecules

Therapeutic regulation of transduction pathways can modulate the programmed cell death (PCD) process, which is considered to play a significant role in IVD cell degeneration [62]. Several studies investigated the anti-apoptotic effect of small molecules in IVD cells (Figure 3). Recent studies showed that naringin and icariin could upregulate the cellular concentrations of anti-apoptotic protein B-cell lymphoma 2 (Bcl-2) and downregulate the apoptotic effect of promoter proteins, including cleaved caspase 3 and BCL-associated X (Bax) to modulate the apoptotic rate of human NP derived cells [63, 64]. Furthermore, after 4 hours of treatment with icarin, the phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) signaling pathway was significantly activated. Based on previous studies, activation of this signaling pathway is correlated to anti-apoptosis and anti-oxidative stress; therefore, the anti-apoptotic effect of icarin is linked to the PI3K/AKT signaling pathway [63, 64]. Chen et al. confirmed the protective effect of CBD on hydrogen peroxide-induced apoptosis. They identified that CBD increased cell viability and reduced apoptosis in NP cells after exposure to hydrogen peroxide by reducing the expression level of caspase 3 and promoting the Bcl-2 protein expression [58].

Figure 2. The modulatory effect of small molecules on inflammation, anabolic and catabolic processes and their impacts on IDD. Pro-inflammatory cytokines including IL-1 and TNF-α, are the key cytokines triggering IVD matrix degeneration through activation of NF-κB and P38/MAPK pathways. Moreover, these cytokines activate MMPs and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTs) which finally will elevate ECM degradation. Small molecules can prevent IDD progression by inhibiting the activity of pro-inflammatory cytokines and the subsequent factors (NF-κB and P38/MAPK), or modulating the anabolic, catabolic and even some alternative pathways such as BMP-2 or Vitr pathways. IL: interleukin; ADAMTs: a disintegrin and metalloproteinase with thrombospondin motifs; MMPs: matrix metalloproteinase; CBD: cannabidiol; ECGC: Epigallocatechin gallate; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; MAPK: MAP Kinase; UA: urolithin A, TGF-β: transforming growth factor-beta; Cox: cyclooxygenase; PGE2: prostaglandin E2; IFN-γ: interferon-gamma; TNF-α: tumor necrosis factor-alpha.
Figure 3. The effect of small molecules on programmed cell death (apoptosis) and oxidative stress in degenerated IVD cells. Both intrinsic and extrinsic pathways of apoptosis are playing critical roles in IVD cell degeneration. Small molecules can increase the expression level of Bcl-2, leading to inhibition of the intrinsic pathway by its inhibitory effect on BAX and BAK1. BAX and BAK1 control the release of cytochrome C from mitochondria to cytosol or bind to Apaf-1, leading to inhibition of caspase 9 activity. Several small molecules can also decrease the expression level of caspase 3 in disc cells and subsequently inhibit apoptosis. Inflammatory cytokines and oxidative stresses can also increase the number of apoptotic cells in degenerated IVD cells by activating the ROS-mediated PI3K/Akt pathway. Production of ROS and the expression level of related products such as iNOS can be suppressed by different small molecules. TNF-α: tumor necrosis factor-alpha; IL: interleukin; NF-kB: nuclear factor kappa-light-chain-enhancer of activated B cells; MAPK: MAP Kinase; Bcl2: B-cell lymphoma 2; Bax: BCL-Associated X; BAK1: BR1-associated receptor kinase 1; PI3K: phosphatidylinositol 3-kinase; Akt: Protein Kinase B; ROS: reactive oxygen species; iNOS: inducible nitric oxide synthase; CBD: cannabidiol.

The effect of 17 beta-estradiol (E2) was also assessed on isolated NP cells from healthy rats and their intact IVDs, which were cultured with or without TNF-α. It was shown that in NP cells, E2 significantly increased matrix macromolecules expression, telomerase activity, and cell proliferation potential but attenuated senescence markers (p53 and p16). P53 plays a critical role in intrinsic pathways of apoptosis; therefore, the reduction of this marker could lead to a decrease in apoptosis [65]. Rhein acts through several closely interacting pathways affecting apoptosis. Rhein blocks the p38/MAPK pathway which in turn activates the PI3K/AKT parallel signaling pathways. Consequentially several downstream pathways are activated which regulate the cell cycle and apoptosis [66]. Therefore, the therapeutic potential of rhein as a multitarget molecule is due to its synergistic modulation of multiple pathways [67].

Resveratrol is another small molecule which could induce anti-apoptotic genes (e.g., Bcl-2) and simultaneously reduce the expression level of pro-apoptotic genes such as Bax or caspase 3. Moreover, when LY294002 was used as a strong inhibitor of PI3K/AKT, the anti-apoptotic effects of resveratrol in IL-1β-primed NP cells were attenuated [68]. Therefore, it can be concluded that resveratrol activated the PI3K/AKT signaling pathway, which in turn downregulated NP cell apoptosis. Furthermore, another study showed that resveratrol could activate sirtuin 1 (NAD (+)-dependent deacetylase), which reduces apoptosis in degenerated human NP cells.
[69]; while treatment with LY294002 again increased the rate of apoptosis. This study also suggested that resveratrol could increase the survival rate of degenerative human NP cells by activation of sirtuin 1 through the PI3K/AKT anti-apoptotic signaling pathway [69].

The protective effect of berberine by inhibiting NF-κB activation has been shown on IL-1β stimulated human NP cells undergoing apoptosis [70]. The modulatory effects of berberine on the expression levels of anti-apoptotic protein (Bcl-2), activation of caspase 3, pro-apoptotic Bax, Bak, and release of cytochrome c have also been reported [71].

Pyroptosis is another form of programmed cell death which can lead to production of pro-inflammatory mediators [72]. This process is mediated by nod-like receptor protein 3 (NLRP3) inflammasome, and it has been shown that suppressing the activation of NLRP3 inflammasome could diminish the IDD process [73]. New evidence indicates that microorganisms such as Cutibacterium acnes can induce inflammatory response (IL-1β) and initiate IDD. The number of NLRP-positive cells significantly increased in C. acnes infected disc tissue, which suggested that pyroptosis activation may be induced by C. acnes [73]. A recent study showed that dexmedetomidine, a sedative small molecule drug, inactivated NLRP3 through the suppression of NF-κB and JNK signals, subsequently alleviating pyroptosis during inflammation and IDD [74].

Anti-oxidative effect of small molecules

During IDD, usually excessive reactive oxygen species (ROS) are produced and released locally, suggesting a contribution of oxidative stress to the degeneration process and opening a new horizon regarding the pathogenesis of IDD. ROS, as active mediators, are involved in various cell signaling pathways and cell metabolisms, including matrix degradation, inflammation, apoptosis, autophagy, and senescence of IVD cells. Moreover, ROS can change the structure of matrix proteins in NP, AF, and CEP leading to the impairment of the IVD’s mechanical function and acceleration of IVD degeneration processes [75]. Therefore, a therapeutic option for regulation of oxidative stress in disc cells could be a novel strategy for IVD regeneration. Apoptosis can be triggered by oxidative stress during IDD which has been elaborated in several studies. IL-1β treated NP cells produce more ROS in comparison to untreated cells, which consequently decreases the PG levels and triggers apoptosis [76]. Moreover, the ratio of apoptosis is increased in NP cells exposed to hydrogen peroxide and the expression levels of ECM proteins such as aggregan and type II collagen are decreased [77]. Notably, the detrimental effects of oxidative stress on the cells may be efficiently prevented using anti-oxidative agents, such as different small molecules that protect IVD cells from apoptosis (Figure 3). For instance, the effect of naringin on oxidative stress-induced apoptosis was investigated in rat NP-derived mesenchymal stem cells (MSCs). The findings showed that naringin had protective effects against hydrogen peroxide induced NP cell apoptosis. The potential mechanism of naringin to alleviate apoptosis may be due to the activation of the ROS-mediated PI3K/AKT pathway [63]. In the same way, the protective effect of CBD on NP cells against oxidative stress was also reported. The results demonstrated that the pre-treatment with CBD suppressed the expression level of iNOS, which activated PI3K/AKT signaling pathway [58]. Another study by Krupkova et al. evaluated the anti-oxidative effect of EGCG on human IVD cells exposed to hydrogen peroxide. Their results demonstrated that survival of the treated disc cells by EGCG under severe oxidative stress was considerably enhanced in comparison to the control cells, which happened through activation of PI3K/AKT pathway and inhibition of cytochrome c release from mitochondria [78].

The inhibitory effect of E2 on ROS generation was studied by several investigators. ROS/NF-κB pathway of rat NP cells is affected by the interaction of estrogen receptor and E2, which inhibits TNF-α-induced premature senescence [65].

Cryopreservation can be used to allow the storage of cells over prolonged periods of time. While cryopreservation at -196°C would render IVD cells metabolically inactive, cells usually suffer insults during freeze-thawing such as the generation of ROS [79]. For this reason, the effect of icariin (25 µM) as an addition to cryopreservation media was investigated by Chan et al. They found that icariin improved the viability and function of human NP derived stem cells by preserving the phenotype after thawing the cells [80]. The increased activity of glutathione peroxidase (GPx) and superoxide dismutase (SOD) can explain the oxidation resistance, which provides oxidative stress protection to the cryopreserved cells. Several studies investigated the anti-oxidative effect of resveratrol on human, rat, and bovine NP cells in vitro. The protective effect of resveratrol is due to the stimulation of sirtuin 1 and the PI3K/AKT pathway which was activated in different settings [81-85]. Also, icariin could inhibit induced oxidative stress in NP cells in vitro [64]. Luo et al. observed that the production of ROS under hydrogen peroxide exposure was down-regulated with berberine, which protected human NP cells against oxidative stress-
induced apoptosis [86]. A recent *in vitro* study showed that luteoloside could successfully suppress iNOS and modulate ROS production in IL-1β treated NP cells [57].

Although most of the studies showed an anti-oxidative effect of these small molecules on IVD cells, there is not enough *in vivo* and preclinical evidence to support the efficiency of these molecules to retard the process of IDD. Further *in vivo* and clinical studies are required to develop effective anti-oxidative therapies for IVD regeneration.

**Anabolic and anti-catabolic effect of small molecules**

Several studies assessed the anabolic and anti-catabolic effect of small molecules on IVD cells and reported their beneficial impacts on IDD ([Figure 2](#)). Li *et al.* evaluated the influence of naringin on the growth of degenerative NP cells and its regenerative effects on protein and gene expression. Naringin treatment elevated the protein expression of collagen type II, aggrecan, and SOX6, and decreased the gene expression of MMP3 [87]. Another study showed that naringin could promote the expression of anabolic genes such as collagen II, aggrecan, and reduce catabolic gene expression such as MMP13 to sustain the ECM [63].

Through an *in vitro* study, the TNF-α-induced production of MMPs and degradation of collagen II have been investigated, and the an-senescence and anti-catabolic effects of urolithin A have been confirmed [88]. Estradiol can increase the anabolic activity of NP cells and induce the downregulation of MMPs, indicating protective capabilities of estradiol, as shown *in vitro* [89]. The down-regulation of the protein level of caspase-3, MMP3, and MMP13 and up-regulation of the protein level of type II collagen were closely related to the anti-degenerative mechanism [89]. With the therapeutic application of E2 to degenerated CEP cells, increased expression of collagen II and aggrecan was noted. In addition, an increase in the TGF-β secretion was reported [90]. Hua *et al.* demonstrated the anti-catabolic effect of icariin by decreased MMP and ADAMTS gene expression in human NP cells stimulated by IL-1β [51]. An anabolic effect of 200 μM resveratrol and decrease of the catabolic effects of pro-inflammatory stimuli (IL-1β) added to bovine NP cells was also reported by Li *et al.* [82]. The anabolic effect of resveratrol in cell culture could be reproduced in an *in vivo* study by Kwon *et al.*, who induced degeneration by annulotomy in rabbit discs followed by two intradiscal injections of resveratrol or carrier (DMSO) percutaneously. The regeneration of discs was assessed by magnetic resonance imaging (MRI), real-time polymerase chain reaction (RT-PCR), and histological analysis. An increased aggrecan and decreased MMP13 gene expression in the treatment group compared to the carrier was observed, and increased matrix PG production was confirmed by histology [91]. The effect of statins on the homeostasis of disc cells was also investigated in previous studies. It has been shown that hydrophilic statins had more regenerative potential on NP cells than lipophilic statins. They also showed that hydrophilic statins increased the expression of type II collagen and SRY-box transcription factor 9 (SOX9) in a lower dosage than lipophilic statins.

It has been shown that the expression level of anabolic genes in NP cells increased by metformin, while the expression of catabolic genes considerably decreased [92]. Gefitinib, a small molecule which inhibits epidermal growth factor receptor (EGFR), was also investigated for its potential effects on IDD regeneration. In an *in vitro* study, gefitinib at 10 μM was administered 30 minutes before the treatment of rat NP cells with 10 ng/ml TGF-α. After 48h of treatment with TGF-α, RNA and protein analyses were performed. It was concluded that gefitinib caused therapeutic inhibition of EGFR signaling thereby inhibiting IVD degeneration and enhancing IVD matrix synthesis in TGF-α treated NP cells [20]. The modulating effect of tofacitinib on anabolic and catabolic processes was also investigated in rat and human degenerated IVD cells. Tofacitinib was used to pre-treat disc cells for 30 minutes, followed by incubation with soluble IL-6 receptor (Sil-6R) and IL-6. Tofacitinib decreased the expression of catabolic factors such as MMP13. The study showed the therapeutic potential of tofacitinib in lessening the development of IDD through suppressing the catabolic effects of IL-6 [60]. Two similar *in vitro* studies showed that luteoloside and APO 866 (daporinad) increased the content of ECM-related proteins such as type II collagen and aggrecan and reduced the expression level of MMP13 and ADAMTS5 in IL-1β primed disc cells [57, 93].

**Additional targets**

**Autophagy**

Several investigations have demonstrated that autophagy occurs in IVD cells [94-96]. Autophagy is a conserved cellular process that continues to occur in all types of cells throughout life. Through this well-coordinated and multi-step process, cells remove unnecessary or dysfunctional components. Usually, a low basal level of autophagy occurs in IVD cells, which was confirmed in cells isolated from non-degenerative adult rat discs [94]. However, autophagy can considerably increase in degenerative
IVD cells [97]. This process can successfully reduce apoptosis in both NP and AF cells, leading to the attenuation of IVD degeneration [94]. It has been demonstrated that nutrition deprivation markedly induced IVD cell apoptosis through the intrinsic pathway, whereas this process can be blocked by sirtuin 1 via acceleration of autophagy [98]. Moreover, the suppression of autophagy by exposure of the cells to 3-methyladenine (an autophagy inhibitor) increased the apoptosis of the cells [99].

The impact of small molecules on autophagy was assessed in different in vitro studies. Shi et al. used autophagy markers (LC3 and Beclin-1) to assess the impact of APO866 on autophagy of NP cells as well as their apoptosis. The results showed that APO866 inhibited IL-1β-induced NP cell apoptosis by induction of autophagy. These findings showed the therapeutic potential of APO866 for IDD [93]. Metformin activates the upstream regulator AMPK, which directly induces autophagy in NP cells in a dose- and time-dependent manner to block apoptosis [92]. In a rat IDD model, the controlled-release of gefitinib protected IVDs from degeneration possibly through the modulation of the EGFR-autophagy axis which was shown to not only suppress cartilage matrix degradation but also boost type II collagen synthesis [20].

Mammalian target of rapamycin (mTOR)

In molecular signaling, the mammalian target of rapamycin (mTOR) acts as a negative regulator of autophagy. The mTOR is a serine/threonine kinase which regulates cellular activation such as cell growth and division, cell motility and cell survival. The mTOR exists in two distinct protein complexes including mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). Protein kinase B (known as AKT), an essential pro-survival mediator by suppressing apoptosis, regulates mTORC1 and mTORC2 [100]. It has been shown that the IVD cells would utilize the mTOR signaling and autophagy to cope with stressful conditions such as low oxygen, pH and nutrient concentration [101]. Rapamycin is the primarily isolated mTORC1 inhibitor which can extend mammalian lifespan via inhibiting the cell cycle progression and lethal neoplastic diseases. Today, serious adverse effects of rapamycin including immunosuppression limited its extensive clinical use. A very recent study assessed the effects of mTOR inhibitors on human IVD cells. In this study, four different small molecules and mTORC inhibitors including INK-128, NVP-BEZ235, MK-2206 and curcumin were examined; the results showed the pharmacological modulation of mTOR signaling and autophagy increased the survival rate of IVD cells by suppression of apoptosis [102].

Anti-senescence effect

Senescent cells are non-dividing cells, which are still metabolically active. These cells have been shown to contribute to the catabolic shift in IVD tissue during degeneration by secreting the senescence-associated secretory phenotype (SASP) which contributes to a pro-inflammatory milieu [103]. Curcumin and o-vanillin have been shown to both exert anti-inflammatory and anti-oxidative effects and act as senomorphic drugs. Cherif et al. observed a reduced number of senescent disc (NP and AF) cells and decreased SASP factors as well as an increase in cell proliferation after treatment with curcumin or o-vanillin. This effect was due to the selective induction of apoptosis in senescent cells without negative effects on the proliferating cells [103, 104].

Bone morphogenetic protein activators

Bone morphogenetic proteins (BMPs such as BMP-2 and BMP-7) have shown promise in IVD regeneration. Li et al. evaluated the effect of BMPs for regeneration of IVD both in vitro and ex vivo. Their results showed that BMP heterodimers could successfully upregulate the aggrecan and type II collagen gene expression, as well as glycosaminoglycan (GAG) synthesis of NP cells [93]. The investigations of kaempferol and statins in the treatment of IDD have shown a potential regenerative effect via the BMP-2 signaling pathway [105, 106]. Lovastatin (at concentrations ≥1 µM) could significantly enhance the expression of anabolic genes encoding BMP-2 and BMP-7 [107]. Although these small molecules show great potential in slowing down the progression of IVD degeneration via activation of the BMP-2 signaling pathway, more studies are needed to further elucidate their effects as BMP activators.

Wnt pathway Inhibitor

In IDD, increased Wnt signaling suppresses progenitor cell proliferation and induces apoptosis of NP cells [108]. Moreover, it has been suggested that the Wnt signaling pathway is involved in fibrosis of the AF. SM04690, a small molecule inhibitor of the Wnt pathway, has been utilized in drug development for the treatment of IDD and osteoarthritis. SM04690 demonstrated regenerative properties in preclinical studies, including reduction of inflammation, inhibition of fibrosis, activation of NP cell proliferation, and production of ECM in a rat IVD model. These findings suggest SM04690 as a potential treatment for IDD [109].
**In vivo and Clinical studies**

To date, several *in vivo* studies using small molecules and targeting IDD and related degenerative processes have been published (Table 3, Figure 4). All these *in vivo* studies showed promising results of different small molecules on IDD; however, there is still limited evidence for a regenerative impact of small molecules on degenerated disc tissue. Most of the cited studies showed the preventive effects of the drugs on IDD progression. Only one study showed that EGCG could reach the MRI and histological scoring levels measured in the sham group [50]. Other studies showed that the symptoms or progression of IDD were alleviated in comparison with the vehicle-treated animals (negative control). Approximately 90% of *in vivo* investigations (Table 3) followed the animal cases up to 12 weeks, which is a very short-term follow-up and could explain such insignificant results [110].

Most of the *in vivo* investigations used standard methods to analyze the regenerative process or the inhibition of IDD progression, including X-ray, MRI, histopathology and immunohistochemistry (IHC) (Figure 5A). However, these methods have some limitations to assess the beneficial effects of small molecules on IDD. For instance, MRI was used as a technique to analyze the disc height index (DHI) or the Pfirrmann grade in different treatment groups, whereby 3.0 Tesla (T) or lower (1.5T) clinical MRIs were frequently used (7 out of 10 *in vivo* studies on...
rodents). The 1.5T or 3.0T MRI and related image quality are not suitable for small animal imaging (mouse, rat, and rabbit), which may lead to an irreproducible scoring by the radiologists [111].

**Table 3. An overview of the in vivo studies for individual small molecules**

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Study</th>
<th>Aim</th>
<th>Animal/Patient-model</th>
<th>Intervention</th>
<th>Analysis</th>
<th>Results/Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naringin</td>
<td><strong>Zhang et al. 2018 [63]</strong></td>
<td>To assess the potential therapeutic effect of Naringin on IVD regeneration</td>
<td>Sprague-Dawley Rat (C, NL, n=36), puncture-induced rat IDD model</td>
<td>Intraperitoneal injection of Naringin (80mg/kg/day)</td>
<td>Histology and MRI at 4 and 12 weeks after surgery</td>
<td><strong>Histology</strong>: protection of CEP, no significant difference between saline (vehicle) and Naringin treated groups regarding the damage to NP cells after 12 weeks; <strong>MRI</strong>: There is a significant difference between (vehicle) and Naringin treated groups in terms of Pfirrmann MRI grade scores. <strong>Conclusion</strong>: Naringin may exert a protective effect on IVD after an initial injury.</td>
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<tr>
<td>Naringin</td>
<td><strong>Silveria et al. 2014 [127]</strong></td>
<td>To assess the protective effect of CBD on lesion-induced IDD</td>
<td>Wistar Rat (C: male, n=19), puncture-induced rat IDD model</td>
<td>Intradratical injection of CBD (60-80-120nm)</td>
<td>Histopathology (15 days after surgery), MRI (2 and 15 days after surgery)</td>
<td><strong>Histology</strong>: CBD (120 nmol) prevented the typical histological changes in the AF, no significant protective effect seen on NP. <strong>MRI</strong>: Injection of CBD (120 nmol) immediately after lesion significantly improved MRI pixel intensity. <strong>Conclusion</strong>: Considering that CBD presents an extremely safe profile, only high dose of CBD (120 nmol) could halt the IDD progression.</td>
</tr>
<tr>
<td>Estradiol (E2)</td>
<td><strong>Jin et al. 2018 [121]</strong></td>
<td>To analyze the effect of E2 on IDD in the model of menopause rats</td>
<td>Sprague-Dawley Rat (C: female, n=30), oophorectomy (OVX) to induce menopausal in rats</td>
<td>10 μg/kg/day E2 supplementation for 12 weeks</td>
<td>MRI, histopathology, IHC (LC3 for autophagy) (12 weeks post-surgery)</td>
<td><strong>Histopathology</strong>: The OVX + E2 treatment group showed the NP tissues were similar to those observed in the sham group. <strong>IHC</strong>: There are no significant differences between OVX + E2 treatment and sham group in terms of autophagy. <strong>Conclusion</strong>: E2 via regulating the redox balance (autophagy) of IVD could be a potential therapeutic agent for IDD in the postmenopausal women.</td>
</tr>
<tr>
<td>Estradiol (E2)</td>
<td><strong>Liu et al. 2018 [132]</strong></td>
<td>To further explore whether estradiol (E2) had protective effects on IDD in OVX rats</td>
<td>Sprague-Dawley Rat (C: male, n=40), puncture-induced OVX-rat IDD model</td>
<td>Subcutaneous injection of 20 μg/kg/day E2 for 28 d</td>
<td>X-ray (disc height index-DHI), histopathology, IHC, western blot (WB) (30 d post-surgery)</td>
<td><strong>Histopathology</strong>: Mean histological scores in Sham and OVX + E2 group were significantly lower than OVX+ vehicle group. <strong>IHC</strong>: E2 downregulated caspase-3, MMP-3 and MMP-13 proteins level but upregulated collagen Type II <strong>WB</strong>: Confirmed IHC results <strong>Conclusion</strong>: E2 shows protective effects against IDD by down-regulating catabolic proteins and up-regulating anabolic ones in OVX-animal models.</td>
</tr>
<tr>
<td>Icarin</td>
<td><strong>Hua et al. 2020 [146]</strong></td>
<td>To explore the effect of icariin on IDD</td>
<td>Sprague-Dawley Rat (C: male, n=36), puncture-induced rat IDD model</td>
<td>Intraperitoneal injection of Icarin (80mg/kg/day)</td>
<td>Histology: protection of CEP, no significant difference between saline (vehicle) and Icarin treated groups regarding the damage to NP cells after 12 weeks; <strong>MRI</strong>: There is a significant difference between (vehicle) and Icarin treated groups in terms of Pfirrmann MRI grade scores. <strong>Conclusion</strong>: Icarin may exert a protective effect on IVD after an initial injury.</td>
<td></td>
</tr>
</tbody>
</table>
### Celecoxib (CXB)

#### Study

**Willems et al. 2015 [141]**

**Aim**

To assess the effect of controlled delivery of CXB on IVD regeneration

**Animal/Patient-model**

Dog (C: female, n=18), canine model of spontaneous mild IDD

**Intervention**

A bolus intradiscal injection of CXB (7.7 μM), intradiscal injection CXB loaded hydrogel (77 μM and 770 μM)

**Analysis**

Histology and IHC: No significant differences were found between the injected treatments

**Results/Conclusion**

Histology and IHC: CXB-loaded hydrogel resulted in limited inhibition of PGE2 production in dogs with spontaneous IDD

**Conclusion:**

The delivery of CXB resulted in limited inhibition of PGE2 production in dogs with spontaneous IDD.

#### Study

**Tellegen et al. 2018 [61]**

**Aim**

The effect of control release of CXB on IVD regeneration

**Animal/Patient-model**

Dog (C: male, n=6), canine IDD model

**Intervention**

One month after surgery, Intradiscal delivery of 40 µl CXB loaded microsphere (CXB-M), low (8.4 µg CXB) and high dose (280 µg CXB)

**Analysis**

MRI (0, 4, 8, 16 weeks after injection), clinical examination of discogenic pain (16 weeks after the initial injection)

**Results/Conclusion**

MRI: IVD was maintained in the disc treated with either low or high dose CXB-M, Predominance score was lower in CXB-M treated groups compared to the negative control

**Conclusion:**

Controlled release of CXB inhibited progression of IDD, the development of osteophyte formation, and decreased the immunonpositivity of nerve growth factor

#### Study

**Tellegen et al. 2018 [115]**

**Aim**

To assess the impact of sustain delivery of CXB on discogenic pain

**Animal/Patient-model**

Dog (C: female, n=10), canine patients with low back pain

**Intervention**

Intradiscal injection loaded hydrogel containing 2.93 μg/mL CXB

**Analysis**

MRI (0, 4, 12 weeks after injection), clinical examination of low back pain (12 weeks after the initial injection)

**Results/Conclusion**

MRI: No evident of CXB regenerative effects on MRI

**Conclusion:**

The majority of the treated canine patients, quality of life improved without evident regenerative effects

**Limitations:**

Small group size, absence of a placebo group.

### Berberine

#### Study

**Luo et al. 2019 [86]**

**Aim**

The effects of berberine on IDD were investigated

**Animal/Patient-model**

Sprague-Dawley Rat (C: female, n=24), needle puncture model

**Intervention**

Intraperitoneal administration of berberine (150 mg/kg/day) for 8 weeks post-surgery

**Analysis**

MRI and histopathology (8 weeks post-surgery)

**Results/Conclusion**

MRI: Pfirrmann scores were significantly lower in the berberine treatment group than the saline treatment

**Conclusion:**

Berberine could attenuate puncture-induced IDD in animal model.

### Metformin

#### Study

**Chen et al. 2016 [92]**

**Aim**

To assess the effects of Metformin on IDD
Analysis of Animal/Patient

**Curcumin**

**Aim**
To observe the effect of curcumin on IDD

**Intervention**
Intraperitoneal injection of 50 mg/kg/day for 16 weeks post-surgery

**Analysis**
MRI and histopathology (8-16 weeks post-surgery)

**Results/Conclusion**

- **MRI:** Metformin treated group showed lower Pfirrmann scores compared to the vehicle-treated animals
- **Histopathology:** The histologic score of the metformin group was significantly lower than those of negative control both at 8- and 16-weeks post-surgery

**Conclusion:** Metformin showed a protective effect against progression of IDD.

**SM04690**

**Study**
Barroga et al. 2017 [109]

**Aim**
To investigate the effects of SM04690 on IDD

**Animal/Patient-model**
Rat (C: NI, n=272), puncture-induced IDD model

**Intervention**
Three µl aliquots intradiscal injection of SM04690 (0.066 mg/disc)

**Analysis**
X-ray and histopathology (6 weeks post-surgery)

**Results/Conclusion**
X-ray: % DHI in SM04690 treated animals significantly increased compared to vehicle control

**Histopathology:** Treatment by SM04690 increased number of NP cells and increased ECM vs. vehicle control

**Conclusion:** SM04690 has potential as a modifying therapy for IDD.

**Gefitinib**

**Study**
Pan et al. 2018 [20]

**Aim**
to investigate the therapeutic potential of gefitinib in ameliorating IDD

**Animal/Patient-model**
Sprague-Dawley Rat (C: female, n=18), puncture-induced IDD model

**Intervention**
Three µl aliquots intradiscal injection of gefitinib (30 mM)

**Analysis**
MRI and histopathology (4 weeks post-surgery)

**Results/Conclusion**

- **MRI:** DHI% values of the gefitinib-treated group were significantly higher than those of the IDD control. The Pfirrmann scores also showed that the degree of disc degeneration was markedly lower in the gefitinib-treated group as well.
- **Histopathology:** The gefitinib treatment considerably decreased the histological scores in comparison to IDD control group.

**Conclusion:** The results suggest the potential application of gefitinib for treating IDD.

**Statin**

**Study**
Than et al. 2014 [142]

**Aim**
To find a new conservative treatment for IDD and related discogenic pain

**Animal/Patient-model**
Sprague-Dawley Rat (C: NI, n=272), puncture-induced IDD model

**Intervention**
Six weeks post-surgery, intradiscal injection of 2 µL simvastatin (SIM) at 3 different doses (5, 10, or 15 mg/mL) in either a saline or hydrogel carrier

**Analysis**
MRI and histopathology and IHC (2, 4, 8, 12 and 24 weeks after the initial injection)

**Results/Conclusion**

- **MRI:** MRI analysis showed a higher index (better results) for treatment with 5 mg/mL SIM administered in comparison to the higher doses (15 mg/mL).
- **MRI index:** 5 mg/ml hydrogel>10 mg/ml saline>15 mg/ml saline
- **Histopathology and IHC:** histological grades confirmed the MRI results

**Conclusion:** Intradiscal injection of simvastatin into IDD may result in retardation of degeneration process (5 mg/ml simvastatin in a hydrogel carrier)

**Limitation:** unbalanced time point analysis for all groups, Control group was assessed only histologically.

**Luteoloside**

**Study**
Lin et al. 2019 [57]

**Aim**
To investigate the protective potential of luteoloside in IDD

**Animal/Patient-model**
Sprague-Dawley Rat (C: NI, n=272), puncture-induced IDD model

**Intervention**
Intraperitoneal injection of 10 mg/kg/day luteoloside for 4 and 8 weeks post-surgery

**Analysis**
MRI, X-ray and histopathology (4, and 8 weeks post-surgery)

**Results/Conclusion**

- **MRI:** Pfirrmann MRI grade scores were significantly lower in the luteoloside group than in the IDD group
- **X-ray:** DHI was significantly lower in the IDD group than in the luteoloside treatment group

**Histopathology:** Both ECM and NP tissues were better preserved in the luteoloside-treated group when compared to the IDD group

**Conclusion:** Luteoloside only ameliorate IDD progression during long-term follow-up (8 weeks).

**Curcumin**

**Study**
Ma et al. 2015 [145]

**Aim**
To observe the effect of curcumin on IDD

**Animal/Patient-model**
Sprague-Dawley Rat (C: male, n=60), Surgically induced IDD model in the lumbar area (removal of the spinous processes, the articular processes, the supraspinous ligaments and the interspinous ligaments).

**Intervention**
Intraperitoneal injection of 50 mg/kg and 100 mg/kg curcumin (single dose)

**Analysis**
MRI, Electron microscopy (EM), RT-PCR, and western blot (WB) (6 weeks post-surgery)

**Results/Conclusion**

- **MRI:** The IVD signals of curcumin-treated animals (L1-6) were slightly lower than those in the normal group but were considerably higher than those of NP models.
- **EM:** The degree of degeneration related to NP, AF and ECM structure of IVD samples was better in curcumin-treated animals in comparison to the IDD models
- **RT-PCR:** The expression levels of NF-κB-p65 and TNF-α were significantly lower in curcumin-treated animals than the other groups.
- **WB:** curcumin-treated animals had significantly lower NF-κB-p65 and TNF-α expression levels than IDD animal models.

**Conclusion:** curcumin can decelerate the IDD process by blocking the NF-κB-p65 pathway and reducing inflammatory factors

**Limitation:** 1. No statistical analysis was performed on the differences between each group regarding the MRI test. 2. Lack of further verification of the type of lumbar IDD and related IDD categorization.

G: gender, NI: no information.
Small laboratory mammals are the main animal models for IDD [112, 113]; according to Table 3, 16 out of 18 studies utilized rodent and lagomorph animal models (rat and rabbit) to explore the effect of small molecules on IDD regeneration or discogenic pain. However, significant limitations exist with using such animal models for IVD regeneration studies. For instance, in the rat tail models, the mechanism of disc injury is different due to different mechanical loading and persistence of notochordal cells which affect the regeneration processes. Furthermore, due to the smaller size of the disc, the diffusion of nutrition is different in comparison with the human disc. Another limitation is the lack of human-like longitudinal compression in the quadrupedal animal models [114]. In previous studies, chondrodystrophic dogs (beagle) were used to analyse the effect of local delivery of celecoxib (CXB) on IDD [61, 115]. Tellegen et al. studied the therapeutic effect and the safety of celecoxib-loaded microspheres administered in a canine IDD model. This study showed that there was no evidence of adverse effects on MRI or macroscopic evaluation of IVDs. The diagnostic analysis of NP PG content revealed that site-targeted and sustained
administration of CXB inhibited IDD. Local delivery of the COX-2 inhibitor showed decreased neuronal growth factor and PGE2 tissue levels, which led to an inhibition of the inflammation and alleviation of pain [61]. In another related study, the biocompatibility, safety and feasibility of poly(e-caprolactone-co-lactide)-b-poly(ethylene glycol)-b-poly(e-caprolactone-co-lactide) (PCLA-PEG-PCLA) hydrogel releasing celecoxib was investigated. The biocompatibility was evaluated by administering a subcutaneous injection in rats. The feasibility and safety were evaluated by administering an intradiscal injection to dogs suffering from early spontaneous IDD. Clinical improvement was achieved by reduction of back pain in 9/10 dogs, which was shown by clinical examination and owner questionnaires. The study demonstrated the effectiveness and safety of hydrogel-based celecoxib delivery [115]. Chondrodystrophic dogs, as a large animal model, not only lose the notochordal cells following birth, but they also have a special phenotype (short and curved limbs) predisposing them to spontaneous disc degeneration. With respect to ethical concerns, the number of animals (N ranging from 12 up to 40, Table 3) is another limiting factor for the majority of in vivo studies, which can make it difficult to interpret the results due to high variations.

The behavioral assessment of discogenic pain (i.e., Von Frey filament test) in animal models is still in its infancy [110]. Von Frey filament test provides a quantitative measurement of the paw withdrawal threshold [116]. Krupkova et al. and Lin et al. used this method to analyze the effect of EGCG and resveratrol on IVD regeneration and related discogenic pain [50, 117]. However, in other in vivo studies, it is unknown whether the regeneration of the IVD is correlated with the resolution of discogenic pain.

In a rat tail static compression model, Yurube et al. evaluated the presence of the MMP- and ADAMTS-cleaved aggrecan neoepitopes in vivo, using IHC. These neoepitopes produced by catabolic enzymes in the degeneration process could be used for early detection of IDD [118]. As a proposed diagnostic approach, these antibodies could be modified and labeled with luminescence agents to be detectable via in vivo bioluminescence imaging. This idea might be developed in the future for early diagnosis of IDD and the results could be used for evaluation of the treatment responses in both animal models and clinical cases (Figure 5B).

Until now, there have been no record of clinical trials (clinicaltrials.gov; www.clinicaltrialsregister.eu) and only three clinical studies have been reported regarding the effect of small molecules on IVD regeneration: Five human patients with non-small cell lung cancer and IDD received gefitinib treatment over the past five years, which not only resulted in tumor regression but also ameliorated IDD [20]. Makris et al. conducted a retrospective cohort study to evaluate the use of statins in higher doses for patients suffering from a spinal degenerative joint disease (SDJD). They described an inversely proportional relationship between the dose of statins prescribed to patients suffering from hypercholesterolemia and the risk of SDJD occurrence [119]. Contrary to the previous reports, a higher risk of developing low back pain was associated with the higher dosage of statins, which tended to cause statin-induced myopathy [120]. With respect to the in vivo and clinical studies, the application of biological treatment strategies for the regeneration of IVDs remains a largely undiscovered field which has great potential for further investigations.

Future prospective and Conclusions

Small molecule drugs have shown promising therapeutic potential for the regeneration of IVD tissue and attenuation of the degenerative process. Nowadays, the focus in medicine is on individualized therapies and the factors aggravating the degenerative process in an individual patient need to be considered in more detail. Research towards therapies that induce the intrinsic regenerative potential of the IVD and CEP cells are of high interest due to the long-lasting effects and limited disturbance of the tissue homeostasis which happens with external manipulation. Several in vitro studies have investigated the potential of small molecules to induce autophagy, for stimulating the natural process of IVD cells to adapt and regenerate in a stressful environment [83, 88]. To effectively control the IVD process, emphasis should be put upon an individualized therapeutic approach, like estradiol supplementation which has been advised for menopausal women [121]. Studies on senomorphic drugs show the promising potential of curcumin or o-vanillin in reducing the number of senescent cells. A selective apoptotic effect on senescent cells, while sparing the actively proliferating cells is of high significance and further work must be followed up to translate this promising potential to organ culture or in vivo studies [104]. An important factor which should be highlighted for utilization of different small molecule agents is the limit point of pharmacological or biological treatments. The selection of degeneration severity-based therapeutic options needs to be assessed before the start of any treatment. Biological or molecular treatments including growth factors, cell, gene therapy, and small molecules, could typically be used to repair and regenerate the
degenerated disc in the early stages of IDD (Pfirrmann grade I-III). However, these methods are not sufficient to treat the advanced stages of diseases (Pfirrmann grade IV-V) and surgery would be required as the last resort. Therefore, detailed diagnostic measures are indispensable to assess the type and stage of IDD. New diagnostic approaches (i.e., in vivo bioluminescence) should be developed to detect early IDD or to evaluate the prompt tissue responses to different small molecules in vivo.

The promising results of the discussed investigations are only the foundation to answer the more complex questions arising from ex vivo and in vivo models. Small molecules have a huge therapeutic potential, as their application can be directed with controlled release formulas and in many cases, the evidence of their efficacy is already proven in the treatment of other diseases in the clinics [61, 92, 119]. It is of great importance to consider the dosing, the systemic effects of the drugs and interactions of the various compounds with each other and with over the counter and prescribed medicines. Combination therapies may further potentiate the positive effects of the small molecules on the regeneration of the IVD cells. In the search for therapies on the degenerated IVD, delivery of the compound to the IVD tissue is a question that will always complicate the path to clinical translation (Table S4). The small molecules administered systemically may be able to reach the disc in some cases; however, in compromised situations such as CEP damage or sclerosis, it may be a challenge to reach an effective concentration of the compound in the IVD. Local delivery of small molecules (i.e., direct injection) is another strategy to overcome the limitations of systemic delivery, such as diffusion problem due to CEP damage, or systemic side effects. The evolving field of hydrogels, microspheres, nanoparticles, and further substances that allow for a controlled release in situ show promising results to resolve this problem in the future [20, 61, 114]. Nevertheless, needle size should be considered to avoid exacerbating degeneration.

In vitro results have shown the beneficial effects of small molecules on regeneration of degenerated IVDs through attenuation of inflammation, oxidative stress, apoptosis, catabolism, and stimulation of anabolic processes via different signaling pathways. Similarly, most in vivo results also indicate beneficial effects of these small molecules on the amelioration of IDD [20, 57, 63, 92, 116]. However, the number of clinical studies in this regard is very low, and no clinical trials on the effect of these small molecules on IDD have been performed so far. In future, a road map for discovery of new small molecules and their clinical translation for IDD treatments should be provided. To date, the major limitations for clinical translation include the insufficient in vivo evidence, due to a lack of representative animal models; the lack of an adequate delivery system for different small molecules; and the lack of patient stratification methods to identify the patients who would respond to and benefit from a small molecule-based therapy. The potential necessity of long-term treatment should also be emphasized to achieve a successful therapy from small molecule agents for regeneration of degenerated IVD. The avascular nature of the IVD may increase the duration of treatment, particularly for oral administration of small molecules which should first be absorbed by digestive tracts, delivered to the target site via the systemic circulation and finally reach the degenerated disc through diffusion.

To bridge the gap between rudimentary animal models and clinical studies, a more clinically relevant animal model should be used to better replicate the complexity of the human IVD and the pathology of IDD. The modeling of pain or inflammation, systemic response to degeneration and related treatment are only represented to a limited extent in the established in vitro models. Considerations for establishing an appropriate animal model include factors like the absence of notochordal cells, size of IVD tissue, body mass relative to humans, mechanical compression forces upon the IVD, type of injury and ethics [113]. Non-human primates (i.e., baboons and macaques) and chondrodystrophic dogs (i.e., beagle and dachshund) closely match the clinical condition of IDD regarding many of the physical and mechanistic criteria (spontaneous models of IDD) [122, 123]. However, ethical considerations should preclude their widespread use in pre-clinical studies. The ovine IDD model could be one of the best animal models due to desirable characteristics such as the absence of notochordal cells, similar body mass to humans, and mechanical compression forces acting upon the IVD [124]. Other study parameters such as follow-up time and animal numbers are also important factors in study design. With short-term follow-up (8-12 weeks), the study is usually limited to investigating the protective effect of small molecules on IDD progression; while longer-term follow-up studies (6 to 12 months) are necessary to detect any regenerative effects [97]. Moreover, if ethical considerations allow for it, a higher number of animals for in vivo studies could be used to decrease the outcome variation and make it easier to draw conclusions [125].

Behavioral pain assessment should be utilized to assess the effect of small molecules on IDD such as von Frey filament test, gait analysis, weight loading, and hot plate analysis. It should be highlighted that the collaboration of neuroscience, orthopedic, and
biomedical sciences is a necessity to extend the current understanding of the complex and multifactorial pathophysiology of IDD and discogenic pain [110]. For in vivo imaging techniques, using equipment with higher sensitivity and resolution is also recommended. Recently, better MRI systems have become available for small animal models that are able to generate magnetic fields of 9.5T or even 21T, offering an excellent tool for small animal preclinical studies [111]. Regarding ethical issues, it should also be noted that all mechanisms, pathways and related beneficial effects of each small molecule on IDD have to be identified as best as possible by in vitro studies, followed by ex-vivo IDD organ culture models to confirm the results. Furthermore, new experimental techniques such as lab-on-a-hip that simulate the activities, mechanics and physiological response of entire organs and organ systems are being developed [126]. Recently, a microfluidic disc-on-a-chip device has been developed that was tailored for laboratory small animal disc organs as a long-term ex-vivo organ culture platform. This device lays groundwork for future studies by simulating the chronic nature of IDD [126].

In conclusion, small molecule therapy is an alternative treatment for conventional therapies and surgical approaches in discogenic pain. Effective regeneration of the degenerated IVD with small molecules has opened an important area of research in IVD regeneration in the last decade. However, it is of great importance to investigate the mechanism of action of small molecules in targeting different signaling pathways towards an effective therapy. Several of these small molecules are effective in inhibiting apoptosis, inflammation, oxidative stress, and senescence, which can prevent degeneration of the disc cells. They also showed anabolic and anti-catabolic effects (intrinsic regeneration) which are critical factors for the regeneration of damaged IVD. Further analysis, especially in large animal and advanced organ culture models and then clinical studies are needed to confirm the preliminary results obtained from in vitro investigations. Moreover, it will be of importance to investigate other regenerative effects of different small molecules, such as the induction of NP-like cell differentiation in endogenous and exogenous stem or progenitor cells.

**Abbreviations**

LBP: low back pain; IVD: intervertebral disc; IDD: intervertebral disc degeneration; CLBP: chronic low back pain; NP: nucleus pulposus; AF: annulus fibrosus; SP: spinous process; TP: transverse process; SC: spinal cord; VB: vertebral body; ECM: extracellular matrix; PG: proteoglycan; IFN-γ: interferon-gamma; TNF-α: tumor necrosis factor-alpha; IL: interleukin; ADAMTS: a disintegrin and metalloproteinase with thrombospondin motifs; MMP: matrix metalloproteinase; CBD: cannabidiol; ECGC: epigallocatechin gallate; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; MAPK: MAP Kinase; JAK3: janus kinase 3; Nrf2: nuclear factor erythroid 2-related factor 2; HO-1: heme oxygenase-1; PGE2: prostaglandin E2; COX2: cyclooxygenase-2; E2: 17 beta-estradiol; Bcl-2: B-cell lymphoma 2; Bax: BCL-associated X; BAk1: BRII-associated receptor kinase 1; PI3K: phosphatidylinositol 3-kinase; AKT: protein kinase B; ROS: reactive oxygen species; iNOS: inducible nitric oxide synthase; SOX: SRY-Box transcription factor; TGF-β: transforming growth factor beta; EGFR: epidermal growth factor receptor; BMP: bone morphogenetic protein; MRI: magnetic resonance imaging; RT-PCR: real-time polymerase chain reaction; IHC: immunohistochemistry; WB: western blot; HIVD: healthy intervertebral disc; DIVD: degenerated intervertebral disc; SMs: small molecules; CXB: celecoxib; PCLA-PEG-PCLA: poly(ε-caprolactone-co-lactide)-b-poly(ethylene glycol)-b-poly(ε-caprolactone-co-lactide); mTOR: mammalian target of rapamycin; NLRP3: nod-like receptor protein 3; IGF-I: insulin-like growth factor 1; PDGF: platelet-derived growth factor; FGF: fibroblast growth factor; ERK: extracellular signal-regulated kinase; SDJD: spinal degenerative joint disease; UA: urolithin A; T: Tesa; DHI: disc height index; Ab: antibody; NE: neo-epitope; DC: disc cell; IP: intraperitoneal injection; ID: intradiscal injection/delivery.

**Supplementary Material**

Supplementary figures and tables.

http://www.thno.org/v11p0027s1.pdf

**Competing Interests**

The authors have declared that no competing interest exists.

**References**


http://www.thno.org


