Anti-tuberculosis drugs decrease viability and stimulate the expression of chondrocyte marker genes in human nucleus pulposus cells

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Abstract. Isoniazid (INH), rifampicin (RIF), ethambutol (ETH) and pyrazinamide (PYR) are first-line drugs used in anti-tuberculosis (TB) therapy. However, no studies have been conducted concerning the effect of anti-TB drugs on the cells of the intervertebral discs (IVDs), the predominant location of the osteoarticular form of TB (OATB). Cells from the nucleus pulposus (NP), which are located in the center of the IVDs, were obtained from 12 adolescent patients who underwent surgery due to idiopathic scoliosis. The NP cells were incubated for 24 h with transforming growth factor β1 (TGF-β1) and each anti-TB drug (INH, RIF, ETH and PYR), separately. Incubation with 2.5 ng/ml TGF-β1 resulted in an 80% decrease in *ACAN* mRNA levels; while 5 μg/ml INH led to a 2.3-fold increase in *COL2A1* and a 2.9-fold increase in *ACAN* mRNA levels. Treatment with 10 μg/ml RIF initiated a 2.2-fold increase in *COL1A1* mRNA levels and 5 μg/ml PYR resulted in an 8-fold increase in *SOX9* mRNA levels. Following 192 h of treatment with INH and RIF, NP cell viability was diminished; however, no drugs modified the concentrations of glycosaminoglycans (GAGs). This study aimed to determine the effect of anti-TB drugs on the expression of chondrocyte marker genes in human IVD cells. Anti-TB drugs increased the expression of chondrocyte marker genes and diminished the viability of IVD cells. This study demonstrated that in addition to the common side effects of anti-TB drugs, these drugs also have an effect on IVD cells.

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Introduction

Successful chemotherapy of pulmonary and extrapulmonary tuberculosis (TB) requires the prolonged administration of at least three anti-TB drugs (1). Prolonged drug therapy is required to eliminate persistent bacilli, which are small populations of metabolically inactive microorganisms. The recommended standard treatment for adult respiratory TB is a regimen of isoniazid (INH), rifampicin (RIF) and pyrazinamide (PYR) for two months, followed by four months of INH and RIF. Ethambutol (ETH) and streptomycin are also commonly added to this regimen (1).

Tuberculosis is one of the most common causes of mortality from curable infectious diseases. The World Health Organization (WHO) estimated that the incidence of TB was 0.13% worldwide in 2010 (2). Osteo-articular TB (OATB) accounts for ~2-3% of all TB cases, and ~35% of extra-pulmonary TB cases (3). TB of the spine is potentially the most damaging form of OATB and is responsible for ~50% of OATB cases (3). Furthermore, spinal infection is hematogenous or postoperative (4).

The cytotoxic effects of certain antibiotics on intervertebral disc (IVD) cells were investigated in animals and humans (5,6). The penetration and distribution of antibiotics into avascular IVDs is significantly dependent on the charge of the antibiotics (7). The concentration of cephazolin, a drug administered intravenously during spinal surgery, reached a maximum concentration in the serum in <10 min, and in the IVDs it reached a concentration 15 times lower than that in the serum in <60 min (8). Although studies have been conducted concerning the serum concentrations of anti-TB drugs, there are no studies that describe the penetration of these drugs into IVDs.

Liver injury, skin reactions, gastrointestinal and neurological disorders have frequently been observed as adverse effects of anti-TB treatment (9). Hepatotoxicity has been identified to be a common adverse reaction to RIF, and peripheral neuropathy affecting muscles, joints and limbs in anti-TB drug-treated patients has been linked to neurotoxicity (10). In addition, INH has been connected to an increased risk of developing hepatitis

and INH has been demonstrated to compete with vitamin B6 in its action as a cofactor in the synthesis of synaptic neurotransmitters (11). In anti-TB treatment using ETH, optic neuritis and retrobulbar neuritis were common toxic effects as well as pruritus, joint pain, gastrointestinal problems and hepatotoxicity (12). Dose-related hepatotoxicity and gastrointestinal imbalance were also adverse effects resulting from treatment with PYR (11). As anti-TB drugs are also applied in the treatment of OATB, they are required to reach infected IVDs. The effect of anti-TB drugs on IVD cells, particularly nucleus pulposus (NP) cells, has not yet been observed in terms of cell viability, gene expression or glycosaminoglycans (GAGs) concentration.

There are clear morphological and physiological differences between articular cartilage and NP tissues, suggesting that there are differences in their cellular phenotypes; however, *COL1A1*, *COL2A1*, *ACAN*, *SOX9* and *TGFB1*, the key marker genes, were observed in the two tissues. The extracellular matrix of the NP is ≤80% hydrated, as a result of large quantities of the aggregating proteoglycan, consisting of aggrecan protein encoded by *ACAN*. This proteoglycan is entangled in a variably orientated scaffold of type II collagen fibers encoded by *COL2A1* (13). Sox9 encoded by *SOX9*, is involved in chondrocyte differentiation and maintenance of the chondrocytic phenotype (14). Transforming growth factor β1 (TGF-β1, encoded by *TGFB1*) is a member of the family of cytokines acting through the Smad protein pathway and regulates chondrogenesis (15). There have been no attempts to characterize the expression of these key chondrogenic genes in NP cells when treated with anti-TB drugs. Thus, this study aimed to demonstrate the impact of anti-TB drugs on IVDs as the incidence of tuberculosis is increasing in countries with AIDS epidemics (2), thus it is important to fully understand the effects of the anti-TB drugs.

NP cells were observed in the present study, as damage to this susceptible section of the IVDs by anti-TB drugs may be irreversible. In this study, by the incubation of human NP cells with INH, RIF, ETH and PYR, the following hypotheses were investigated: i) the expression of *COL1A1*, *COL2A1*, *ACAN*, *SOX9* and *TGFB1* chondrocyte marker genes in NP cells may be sensitive to treatment with anti-TB drugs; and ii) the transcriptional activity of the genes encoding matrix protein in the cultured cells may monitor functional changes during anti-TB therapy. Additionally, anti-TB drugs may influence NP cell viability or GAG synthesis. This study aimed to determine whether anti-TB drugs resulted in adverse side effects in NP cells.

Subjects and methods

Subjects. Human NP cells were collected (using an anterior approach) from 12 patients undergoing treatment to correct thoraco-lumbar or lumbar scoliosis during the routine preparation of the site for anterior spodylodesis. All patients were treated in Poznań Medical University Hospital of Pediatric Orthopaedics and Traumatology and were recruited into the study consecutively.

The following eligibility criteria was adopted: i) 10-19 years of age; ii) adolescent idiopathic scoliosis (AIS); iii) a Cobb angle of >40 degrees; and iv) scoliosis correction from an anterior approach with routine removal of an IVD for preparation of the site for anterior spodylodesis. NP cells were extracted from non-degenerative IVDs.

Patients were excluded from the study if they had taken painkillers, antibiotics or steroids prior to hospital admission; had undergone previous surgery in the spinal area; or had exhibited indications of TB infection.

Patients who fulfilled the inclusion criteria received in-depth information concerning the aim of the study and were assured anonymity. Informed consent from the legal guardians of each patient was obtained prior to requesting permission from the patients to obtain the NP cells. The mean age of the patients was 16±2.3 years (range, 14-19). The Ethics Committee of Poznań University of Medical Sciences (Poznań, Poland; approval no. 838/09) approved the design of this study and confirmed its accordance with universal ethical principles.

Cell culture. Non-degenerate IVD tissue was dissected predominantly from Th12 to L3 vertebrae (in one case lumbar tissue was dissected from L1 to L4) to separate the NP from the annulus fibrosus (AF) tissue. The NP was enzymatically digested overnight at 37˚C with 0.02% collagenase type II (Sigma, St. Louis, MO, USA) in a serum-free medium containing an antibiotic antimycotic solution (100 units penicillin, 100 µg streptomycin and 25 ng amphotericin B per milliliter; Sigma). The digested tissue suspension was filtered through a sterile nylon fabric to remove the remaining tissue debris. Cells were centrifuged at 300 x g for 5 min, seeded onto a tissue culture flask and cultured at 37˚C in 5% CO_2/95% air, in (1:1 v/v) Dulbecco's modified Eagle's medium/Nutrient F-12 Ham (DMEM/F-12; Sigma) supplemented with 10% fetal bovine serum (FBS; Sigma) and an antibiotic antimycotic solution (Sigma). For gene expression experiments, 150,000 cells were placed in each well of 6-well plates; while for cell viability experiments, 25,000 cells were placed in each well of 24-well plates. The activity of caspase-3 and -7 was determined on 96-well plates with 5,000 cells/well. For GAG assays 200,000 cells were placed in each well of 6-well plates. The following concentrations of test substances were used: 2.5 ng/ml TGF-β1 (Promega Corporation, Madison, WI, USA), 5 µg/ml INH, 10 µg/ml RIF, 2 µg/ml ETH and 5 µg/ml PYR (Sigma).

Gene expression. Following the 24-h incubation of NP cells with test substances, total RNA was extracted from the cultured cells using TRItidy G, according to the manufacturer's instructions (Applichem GmbH, Darmstadt, Germany). Total RNA (1 µg) was reverse-transcribed using the Superscript Reverse Transcriptase kit (Invitrogen Life Technologies, Carlsbad, CA, USA). Oligo$(dT)_{15}$-primed cDNAs were amplified by quantitative PCR (qPCR) using the primers listed in Table I and the Light Cycler FastStart DNA Master SYBR-Green I kit (Roche Diagnostics GmbH, Mannheim, Germany). Crossing-point values were calculated automatically, based on the second derivative algorithm, and the results were analyzed by the relative expression method. *HMBS* and *MRPL19* were used as reference genes.

Cell viability and caspase activity. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-based

Table I. Primers used in qPCR.

Gene	Primer sequence	Amplicon length (bp)	GenBank Accession nos.
ACAN	5'-ACCAGACTGTCAGATACCCC-3' 5'-CATAAAAGACCTCACCCTCC-3'	156	NM_001135
SOX9	5'-GAAGAACGGGCAGGCGGA-3' 5'-TTTGGGGGTGGTGGGTGG-3'	181	NM_000346
COL2A1	5'-ACCAGGACCAAAGGGACA-3' 5'-GCAGCAAAGTTTCCACCA-3'	246	NM_033150
COL1A1	5'-GAAGGGACACAGAGGTTTCAG-3' 5'-TTCCACGAGCACCAGCAG-3'	179	NM_000088
TGFB1	5'-GAAACCCACAACGAAATC-3' 5'-AATTTCCCCTCCACGGCT-3'	300	NM_000660
MRPL19	5'-TCCAACCGCCGCCGAAAC-3' 5'-AACACGAAGAATACTTCCAACA-3'	197	NM_014763.3
HMBS (PBGD)	5'-CCCTGGAGAAGAATGAAGTG-3' 5'-TCCCCGAATACTCCTGAA-3'	254	NM_000190

Cell Growth Determination kit (Sigma) was used for cell viability analyses. Following 24, 48 or 192 h of stimulation with test substances, the cells in 24-well plates were incubated in serum-free DMEM/F-12 with the addition of MTT (0.5 mg/ml). Following incubation for 4 h, the cell culture medium was removed and solvent was added (1 mM hydrochloric acid in isopropanol anhydride; POCH S.A., Gliwice, Poland). Following gentle mixing, analysis was performed using the Stat-Fax 2100 spectrophotometer (Awareness Technology Inc., Palm City, FL, USA) at a wavelength of 630 nm (background absorbance was measured at a wavelength of 405 nm).

Caspase activity was analyzed by a Caspase-Glo 3/7 assay (Promega Corporation). Following cell stimulation, Caspase-Glo 3/7 Reagent was added (culture medium: Caspase Glo 3/7 reagent, 4:1) to cells in a 96-well plate. Subsequent to incubation for 1 h, luminescence was measured in the TD20/20 luminometer (Turner Designs Inc., Sunnyvale, CA, USA).

GAG level in NP cells. NP cells were grown in culture media with the addition of the test substances and digested with papain after 192 h. Subsequently, Blyscan Sulfated Glycosaminoglycan assays (Biocolor Ltd., Carrickfergus, UK) were performed according to the manufacturer's instructions.

Statistical analysis. For qPCR data, crossing-point values were transformed into relative quantities by the instrument software (LightCycler Software 4.05; Roche Diagnostics GmbH). Gene specific standard curves were applied to calculate the relative levels of respective transcript upon Cp values. The mean values of the target-to-reference ratios from at least three independent experiments were presented as the mean ±SEM. Controls were normalized to one.

P-values for all gene expressions were calculated and $P<0.05$ was considered to indicate a statistically significant difference. Statistical analysis was performed on data transformed into $\log_{10}$ values with the use of MS Excel and GraphPad InStat software (La Jolla, CA, USA) applying the Student Neuman-Keuls post hoc analysis of variance.

Results

Gene expression. Fig. 1 demonstrates that treatment of NP cells with 2.5 ng/ml TGF-β1 for 24 h resulted in an 80% decrease in *ACAN* mRNA levels (P=0.003); while *COL1A1*, *COL2A1*, *SOX9* and *TGFB1* mRNA levels were not significantly changed. The NP cells were treated with 5 μg/ml INH for 24 h, resulting in a 2.4-fold increase in *COL2A1* mRNA levels (P=0.025) and a 3-fold increase in *ACAN* mRNA levels (P=0.045); while those of *COL1A1*, *SOX9* and *TGFB1* were not significantly affected. Treatment of NP cells with 10 μg/ml RIF for 24 h increased *COL1A1* mRNA levels 2.2-fold (P=0.010) but did not result in changes in *COL2A1*, *ACAN*, *SOX9* and *TGFB1* mRNA levels. Treatment of NP cells with 2 μg/ml ETH for 24 h did not change the *COL1A1*, *COL2A1*, *ACAN*, *SOX9* and *TGFB1* mRNA levels. Treatment of NP cells with 5 μg/ml PYR for 24 hours resulted in an 8.7-fold increase in *SOX9* mRNA levels (P=0.017). *COL1A1*, *COL2A1*, *ACAN* and *TGFB1* mRNA levels were not significantly changed by PYR.

Cell viability. NP cells were treated with 2.5 ng/ml TGF-β1 and anti-TB drugs to determine whether these substances induced changes in the cell viability. Fig. 2 demonstrates that following 192 h of treatment with 2.5 ng/ml, TGF-β1 decreased the viability of NP cells by 30%. The same length of treatment with 5 μg/ml INH led to a 15% decrease in the viability of NP cells. Treatment of NP cells with 10 μg/ml RIF for 192 h diminished cell viability by >30%. Treatment with ETH (2 μg/ml) for 24 h increased the viability of NP cells 1.3-fold; however, PYR did not significantly alter NP cell viability. The caspase-3 and -7 activity assay did not demonstrate an increase in activity in cells treated by anti-TB drugs (data not shown).

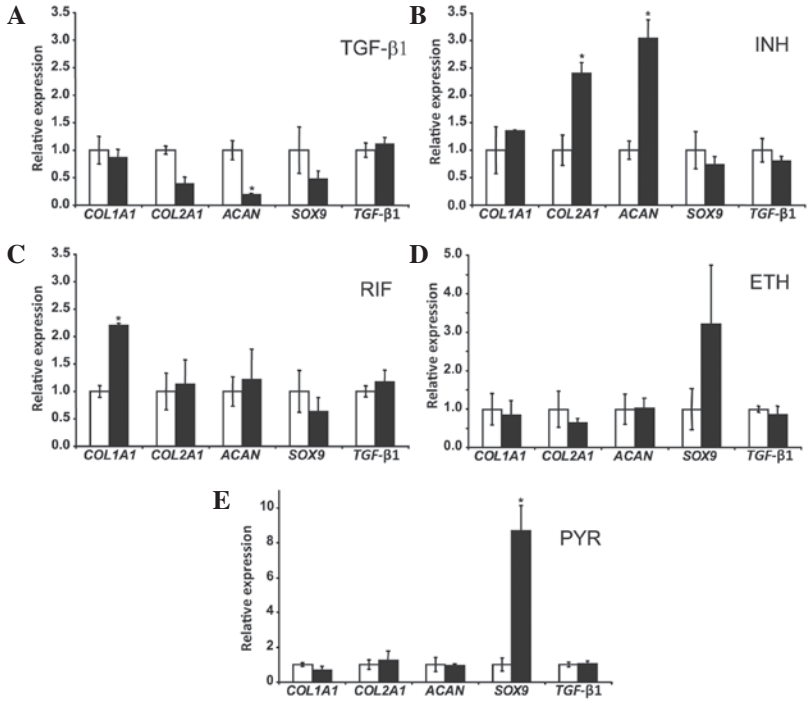

Figure 1. COL1A1, COL2A1, ACAN, SOX9 and TGF-β1 mRNA levels in NP cells treated with (A) 2.5 ng/ml TGF-β1 (B) 5 μg/ml INH (C) 10 μg/ml RIF (D) 2 μg/ml ETH and (E) 5 μg/ml PYR for 24 h (black bars). Untreated control equals 1 (open bars). Error bars respresent ±SEM. *P<0.05. TGF-β1, transforming growth factor-β1; NP, nucleus polposus; INH, isoniazid; RIF, rifampicin; ETH, Ethambutol; PYR, pyrazinamide.

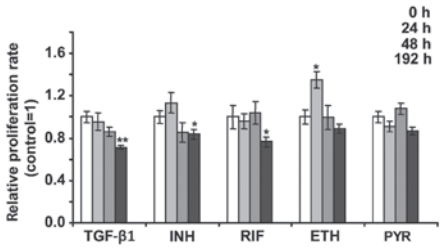

Figure 2. Viability test of NP cells treated 24, 48 and 192 h with 2.5 ng/ml TGF-β1, 5 μg/ml INH, 10 μg/ml RIF, 2 μg/ml ETH and 5 μg/ml PYR. Untreated control equals 1 (open bars). Cells were grown in culture media with addition of test substances and an MTT assay was performed at the indicated time. Error bars represent ± SEM. *P<0.05 and **P<0.01. NP, nucleus polposus; TGF-β1, transforming growth factor-β1; INH, isoniazid; RIF, rifampicin; ETH, Ethambutol; PYR, pyrazinamide; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

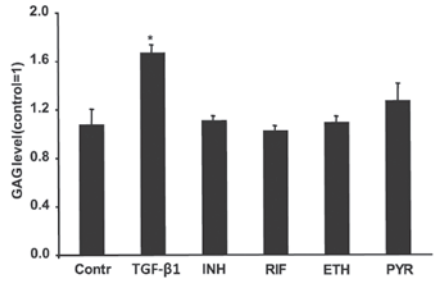

Figure 3. GAG level in NP cells. The NP cells were treated for 192 h with 2.5 ng/ml TGF-β1, 5 μg/ml INH, 10 μg/ml RIF, 2 μg/ml ETH and 5 μg/ml PYR. After 192 h, the NP cells were digested with papain. Subsequently, a Sulfated Glycosaminoglycan assay was performed to analyze the GAG content. Data are shown as a fold of the untreated control value. Error bars represent ± SEM. *P<0.05. GAG, glycoseaminoglycan; NP, nucleus polposus; TGF-β1, transforming growth factor-β1; INH, isoniazid; RIF, rifampicin; ETH, Ethambutol; PYR, pyrazinamide.

GAG level in NP cells. NP cells were treated for 192 h with 2.5 ng/ml TGF-β1, 5 μg/ml INH, 10 μg/ml RIF, 2 μg/ml ETH and 5 μg/ml PYR. A Sulfated Glycosaminoglycan assay (Blyscan) was subsequently performed. TGF-β1 led to a 1.7-fold increase in GAGs production; however, treatment with anti-TB drugs did not result in a significant change in GAG content (Fig 3).

Discussion

The present study demonstrated the specific side effects of anti-TB drugs on human NP cell gene expression, viability and GAG synthesis. The toxicity of anti-TB drugs (INH, RIF, ETH and PYR) has been studied in hepatocytes and other types of cells, but no effects have been demonstrated in cells from the human articular system, particularly in human IVD cells. In the present study the side effects of the anti-TB drugs were divided into three categories: Stimulating chondrocyte marker genes and decreasing cell viability (INH and RIF), stimulating chondrocyte marker genes and non-regulating viability (PYR) and drug non-stimulating chondrocyte marker genes with observed viability effects (ETH).

Prior to investigating the impact of INH, RIF, ETH and PYR on NP cells, the identity of the cultured primary cells obtained during surgery was validated by the expression of the following chondrocyte marker genes: *COL1A1*, *COL2A1*, *SOX9*, *ACAN* and *TGFB1*. In addition, TGF-β1 was also used, as its impact on chondrocyte marker gene expression in NP cells has been determined previously (16). In the present study, the expression of *ACAN* in NP cells decreased with the addition of 2.5 ng/ml TGF-β1, which was concordant with previous observations concerning the effect of 10 ng/ml TGF-β1 on *ACAN*

expression in adolescent and adult NP cells (17). However, in similar conditions, in a previous study, 72 h of treatment with 10 ng/ml TGF-β1 led to an increase in *ACAN* expression and *COL1A1*, *COL2A1* and *SOX9* mRNA levels were constant (18). The presence of 1 ng/ml TGF-β1 in platelet-rich plasma induced an increase in *ACAN*, *COL2A1* and *SOX9* expression (15). Moreover, the effect of TGF-β1 on NP cells was limited at 1 ng/ml and 2 ng/ml, whereas concentrations of ≤1 ng/ml TGF-β1 increased the viability of NP cells but a concentration of ≥2 ng/ml TGF-β exhibited no effect (15). In the present study, TGF-β1 led to a decrease in NP cell viability after 192 h and this antiproliferative effect of the growth factor confirms its involvement in cell growth. Although TGF-β1 is an inhibitor of cell proliferation in the majority of cell types and has been shown to stimulate the growth of human nasal septal chondrocytes (19), it exhibited no visible effect on the growth of NP cells or stimulated NP cells (18,20). It was hypothesized that TGF-β1 requires a proportional quantity of other growth factors to be present in platelet-rich plasma to stimulate *ACAN*, *COL2A1* and *SOX9* as well as promote cell viability. It appears that the effects of TGF-β1 on chondrocytes are dependent upon the culture system to which it is added; therefore results may differ between studies (18).

To determine whether apoptosis may be responsible for the TGF-β1 effect, the activity of caspase-3 and -7 were analyzed. Caspases-3 and -7 belong to the group of caspases termed executioner caspases and exist within the cytosol as inactive zymogen dimers that are activated by initiator caspases-8 and -9 (21). As no changes were observed in the caspase-3 and -7 activity it was concluded that apoptosis was not responsible for the decrease in NP cell viability in the presence of TGF-β1 (data not shown).

Proteoglycan aggregates are commonly located in the structure of cartilage and IVDs. GAGs attach covalently to proteins, such as aggrecan, form proteoglycans. In the present study, similar to the study by Yang *et al* (17), the level of GAGs was increased by the addition of TGF-β1 in NP cells. As TGF-β1 induced a simultaneous decrease in the expression of the ACAN gene, it was concluded that the entire effect of TGF-β1 on proteoglycan content in the extracellular matrix of NP remains unknown. This data implies that the proteoglycans, due to the action of TGF β1, may contain less ACAN protein which is highly glycosylated (22). The present data are consistent with the observation that TGF-β1, via Smad proteins, stimulates the expression of glucuronosyl transferase I (GlcAT-I), an important enzyme in the GAG biosynthesis pathway (23). Although NP cells from adolescents with idiopathic scoliosis may reveal some degenerative changes, we demonstrated that our NP cells were not degenerated as disc degeneration eliminates the regulation of GAGs synthesis by TGF-β1 (17,23)

NP cells that expressed marker genes have been used to investigate the effect of anti-TB drugs. The INH concentration in the plasma following oral administration of a 700-mg dose has been demonstrated to reach 3-5 μg/ml in 6 h (24). In the present study, NP cells were incubated with 5 μg/ml INH for 24 h, resulting in increased *ACAN* expression, which was the opposite effect to that of TGF-β1. Moreover, INH stimulated *COL2A1* gene expression, which may result in the biosynthesis of extracellular matrix. The mechanism of these anti-aging effects of INH remains to be elucidated, as it is not clear whether the drug itself, or its metabolites (such as hydrazine), act on *COL2A1* and *ACAN* gene expression (1). It has previously been suggested that the predominant toxic effect of INH is a result of its metabolites, monoacetyl hydrazine and hydrazine, as well as related compounds, which induce changes in the expression of numerous genes in the rat liver (1,6,25,26). We suggest that a similar mechanism may be associated with the antiproliferative effect of INH on NP cells; however, this requires further investigation, as caspase-3 and -7 were not activated (data not shown) and the synthesis of GAGs was not affected by treatment with INH.

In a study by Boman (27), the RIF concentration in the plasma decreased to 4 μg/ml in 6 h following oral administration of a 10-mg/kg dose, and the peak serum concentration was 8 g/ml. Similar to the results of the present study, RIF was previously observed to inhibit the growth of different types of cancer cells and osteoblast cells *in vitro* (28-30). The predominant elimination pathway is deacetylation into desacetyl rifampicin, while a 3-formyl rifampicin is produced separately by hydrolysis (20,28,29). RIF acts through the pregnane X receptor (PXR) and mediates the induction of the *CYP2C9*, *CYP3A*, *CYP2* and *MDR1* genes (1,28,31). In the current study, RIF stimulated COL1A1 expression though a

typical PXR motif, TGAACT, is not present in the 1000bp located upstream of the transcription start site of *COL1A1*. Therefore, the mechanism of *COL1A1* regulation by RIF remains to be elucidated. In the NP cells of aged rabbits, *COL1A1* was upregulated; thus, it was concluded that RIF may promote the aging of NP cells in patients treated with this drug (32). Observations of the antiproliferative effect of RIF on NP cells without a concomitant increase in caspase-3 and -7 activities requires additional investigation.

Following the administration of a single 25 mg/kg dose, the mean maximum concentration of ETH in serum has been demonstrated to reach 4.5 μg/ml (33-35). In the present study, of all the tested anti-TB drugs, ETH had the least effect on gene expression and was the only drug to stimulate cell viability after 24 h. This anti-aging property of ETH subsequent to short-term incubation was not observed after 192 h. The co-administration of therapeutic doses of ETH with other anti-TB drugs led to a marked decrease in male rat fertilizing capacity and fertility, and an increase in pre- and post-implantation embryo lethality (36). As the most serious potential adverse effect of ETH is ocular toxicity, manifested by optic or retrobulbar neuritis (7), the safety of ETH treatment in NP cells is required to be determined for longer therapeutic periods.

PYR achieved a maximum concentration of 49 μg/ml in the blood after 2 h (34). PYR is converted to pyrazinoic acid and further oxidized to 5-hydroxypyrazinoic acid by xanthine oxidase (37). Pyrazinoic acid inhibits translation in *Mycobacterium tuberculosis*; however, the mechanism of the influence of PYR on eukaryotic cells is not known (38). In this study, 5 μg/ml PYR induced the expression of *SOX9*, a transcription factor that is key in chondrogenesis (39). The same concentration of PYR did not affect cell viability, caspase-3 and -7 activity, and GAG synthesis. It was concluded that PYR exerted a moderate anti-aging effect on NP cells. PYR may be beneficial in IVD regeneration and may be used to prevent

the loss of *SOX9* expression in degenerated IVD cells. It may also prevent a decrease in *COL2A1* and *ACAN* expression, as *SOX9* is a common transcription factor for the genes (14,40). Prolonged treatment with PYR is required to determine whether *COL2A1* and *ACAN* expression increases.

We propose that INH and PYR may have a dual role in anti-TB therapy; they decrease *Mycobacterium* levels, as well as stimulate the expression of genes encoding the extracellular matrix proteins (*COL2A1* and *ACAN*) and the *SOX9* protein. It was demonstrated that 192 h of treatment with INH or RIF resulted in cytotoxicity, indicated by a decrease in the number of NP cells. These results are similar to those obtained by Hoelscher *et al* (5) in *annulus fibrosus* cells, in which antibiotics (cefazolin and cefamandole) used in spinal surgery led to lower cell proliferation. The results, albeit on a small number of samples, suggested that the anti-TB drugs generated certain side effects on NP cells *in vitro*. To confirm these results, the collection of data following prolonged treatment with anti-TB drugs is required, as antimycobacterial therapy lasts for several months. Application of TGF-β1 and anti-TB drugs revealed opposite effects on the expression of key chondrocyte genes and therefore should be investigated in combination. To the best of our knowledge, this is the first study to demonstrate that anti-TB drugs may be harmful to articular cells. The potential adverse effects of anti-TB drugs may be of importance to clinicians who use anti-TB drug for the routine chemotherapy of mycobacterial infections. In addition, the vertebral column of patients suffering pulmonary or extra-pulmonary mycobacterial infection, treated with INH, RIF, ETH or PYR, should be monitored periodically.

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