Toxicity of the acetyl-para-aminophenol group of medicines to intact intervertebral disc tissue cells

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Abstract. The present study aimed to investigate the effects of paracetamol, an analgesic and antipyretic that is used in emergency departments and neurosurgery departments for postoperative pain management on intervertebral disc tissue. Paracetamol-treated human primary cell cultures and untreated cell cultures were compared using molecular analyses. Cell proliferation and gene expression were statistically analyzed. Cell proliferation was suppressed on days 10 ($P=0.05$) and 20 ($P<0.05$) in the paracetamol-treated groups. Gene expression of chondroadherin, matrix metalloproteinase (MMP)-7, MMP-13 and MMP-19 was higher in the paracetamol-treated samples while gene expression of Cartilage Oligomeric Matrix Protein and interleukin-1β was lower ($P<0.05$). Paracetamol, which appears innocuous compared with many analgesics, may increase the expression of MMPs, which serve a significant role in catabolic reactions and suppress the proliferation of intact intervertebral disc tissue cells.

Introduction

Paracetamol, also known as acetyl-para-amino-phenol or acetaminophen, is an analgesic and antipyretic that is referred to as a N-(4-hydroxyphenyl) ethanamide, N-(4-hydroxyphenyl) acetamide by the International Union of Pure and Applied Chemistry and has the chemical formula $C_8H_9NO_2$ (1). It is suitable for intravenous administration, and its injectable form has significant ease of use. Accordingly, this drug is used as a moderate analgesic and antipyretic following surgical intervention in almost all areas of medicine, including neurosurgery (2).

Paracetamol is an analgesic drug and an indispensable pharmaceutical preparation in emergency departments that can be administered orally, rectally and intravenously (3). This drug and its precursors are believed to be among the most risk-prone pharmaceutical preparations. It has been reported to cause hundreds of deaths each year via acute liver failure even within industrialized countries, and it has toxic effects on liver and kidney tissues (4).

Many pharmaceutical preparations are used to treat a wide range of diseases. However, any medication may adversely affect the tissues of regions surrounding the targeted tissues (5,6); therefore, drugs may accumulate in fluid compartments and tissue layers. The majority of analgesics are composed of organic acids with high plasma protein binding capacity. Paracetamol typically exhibits low levels of binding to plasma proteins, but the binding level increases with higher doses.

Increased binding to plasma proteins facilitates drug accumulation in tissues. As a result, non-ionizing parts of the drug increase, and the interaction level of the drug and the lipid structure of the cell membranes also increase (7-9).

A notable number of studies have been performed to identify an appropriate treatment procedure for damaged osseous joints, facet joints and intervertebral disc structures, and investigations on the toxicity of drugs in non-degenerative, healthy tissues has gained momentum (10,11).

Certain side effects and adverse events associated with paracetamol have been reported previously (12); however, no pharmaco-molecular studies to date have investigated the damage to non-degenerate, intact intervertebral disc tissue cells caused by paracetamol.

The present study investigated the toxicity of paracetamol, an anilid derivate that is frequently prescribed alone or in combination with other non-steroidal anti-inflammatory drugs following major surgical procedures or minimally invasive surgeries for numerous patients with pain or fever, using healthy human intervertebral disc tissue cell cultures.

Proliferation analyses and chondroadherin (CHAD) gene expression levels, as well as continuous expression of the nucleus pulposus (NP) specific marker, were investigated. The

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level of the cartilage oligo matrix protein (COMP) directly or indirectly involved in the catabolic mechanisms of the disc structure was also investigated.

In addition to these markers, the expression of matrix metalloproteinase (MMP)-7, MMP-13 and MMP-19, known to be responsible for the catabolic pathways (13,14), were investigated. The changes in intervertebral disc and extracellular matrix structures were assessed using a reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR). The gene expression levels of IL-1β, a prominent proinflammatory cytokine in disc metabolism (15,16), were also investigated in the present study.

Materials and methods

Ethical approval. The present study was approved by the Namik Kemal University School of Medicine's Local Ethics Committee (dated 28.06.2018 and numbered 2018/107/07/13). Written informed consent was obtained from the patients undergoing surgery whose tissues were used to obtain the primary cell cultures.

Materials. The cell dishes used for the preparation of cell cultures were obtained from Zhejiang Sorfa Life Science Research Co., Ltd. (cat. no. SCD-11-035), and a 96-well FG-Microplate (cat. no. 4346906) was purchased from Thermo Fisher Scientific, Inc. Fetal bovine serum (cat. no. 10500064), L-glutamine (cat. no. 25030024), penicillin-streptomycin (10.000 U/ml; cat. no. 15140122) and amphotericin B (250 μg/ml; cat. no. 12183018) were obtained from Thermo Fisher Scientific, Inc. The paracetamol-active pharmacological agent administered to cell cultures was in the form of a 100-ml vial (10 mg/ml; *Perfalgan*®) and was purchased from Bristol-Myers Squibb Company. The commercial kit used for the cell viability, toxicity and proliferation analyses was Vybrant MTT Cell Proliferation assay (cat. no. V13154; Thermo Fisher Scientific, Inc.). The ELISA with which spectrophotometric analysis was performed was Mindray MR 96A (Georgia Institute of Technology).

ForRT-qPCR,aPureLink™ RNAMinikit(cat.no.15290018), High-Capacity cDNA Reverse Transcription kit (cat. no. 4368814), TaqMan® Gene Expression assay-Hs00154382_m1 (cat. no. 4331182), CHAD TaqMan® Gene Expression assay (cat. no. 4331182), COMP TaqMan® Gene Expression assay (cat. no. 4331182), MMP-7 TaqMan® Gene Expression assay (cat. no. 4331182), MMP-13 TaqMan® Gene Expression assay (cat. no. 4331182), MMP-19 TaqMan® Gene Expression assay (cat. no. 4331182), IL-1β TaqMan® Gene Expression assay (cat. no. 4331182), and internal control gene (housekeeping gene, β-actin) Taqman Fast Advanced mix (5 ml; cat. no. 4444557) were purchased from Thermo Fisher Scientific, Inc.

Inclusion and exclusion criteria. Tissues were obtained from the patients admitted to the Department of Neurosurgery, Namik Kemal University, School of Medicine between July 2018 and August 2019. The tissues of patients with malignancy or pregnancy were not used. Patients who were referred to the emergency department with a complaint of spinal trauma and then diagnosed with spinal instability or traumatic disc hernia following physical, neurological and imaging examinations were enrolled (n=17). The tissues of the patients diagnosed with degenerative intervertebral disc herniation following MRI scans were excluded from the study (n=2).

Paracetamol may interact with beta-adrenergic receptor blockers (n=3), including propranolol, ethyl alcohol (n=3) and oral anticoagulants (n=2), and its efficacy may be inhibited by certain drugs, including chlorpromazine (n=1). Therefore, the tissues of the patients who had taken the aforementioned drugs were excluded.

Intervertebral disc tissues obtained from 6 patients, included 3 males and 3 females (mean body mass index, 27.9 kg/m^2; mean age, 28.62±8.18 years, range, 20-37) were resected and transferred into sterile falcon tubes containing penicillin streptomycin.

Resection of the tissues via surgery and preparation of the primary cell cultures. Non-degenerate healthy disc material [intact NP and annulus fibrosus (AF) tissue] was obtained as surplus surgical material. Human primary intervertebral disc cell cultures were established according to standard protocols (15,16).

Physical and neurological examinations of the patients with spinal trauma who were referred to the emergency department were performed. Subsequently, surgical intervention was decided on. All patients were placed in the prone position under endotracheal general anesthesia. Surgical field antisepsis was provided with 10% povidone-iodine solution and covered in a sterile manner. A median skin incision was performed on the skin and under the skin. Paravertebral muscles were dissected subperiosteally from osseous structures. The regions with neural compression were decompressed via laminectomy and traumatic disc excision. Spinal stabilization was performed through the transpedicular screw-rod system (17). The resected disc tissues were transferred to sterile Falcon tubes at 40˚C, and letter coding was conducted.

Preparation of the primary cell cultures and application of paracetamol to the cultures. The tissues placed in the flow cabinet were transferred to Petri dishes in a sterile manner and were irrigated with 0.9% isotonic sodium chloride solution to differentiate them from the red blood cells. Subsequently, the tissues were mechanically minced. The minced tissues were transferred to Falcon tubes containing 50 ml Hank's Balanced Salt Solution and 0.375 μg collagenase type II and then incubated overnight in an incubator set to a temperature of 37˚C and 5% CO_2. The samples were centrifuged at 4˚C and 161 x g consecutively twice for 10 min. Following centrifugation, the tubes were returned to the flow cabinet. The tube caps were opened in a sterile environment. The supernatant at the top of the tubes was discarded. The pellet located at the bottom of the tube was resuspended with the freshly prepared culture medium. This freshly prepared culture medium consisted of Dulbecco's Modified Eagle's Medium (cat. no. D5796), 15% FBS (cat. no. 10500064), 1% L-glutamine (cat. no. 25030024), penicillin-streptomycin (10,000 U/ml; cat. no. 15140122) and amphotericin B (250 μg/ml; cat. no. 12183018) (all Gibco; Thermo Fisher Scientific, Inc.). The cells, cultured in a T25 flask, were incubated for 72 h in an incubator set to 37˚C and 5% CO_2. Standard human primary cell cultures were performed (6,9,13,16). Cells were trypsinized with trypsin-EDTA (0.25%) (cat.

no. T3924; Sigma-Aldrich; Merck KGaA), and viable cells were counted were counted with Inverted light microscope (CKX41, Olympus Europa SE & Co. KG) at a magnification of x10, using the *Neubauer* chamber following trypan blue staining at 22.4˚C for 12 min. For MTT analysis and acridine orange/propidium iodine (AO/PI) staining, cells were cultivated with 15,000 cells/well in 96-well plates, and for RNA isolation, cells were cultivated with $1.2x10^6$ cells/well in 6 well plates. At the end of the 24-h incubation period, paracetamol content was applied to the cell cultures in the experimental group. A 10 mg/m paracetamol stock solution was used by diluting it with Dulbecco's modified Eagle's medium to a final concentration of 15.22 μl/ml. The final concentration was calculated based on the doses reported in previous studies (18,19).

MTT analysis. MTT analyses were performed on the day of drug applications and on the 10th and 20th days following the applications, with at least three experimental and three technical repetitions. The commercial Vybrant MTT cell proliferation assay kit (V-13154) was used. The 12 mM MTT stock solution was prepared by adding 1 ml sterile PBS to the vial containing 5 mg MTT. Next, 90 μl fresh aforementioned medium was added to the confluent cells in 96-well plates. A total of 10 μl MTT stock solution was added, protected from light, and incubated at 37˚C for 2 h. At the end of this period, 25 μl of the samples were discarded and the same amount of DMSO was added. This was incubated for an additional 10 min at 37˚C and absorbance measured for each well at 540 nm using the ELISA reader (Mindray MR 96A; PRC; Georgia Institute of Technology).

Inverted light microscopy analyses and Acridine orange and propidium iodide (AO/PI) staining. Cell morphology and confluency were analyzed using an inverted microscope. Microphotographs of the cell organizations were obtained in the confocal/contrast phase prior to and following the application of chemical agents at x10 and/or x20 magnifications (Fig. 1; first lane). The cells were stained with Giemsa (GS500; Sigma-Aldrich; Merck KgaA; 37˚C for 10 min) stain to demonstrate the cells' organization more clearly (Fig. 1; third lane). In order to determine the presence of cell death and whether the death was apoptotic, cells were stained (at 22.4˚C for 10 min) with AO/PI and examined using a fluorescent microscope. Microphotographs of cell structures were obtained (Fig. 1; second lane), and the images were evaluated using the CytoVision® v 7.0 (DM6000B; Leica Microsystems GmbH) capture station imaging program (6-16).

AO/PI stain is a cell viability dye that causes viable nucleated cells to fluoresce green and non-viable nucleated cells to fluoresce red. The AO/PI stain was prepared with 5 g sodium-EDTA, 2 mg PI, 25 ml FBS and 2 mg AO dissolved in 1 ml 99% ethanol; sterile distillated water was added to reach a final volume of 100 ml. Cell cultures were stained with AO/PI at room temperature for 10 min, and then cell death and cell viability were monitored at a magnification of x10.

RT-qPCR analysis. Prior to performing the RNA isolation, a live cell count was conducted by staining with trypan blue using the *Neubauer* chamber. RNA isolation was performed on $5.3x10^6$ cells using the Pure Link, Ambion kit (Thermo Fisher Scientific, Inc.). RNA samples obtained were converted into cDNA copies by reverse transcription (Applied Biosystems; Thermo Fisher Scientific, Inc.). The Applied Biosystems 7300/7500 RT-PCR system was used with the following reaction protocol: Hold at 50˚C for 2 min, hold at 95˚C for 10 min, and alternate between 15 sec at 95˚C and 1 min at 60˚C for 40 cycles. RT-qPCR was performed using primers and probes specific to COMP, MMP-7, MMP-13, MMP-19, IL-1β and CHAD genes with the obtained cDNA samples. Commercial Taq-Man gene expression kits (cat. no. 4331182, Thermo Fisher Scientific, Inc.) were used for each gene region. Hs00164359_m1 was used for the COMP gene region, Hs01042796_m1 for the MMP-7 gene region, Hs00419424_m1 for the MMP-13 gene region, Hs00419424_m1 for the MMP-19 gene region, and Hs001541101-coded products were used for the CHAD gene region (information regarding primers used here: https://www.thermofisher.com/order/genome-database/). Relative quantification (RQ) was performed according to the average Ct values obtained using the b-actin (Hs01060665_g1) gene as an endogenous control and the control group as a calibrator (7,8). Using this method, the difference in gene expression between the control group and experimental groups was determined as a fold-change.

Statistical analyses. The program Minitab R18 was used for the statistical analysis. The results are expressed as the mean ± standard deviation. The results were evaluated using an analysis of variance (ANOVA; at a 95% confidence interval to assess whether there were significant differences across groups. When differences across groups were observed, Tukey's honest significant difference (HSD) post hoc test was used for multiple pairwise comparisons. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Cell morphology, confluency and viability. The surface morphologies of the samples were preserved, and the cells were healthy, viable and had proliferated in all microscopic analyses performed at all time periods in the control group (Fig. 1A, C, E, F, H, K, M and O).

Cell proliferation based on MTT analysis. Proliferation was suppressed on the 10th day ($P=0.05$) and the 20th day ($P<0.05$) in the paracetamol supplemented groups and the non-drug-administered control group samples, respectively, and this difference is statistically significant ($P<0.05$ Figs. 1B, D, G, I, L, N, and 2). However, in addition to cell proliferation suppression, it is notable that no round-shaped cells, indicating cytotoxicity, were identified. Furthermore, the images obtained via fluorescent microscopy also supported these results. The dead cells stained red were not identified in the images from the 10th day (Fig. 1G) or the 20th day (Fig. 1I). Additionally, on the 10th day (Fig. 1L) and the 20th day (Fig. 1N), analyses of the microscopic images with Giemsa staining, the numbers of dead cells and the matrix structures around the cells deteriorated and broke down in the experimental group, compared with the cell samples of the control group (Fig. 1M and O).

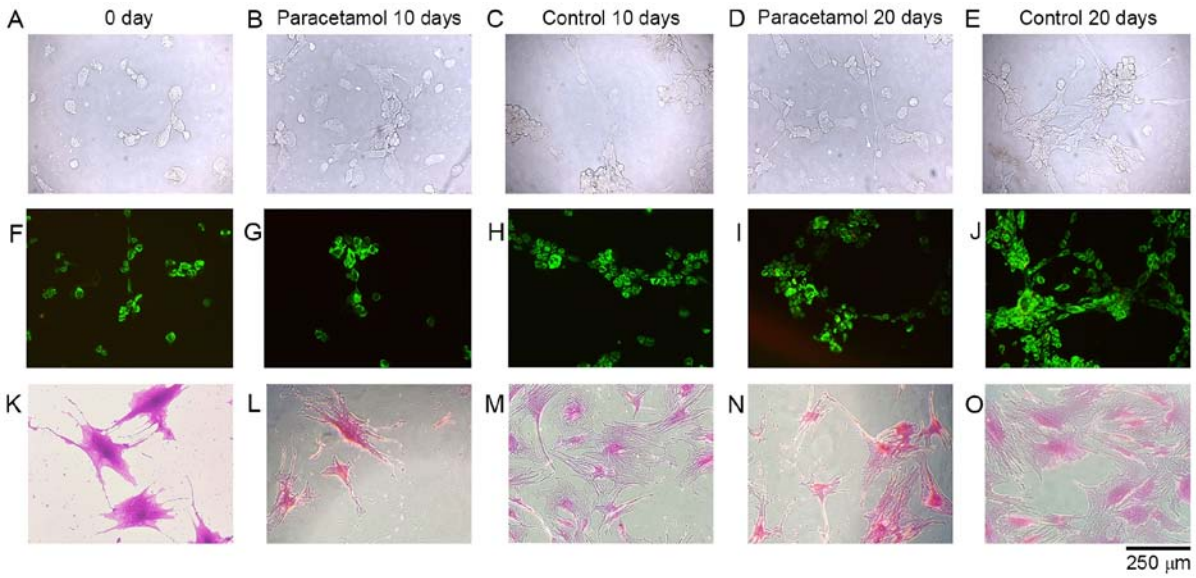

Figure 1. Microscopic evaluation of cultured cells. Lane 1, inverted microscopy of (A) 0 day, (B) 10 days of paracetamol application, (C) 10 day control, (D) 20 days of paracetamol application and (E) 20 day control without staining. Lane 2, Acridine orange and propidium iodide stained cell cultures of (F) 0 day, (G) 10 days of paracetamol application, (H) 10 day control, (I) 20 days of paracetamol application and (J) 20 day control. Lane 3, inverted microscopy of Giemsa-stained cultures of (K) 0 day, (L) 10 days of paracetamol application, (M) 10 day control, (N) 20 days of paracetamol application and (O) 20 day control.

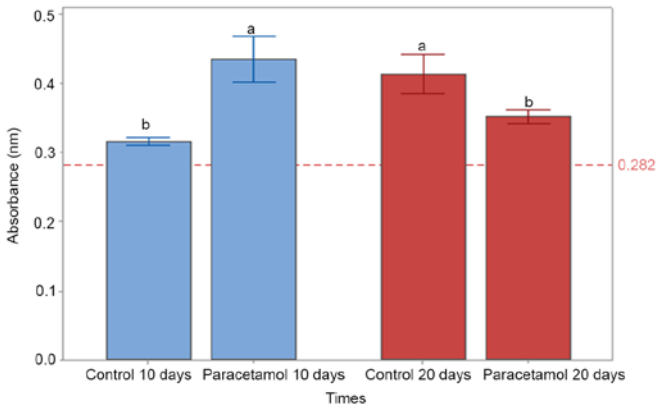

Figure 2. Effects of paracetamol treatment on the proliferation of intervertebral disc tissue cells. Data were analyzed using one-way analysis of variance followed by Tukey's post hoc test. The dotted line indicates the mean absorbance in the control group at 0 h. [a]Highest rate of viability and proliferation. [b]Lowest rate of viability and proliferation.

RT-qPCR analysis. RT-qPCR analysis revealed that the RQ values for CHAD, MMP-7, MMP-13 and MMP-19 increased in the paracetamol-administered samples compared with that in the control group as follows: From 4.29-fold to 5.29-fold, from 0.13-fold to 0.34-fold, from 0.12-fold to 2.39-fold, and from 1.52-fold to 1.63-fold, respectively. However, the RQ values for the COMP and IL-1β markers decreased from 1.32-fold to 0.34-fold and from 7.03-fold to 4.57-fold, respectively (Fig. 3).

Discussion

Paracetamol is a metabolite of phenacetin (20), and the mechanism of its analgesic action has not yet been fully elucidated (20). However, it is known to be effective in inhibiting prostaglandin synthesis in the central nervous system (21). Paracetamol has been widely used since 1950 (22). The increasing use of this drug has resulted in certain adverse events, including an increased mortality rate due to liver toxicity originating from excessive dose uptake (23).

Paracetamol is rapidly and uniformly distributed in body tissues. It is frequently prescribed as an analgesic and antipyretic in the treatment of headache, toothache, migraine, dysmenorrhea, myalgia, neuralgia, musculoskeletal, tonsillectomy pain, the common cold, influenza, and other bacterial and viral infections. It can also be used as a pain controller for numerous cases in neurosurgery and emergency departments, including posterior lumbar laminectomy (24), vertebral compression fractures (25), spinal primary and metastatic tumors (26), post-craniotomy in intracranial pathology surgery (27), traumatic fractures of bones (28), urinary colic (29) and acute appendicitis (30).

In addition to liver and kidney toxicities caused by paracetamol (31,32), certain studies have reported that it may lead to maculopapular rashes (33) or nausea in very rare cases (34), and its long-term use may cause hemolytic anemia (35), thrombocytopenic purpura (36) and agranulocytosis (37). However, no studies have investigated the side effects and adverse events of paracetamol on intervertebral disc tissue cells.

Ramachandran *et al* (38) reported that more recent investigations into acetaminophen hepatotoxicity provided insights into the critical role of mitochondrial dysfunction in mediating liver injury. The authors stated that some studies clarified the mechanisms of acetaminophen-induced hepatocyte cell death. They suggested that a significant controversy on the role of innate immunity in APAP-induced hepatotoxicity is ongoing, even though mitochondrial oxidative and nitrosative stress was known to be a key mechanistic feature involved in downstream signaling following acetaminophen overdose (38).

An *in vitro* toxicity study reported that differentiated HepaRG cells preserved liver-specific functions, including drug-metabolizing enzymes (39). In that study, the feasibility

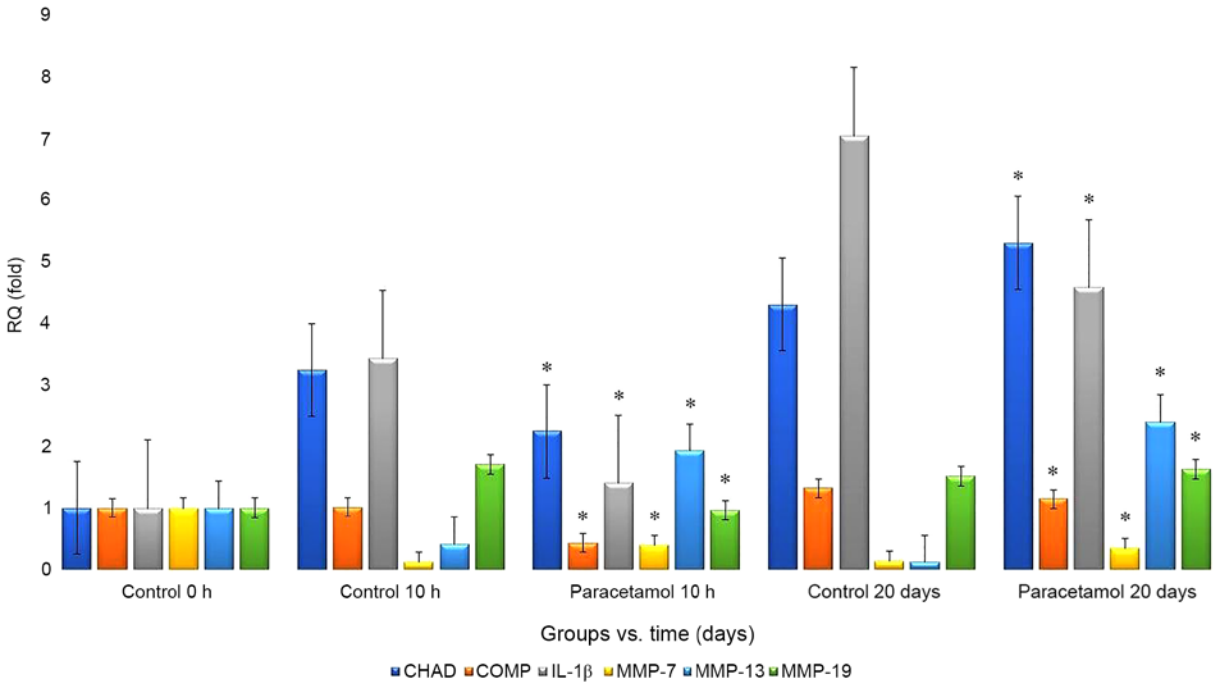

Figure 3. Graph of fold-changes (RQ) in gene expression of CHAD, COMP, IL-1b, MMP-7, MMP-13 and MMP-19. *P<0.05. CHAD, chondroadherin; COMP, Cartilage Oligomeric Matrix Protein; IL-1b, interleukin-1b; MMP, matrix metalloproteinase.

of HepaRG cells as a human hepatocyte was investigated using selected hepatotoxic compounds. The possible adverse effects of acetaminophen have also been investigated in another study (40). It was reported that acetaminophen, commonly used in clinics, had time- and dose-dependent side effects; therefore, further animal studies are required to accurately investigate possible adverse effects (40). It was concluded that acetaminophen-mediated specific cytotoxicity was associated with the molecular mechanisms facilitating apoptosis and inflammatory stress in the liver and kidney.

Knockaert *et al* (41) investigated the role of mitochondrial CYP2E1 in the toxicity of acetaminophen and ethanol. The effects of these two compounds were compared in cells expressing CYP2E1 in either mitochondria only or both endoplasmic reticula and mitochondria and in mock-transfected cells (41). It was reported that when acetaminophen or ethanol was used as a CYP2E1 substrate, the exclusive localization of CYP2E1 within mitochondria may cause reactive oxygen species overproduction, depletion of reduced glutathione, increased expression of mitochondrial Hsp70, mitochondrial dysfunction and cytotoxicity (41). The study emphasized that these deleterious events occurred despite the lower cellular level and activity of CYP2E1 compared with cells expressing CYP2E1 in endoplasmic reticula and mitochondria and that this was particularly apparent in the case of acetaminophen (41). The authors proposed that mitochondrial CYP2E1 may serve a significant role in oxidative stress and cell death (41).

Patel *et al* (42) reported that acetaminophen administered to rats during chronic exercise decreased skeletal collagen and cross-linking. It was suggested that the effect of acetaminophen on the muscle extracellular matrix (ECM) may be mediated by the dysregulation of the balance between MMPs and their tissue inhibitors (TIMPs). In a double-blind, placebo-controlled, randomized cross-design study, two male volunteers performed two trials of knee extension (42). A placebo (PLA) and acetaminophen were administered (1,000 mg/6 h) for 24 h prior to and following resistance exercise (RE). Vastus lateralis biopsies were performed at 0, 1 and 3 h following RE (42). They reported that mRNA expression of MMP-2, type I collagen and type III collagen was not changed by exercise or acetaminophen (P>0.05). They also stated that, compared with the control group, the TIMP-1 expression was lower at 1 h post-RE with acetaminophen but higher at 3 h, and this difference was statistically significant (P<0.05). They emphasized that MMP-9 expression and protein levels were increased at 3 h post-RE independent from treatment and that lysyl oxidase expression was significantly higher at 3 h post-RE with acetaminophen consumption, compared with the control group (P<0.05). It was suggested that short-term acetaminophen consumption prior to RE has a small impact on the measured ECM molecules in human skeletal muscle following acute RE (42).

Commercial cell lines or cell cultures established with animal tissues have been commonly used in previous studies (6-11,13,14). The sensitivity of animal tissue is known to differ from that of human tissue (6-11,13,14). Therefore, the results obtained from assays using animal tissues may diverge from those using human tissues, which may result in misleading outcomes (6-11,13,14). Commercial cell lines are known to comprise only a single type of cell, and there are no complicated coordination mechanisms in the microenvironment of the cells (6-11,13,14). They do not have the same genotypic and/or phenotypic characteristics as in the human body; therefore, the results of studies using cell lines may also be misleading (6-11,13,14).

The surface morphologies of the samples were maintained, and microscopic analyses of healthy and viable cells were performed. Proliferation was suppressed on days 10

(P=0.05) and 20 (P<0.05) in the paracetamol-treated groups, compared with the untreated control group samples. However, no round-shaped cell images were observed. The dead cells that were stained red were not observed in images obtained through fluorescent microscopy. AO/PI results revealed a decrease in the number of cells and a deterioration of matrix structures around the cells in the paracetamol-treated samples at days 10 and 20.

CHAD, as a constantly expressed NP-specific marker, is secreted from NP/AF cells serving a key role in the organization of ECM and contributes toward the formation of a healthy microenvironment in the IVD tissue. ECM aid in keeping cells and tissues together and supports the cell structure and function in the continuity of cell viability and migration of cells (6-8). Cell-matrix interactions are regulated by proteolytic enzymes, including MMP-7, MMP-13 and MMP-19 (6-8).

Consequently, the present study considered the gene expressions of MMP-7, MMP-13 and MMP-19 in addition to CHAD, as they may be associated with the degenerated cell morphology and decreased cell proliferation that may be indicative of IVD degeneration. The RQ values of CHAD, MMP-7, MMP-13 and MMP-19 were higher in the paracetamol-treated samples while the RQ values of the COMP and IL-1β markers were decreased. These results were statistically significant (P<0.05).

Paracetamol is metabolized to N-acetyl-β-benzoquinoneimine, a toxic metabolite by the cytochrome P450 enzyme system in the liver following oral ingestion. This metabolite is detoxified by endogenous glutathione at a normal dose of paracetamol. Glutathione is depleted at its overdose use; thus, n-acetyl-β-benzoquinoneimine cannot be detoxified, which results in liveroxicity (43). The present study has a number of limitations. It is an *in vitro* experimental study; therefore, there is no compensatory mechanism that may be detoxified by endogenous glutathione against N-acetyl-β-benzoquinonimine. Further limitations include the consideration that all the patients were from the same ethnic group and that the cell cultures were obtained from a small number of patients.

Paracetamol is considered to be in the category of innocuous drugs, and its side effect profile has not been fully elucidated. As with every drug, this pharmacological agent may be a highly toxic chemical. Therefore, clinicians should be aware of the potential side effects and adverse events associated with this drug.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

NK and IY conceived and designed the present study. NK selected the patients who met the inclusion criteria and acquired subjects and data. NK also acquired subjects and data, and analyzed/interpreted the data, diagnosed and operated on patients and removed tissues. IY performed inverted light microscopy, conducted/analyzed ELISA and prepared/stored culture drugs. IY, prepared drugs and adapted clinical doses to cell cultures, performed statistical analyses and wrote the manuscript. DYS prepared the human primary intervertebral disc cells culture and performed/analyzed PCR. NK, IY and DYS, contributed to the preparation and critical revision of the manuscript. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was undertaken with the approval of the Ethics Committee of the Namik Kemal University School of Medicine, Tekirdag, Turkey (2018/107/07/13). Written informed consent was obtained from all patients.

Patient consent for publication

Written informed consent for publication was obtained from all enrolled patients.

Competing interests

The authors declare that they have no competing interests.

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