PATHOMECHANISM AND THERAPEUTIC POSSIBILITIES OF ARTHRITIS

Ph.D. Thesis

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List of original papers

List of full papers relating to the subject of the thesis

Hartmann P*, **Butt E*,** Feher A, Szilagyi AL, Jasz KD, Balazs B, Bakonyi M, Berko S, Eros G, Boros M, Horvath G, Varga E, Csanyi E, Electroporation-enhanced transdermal diclofenac sodium delivery into the knee joint in a rat model of acute arthritis. DRUG DESIGN, DEVELOPMENT AND THERAPY 12:1917-1930. doi: 10.2147/DDDT.S161703. (2018) **IF: 3.208**

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Horvath T**,** Hanak L, Hegyi P, **Butt E,** Solymar M, Szucs A, Varga O, Thien B., Szakacs Zs, Csonka E, Hartmann P, Hydroxyapatite-coated implants provide better fixation in total knee arthroplasthy. A meta-analysis of randomized controlled trials. PLOS ONE 12;15:e0232378. doi: 10.1371/journal.pone.0232378. (2020) **IF: 2.740**

Jávor P, Mácsai A, **Butt E,** Baráth Bá, Jász DK, Horváth T, Baráth Be, Csonka Á, Torok L, Varga E, Hartmann P, Mitochondrial dysfunction affects the synovium of patients with rheumatoid arthritis and osteoarthrosis differently (under review in PLOS ONE)

List of full papers not-relating to the subject of the thesis

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I. INTRODUCTION

Arthritis is a collective term encompassing many diseases with distinct etiologies but common symptoms, such as joint pain and inflammation. Osteoarthritis (OA) and rheumatoid arthritis (RA) are both common forms of arthritis despite of the substantial differences in their etiology and pathomechanism. Although epidemiological studies have revealed both endogenous and exogenous risk factors for OA, it is basically a degenerative joint disease, initiated by an injury or repetitive joint stress of the joint. The mechanical damage activates mechanosensitive intracellular signalling in cartilage, which involves the activation of inflammatory processes that may develope structural and symptomatic course of disease. With regards to the etiology, RA is considered as a multifactorial, multigenic autoimmune disease. Although the exact pathomechanism is still unknown, the importance of genetic predisposition has been revealed, as polymorphisms of the HLA-DR4 and DR1 locus and cytokine genes have been associated with RA. As a result, abnormal activation, adhesion and migration of T- and B-lymphocytes and macrophages occur, which is accompanied by increased proinflammatory cytokine production (TNF-α, IL-1). As a consequence of the chronic autoimmune inflammation, intensive angiogenesis and pannus formation in the synovium and cartilage damage may develop.

Beside these well-known factors in the pathogenesis of arthritis, a growing body of evidence suggests the role of mitochondrial damage and dysfunction in synoviocytes and chondrocytes of OA and RA patients. On the one hand, mitochondrial dysfunction has been implicated in the pathogenesis of primary and post-traumatic OA. On the other hand, some haplogroups of mitochondrial respiratory genes are closely associated with higher prevalence of OA and RA meaning an increased genetic predisposition to the development of arthritis. As a consequence of the altered mitochondrial genes, mitochondrial respiratory activity is impaired in arthritis.

Today, the “gold standard” therapy for alleviating arthritis-related pain is diclofenac, a nonsteroidal anti-inflammatory drug (NSAID) of the phenylacetic acid class. Unfortunately, the side effects of such an effective compound are also significant. Habitual use of diclofenac is associated with the NSAID category risk of dose-related gastrointestinal, cardiovascular, and renal adverse effects; consequently, topical preparations were developed to limit the potentially serious complications of systemic treatments. The absorption of the topically applied diclofenac dose is 3-5 % of the oral formulation and the dose reaches the site of action ten times later than the oral dose. New dosing regimens are required to achieve adequate local drug concentrations directly at the site of application. Different penetration techniques have

been developed to increase bioavailability and transdermal drug delivery, including ultrasound and EP. Upon EP, the applications of short, high voltage pulses cause transitory structural perturbations in the lipid bilayer of the membranes. In this way, lipophylic or hydrophylic molecules, neutral or highly charged compounds can be transported across or into the membranes of bacteria or mammalian cells up to 40 kDa molecular weight. Common fields of indication for EP are biological and artificial membranes, but it can also be used on complex structures such as the synovium.

After the exhaustion of conservative therapeutic options, resurfacing arthroplasty and prostheses for total knee arthroplasty (TKA)are recommended as treatment options for people with end-stage arthritis of the knee. Cemented fixation of prosthesis stems is widely used in all patients and is the gold standard for TKA. However, observed signs of osteolysis at the cement–bone interface has raised questions about the long-term durability of cemented TKAs. Therefore, cementless TKAs have been developed with several coating materials. Among them, hydroxyapatite (HA)-coating was a promising coating material with the potential to achieve biological fixation of implants. There are several studies comparing the outcomes of TKA using HA-coated and non-HA coated prosthesis stems but with conflicting results. This can only be answered by a randomized trial comparing the two methods of fixation.

2. MAIN GOALS OF THE STUDIES

Our main goal in these studies was to characterize the pathomechanism of the main groups of arthritic disorders, namely the OA and RA, and to find optimal conservative and operative treatments.

- As a first step, in study I., we characterized the mitochondrial changes in the synovial membrane in OA and RA patients. For this purpose, we used intraoperative samples, that were subjected to HRR, biochemical analysis and histology.
- Then, in study II., we aimed to improve the efficacy of transdermal drug delivery in conservative treatment of arthritis. We hypothesized that EP can amplify the transport of topical diclofenac into the joints and thus the effectiveness of local administration increases. Our aims were to compare the penetration properties of diclofenac hydrogel into the synovial fluid after different administration routes, and to estimate the analgesic and anti-inflammatory reactions after oral and topical drug deliveries in a standardized rat model of carrageenan-kaolin (C/K)-induced knee joint monoarthritis.

- Our final aim was to investigate the current innovations of prosthesis implantation in the operative treatment of arthrosis. Therefore, we conducted a meta-analysis to compare the outcomes of TKA using HA-coated and non-HA coated prosthesis stems. The main purpose was to update current knowledge and compare data evidence on the quality of fixation of the tibial component evaluated with radiostereometric analysis (RSA) in TKA under two conditions: with HA-coated cementless prosthesis, or with non-coated cementless or cement fixation. RSA is able to accurately measure early migration of the stem and maximum total point motion (MTPM), which is the 3-dimensional vector of micromotions, has a predictive value of future loosening as primary outcome in our analysis. The secondary endpoints were clinical outcomes of TKAs including the Knee Society score (KSS) and the Knee Functional score (KFS), which are validated scoring systems for measuring the ability of the patient to function in everyday living and assessing the magnitude of tolerated pain. Other outcomes, such as the Hospital for Special Surgery (HSS) score were also evaluated together with the characteristics of donors and recipients.

3. MATERIALS AND METHODS

3.1. Protocol in Study I.

Prospective clinical study was conducted at a single, level I trauma centre (Department of Traumatology at the University of Szeged) between 01 September 2019 and 30 November 2020. Data were collected from consecutive adult patients (age > 18 years) with signed informed consent undergoing open or arthroscopic knee or hip joint surgery. Participants were allocated into RA, OA and control groups based on their medical documentation, 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) rheumatoid arthritis classification criteria and Kellgren-Lawrence (KL) classification.

Synovial fluid and tissue samples for mitochondrial functional and biochemical analysis and hystology from the synovium of RA, OA and control patients were taken intraoperatively with the permission and signed consent of the patients.

3.1.1. Mitochondrial functional measurements and cytochorme c release

The tissue samples were homogenized in 200 µl of MiRO5 respiration medium, then the homogenates were weighed into the detection chambers, 50 µl in each, which were calibrated to 200 nmol/ml oxygen concentration in room air. Substrates as glutamate (10 mM) and malate (2 mM), succinate (10 mM), ADP (5 mM) and inhibitors as rotenone (0.5 µM) and oligomycin (1 mM) and the uncoupler carbonyl cyanide p-trifluoromethoxy-phenyl-

hydrazine (FCCP) (0.5 μM) were added to the medium. The intactness of the inner mitochondrial membrane was assessed after adding cytochrome C (10 μM).

3.1.3. Inflammatory enzyme activities and cytokine production

Tissue xanthine oxidoreductase (XOR) and myeloperoxidase (MPO) activity and free nitrotyrosine, as a marker of peroxynitrite generation, were measured by enzyme-linked immunosorbent assay. Pro-inflammatory cytokine (TNFα, RANKL) levels were measured in the synovial fluid samples using commercially available ELISA kit according to the manufacturer's instruction.

3. 1.4. Histology

Intraoperatively harvested synovium biopsies were assessed with light microscopy and confocal imaging with a laser scanning endomicroscope (CLSM) to validate the proper assignment of participants to study groups.

3.2. Protocol in Study II.

This study was performed in three experimental series. The goal of the first experimental series was to assess the serum and the synovial concentrations of topically applied or EP-enhanced topical diclofenac by high-performance liquid chromatography (HPLC). In the second series of experiments, inflammation-related changes to the synovial microcirculation were evaluated directly by intravital videomicroscopy (IVM). The animals divided into four groups according to the administration route of diclofenac: per os diclofenac in Group 1 (n=6), with topical diclofenac gel in Group 2, and with EP-combined topical diclofenac gel (n=6) in Group 3. Group 4 served as a per os saline-treated control. The treatments were always applied 2 h before and 2 h after the arthritis induction.

In the third experimental series, the effectiveness of different routes of diclofenac treatment on nociception and inflammatory oedema formation was compared in C/K-induced arthritis. The animals were divided into four groups (n=6) according to the administration route of diclofenac: (1) per os diclofenac sodium, (2) topical diclofenac gel, (3) EP-enhanced topical diclofenac gel and (4) sham (per os saline-treated). Treatments were applied twice daily (every 12 h). The nociceptive tests were performed 24 h after the C/K injection, while knee joint swelling was evaluated at the peak of oedema formation 48 h after arthritis induction. Synovial washing fluid samples and synovium tissue specimens were collected for biochemical measurements and histology.

3.2.1. Arthritis induction

For arthritis induction a single intra-articular injection of a 75 μL mixture of 2% λ-carrageenan and 4% kaolin (C/K) in saline was administered to the right knee joint. The contralateral knee was injected with saline. This intra-articular injection of C/K is a well-established model of an acute onset monoarthritis resembling osteoarthritis. In this model, arthritis is probably initiated by mechanical damage to the inner surface of the synovium resulting in an inflammatory response indirectly to the activation of the endothelial side of the synovial barrier.

3.2.2. Electroporation protocols

EP treatment was performed with a Mezoforte Duo Mez 120905-D instrument. 230 μL diclofenac hydrogel was used, and the EP treatment time was 8 min.

3.2.3. Diclofenac-containing hydrogel formulation and HPLC measurements

The hydrogel was prepared using the following procedure. 5 w/w% diclofenac sodium was dissolved in a mixture of purified water and 30 w/w% ethanol 2 w/w% hydroxypropyl methylcellulose was added to this solution. Diclofenac content of the synovial washing fluid and plasma samples were analysed using an Agilent HPLC system, equipped with an automated solvent delivery system.

3.2.4. Nociceptive tests and knee joint swelling (morphological assessment)

Mechanical hyperalgesia was quantified using a plantar aesthesiometer and was expressed as paw withdrawal thresholds. Thermal hyperalgesia was detected with the paw withdrawal test using a Hargreaves apparatus. Baseline measurements were performed before the induction of arthritis, while the development of inflammation was investigated at the peak of nociceptive sensitivity 24 h after C/K injection. Joint inflammation was characterized by the changes in the diameter of the joints 48 h after C/K injection. The anteroposterior and mediolateral diameters were measured with a caliper square, and the cross-sectional area was calculated.

3.2.5. IVM examinations

The presence of adhesion molecules on postcapillary endothelial cells in the inflammatory synovial microenvironment has previously been shown and proven to be appropriate markers for estimating inflammation. These reactions can be quantitatively assessed by IVM, and the efficacy of various therapeutic interventions can also be judged objectively. Synovial microcirculation was investigated by means of fluorescent IVM, while the synovial membrane was superfused with 37°C saline. Erythrocytes were labelled with fluorescent isothiocyanate and leukocytes were stained with rhodamine-6G.

3.3. Protocol in Study III.

A systematic literature search was performed using MEDLINE, Scopus, EMBASE and the Cochrane Library databases up to May 31st, 2019. At the end of literature search, 11 RCTs involving 902 patients for primary TKA were enrolled to analysis. The primary outcome was Maximum Total Point Motion (MTPM) of the tibial component. This parameter is determined by radiosterometrical analysis and refers to the migration pattern of the prosthesis stems. The clinical outcomes of the implanted joints were evaluated by the Knee Society Score (KSS) and the Knee Function Score (KFS). We used the Cochrane Risk of Bias Tool to assess the risk of bias for each study.

3.4.13. Statistical analysis

In Study I, the statistical analysis was performed with SigmaStat 13.0 statistical software. One-way ANOVA with Tukey's test was used and the data are expressed as mean ± SD. $P < 0.05$ was considered as statistically significant.

In Study II, data analysis was performed with the SPSS 17.0 software. Changes in variables within and between groups were analysed by two-way ANOVA, followed by the Holm–Sidak test. All data were expressed as means ± standard deviation of the mean (SD). P values <0.05 were considered statistically significant.

In Study III, the statistical analysis was performed using Stata 15 SE (Stata Corp). Pooled weighted mean difference (WMD) with 95% CI was calculated for continuous outcomes. Random effect model was applied at all analysis with DerSimonien-Laird estimation.

4. RESULTS

4.1. Study I.

A total of 528 patients underwent hip or knee joint surgery between 01 September l 2019 and 30 November 2020 at the Traumatology Department of the University of Szeged. Inclusion criteria were met in 71 cases, in which 17 patients suffered from RA and 31 from OA. The control group consisted of 23 patients under 40 years of age, without a history of OA and with a need for surgery due to trauma.

4.1.1. Mitochondrial functional measurements

Changes in mitochondrial respiratory functions were evaluated in the presence of glutamate and malate or succinate in order to differentiate between Complex I- and Complex II-based activity. Arthritis groups displayed significantly reduced C I activity compared to the control group in the presence of saturating amount of ADP. Additionally, OxPhos capacity in

the RA group decreased significantly compared to the OA group as well. Respiratory control ratio (RCR) and coupling control ratio (CCR) were defined to quantify changes in the coupling of the electron transfer chain. In comparison with Control group, Complex I-based RCR and CCR both indicated a significant impairment of the electron transfer chain in the RA group. However, no significant difference was found in C II-related respiratory activity, RCR and CCR between the study groups. OA group exhibited significantly higher Compex I-based response to exogenous cytochrome c, while in the RA group both Complex I- and Complex II-related activity significantly increased.

4.1.3. Pro-inflammatory cytokines and oxydoreductive stress markers

Concentration of RANKL was significantly increased in the RA group compared to the control group, while TNF-α levels in the RA group were significantly higher compared to not only the control group, but the OA group as well. Significantly higher XOR activities were measured in the synovial membrane homogenates of RA and OA patients compared to the control group. The synovial tissues of RA patients displayed a significantly higher MPO activity compared to the synovial tissues of OA patients and healthy individuals. Significant difference in MPO activity occurred also between the OA and control groups. In the RA group, significant elevation of NT was present relative to both the OA and control groups.

4.1.5. Histopathology evaluation

CLSEM and H&E staining were used to validate the proper assignment of participants to study groups. Histological assessment was performed independently and blindly on coded slides by two investigators (P.J. and P.H.) using a previously described 0–4-grade histological scoring system, representing a composite of the extent of angiogenesis and fibrosis. Additionally, on H&E stained sections, a thickened synovial membrane, increased cellularity and mild lymphocytic infiltration occurred in samples from patients suffering from OA. More prominent lymphocytic infiltration, fibrosis, and in some cases even extensive fibrosis could be observed in RA samples.

4.2. Study II.

4.2.1. Plasma and synovial diclofenac concentrations

The plasma and the serum concentrations of diclofenac were the highest 10 min after the EP treatment; they then decreased at 30 min and remained constant at 60 and 120 min. EP-enhanced diclofenac delivery exhibited a significantly higher plasma level of diclofenac as compared with the simple topical application 10 min after the application. There were no significant differences in the diclofenac content of the synovial fluid and the plasma after the

EP-combined application. However, simple topical application did not result in detectable diclofenac content in the synovial fluid at the same point in time.

4.2.2. IVM

The PMN–endothelial interactions (rolling and sticking) in the postcapillary venules of the synovial membrane were determined. The rolling fraction of the PMNs in the postcapillary synovial venules exhibited a large degree of dispersion, and no baseline differences were observed between the C/K- and saline-injected knees or between the groups which participated in the treatment protocols (data not shown). However, the injection of C/K was accompanied by a statistically significant increase in PMN adherence (sticking) to the endothelial layer as compared to the control side. This reaction was considerably reduced with the administration of oral diclofenac. However, it was only moderately ameliorated by the EP-enhanced diclofenac hydrogel, and there were no changes in response to the simple topical application of the hydrogel.

4.2.3. Inflammatory enzyme activities and cytokine production

XOR and MPO activity and TNF-α concentration were significantly increased in response to arthritis induction, in comparison with the saline-injected knee joint. These values were significantly decreased, the oral diclofenac and EP-enhanced topical diclofenac-treated groups displayed the same results, however, conventional topical treatment did not influence the increased MPO activity.

4.2.4. Nociception and inflammatory oedema

The mechanical touch sensitivity was considerably increased in response to arthritis as the C/K-injected limbs responded to a lower level of trigger than the saline-injected control limbs in animals receiving the saline vehicle. This parameter was significantly diminished in response to oral and EP-enhanced topical diclofenac treatments, albeit complete restoration was not achieved. The thermal nociceptive latency was also significantly decreased in the injured leg in the saline-treated group, and oral and EP-enhanced topical diclofenac treatments exerted similar protective effects to those seen with the von Frey test.

The cross-sectional area in the C/K-injected knees was significantly enlarged 48 h after the challenge but was significantly reduced by both the conventional topical and the EP-enhanced topical diclofenac treatments. In the case of oral diclofenac administration, complete restoration to the level for the saline-injected knees was achieved.

4.2.5. Adverse effects of diclofenac intake

The gastric adverse effects of diclofenac intake were assessed by planimetric analysis of ulcers in the gastric mucosa. The topically-applied diclofenac sodium with or without EP had no deleterious effect on gastric mucosa; however, an equivalent dose of oral diclofenac sodium resulted in ulcer formation in 70% of the animals. The location of gastric ulcerations in the rat stomach was variable and included only mild lesions such as oedema, irritation and petechia formation.

4.3. Study III.

Eleven RCTs were included in quantitative synthesis, in which TKA with a HA-coated tibial stem was compared to other tibial fixations (cemented and uncemented prosthesis). All trials were homogenous with respect to demographic characteristics.

4.3.1. Radiological outcome

The MTPM of the tibial stem is the primary outcome in this meta-analysis. We used 2 years follow up for the analysis of the MTPM. The higher MTPM is associated with lower stability of the implant with a 0,2 mm cut-off value. Accordingly, if the MTPM exceeds 0,2 mm, the prosthesis is classified as unstable, which greatly increases the likelihood of other complications such as aseptic loosening. If the MTPM is less than 0,2 mm the prosthesis can be considering as stable, in a long run. Thirteen studies were enrolled to the MTPM analysis. The results showed that the MTPM values of the HA-coated cementless stems are significantly lower than that of the uncemented stems (WMD = 0.28, 95% CI: 0.01 - 0.56, P = 0.045). When HA-coated implants were compared to cemented prostheses, the letter displayed lower MTPM (WMD = -0.29, 95% CI: -0.41 to 0.16, P < 0.001).

4.3.2. Clinical outcomes

The secondary outcomes were KSS and KFS. 4 RCTs were enrolled to the analysis of clinical outcomes. The result showed that KSS of HA-coated cementless prostheses is no significantly higher as compared to the uncemented group (WMD = -0.64, 95% CI: -3.02 – 1.73, P = 0.596).

Of interest, there was no statistically significant difference in the KSS of HA-coated cementless and cemented prosthesis (WMD = -0.29, 95% CI: -2.27 to 1.69, P = 0.775). Similar results could be obtained by the analysis of KFS of studies, however, have limited value due to the lack of the comparison between HA-coated and uncemented groups. As such, no difference could be observed between HA-coated cementless and cemented implants (WMD = -4.95, 95% CI: -13.59 to 3.69 P = 0.069). However, comparison between HA-coated and uncemented groups could not have been done due to the low number of studies in the

uncemented group. KSS data are shown on the funnel plot, but unfortunatelly we couldn't run Egger's test on it.

5. DISCUSSION

5.1.Different mitochondrial functions in the synovia of RA and OA patients

This study provides quantitative clinical data on mitochondrial derangements in RA and OA. To be able to allocate our participants into our study groups adequately, the presence or lack of inflammatory states were confirmed by measuring pro-inflammatory cytokines in the synovial fluid samples of our patients. Additionally, histopathological evaluation and in vivo histology with CLSEM were also performed on tissue samples to detect the histological characteristics of RA and OA. Our results complied with the literature as RA patients had significantly higher levels of pro-inflammatory cytokines, hyperactive pathways of ROS production and displayed signs of chronic inflammation, neoangiogenesis and fibrosis by histopathology.

The capacities of the ETC complexes in the study groups were tested with HRR. Interestingly, C I activity was strongly diminished in the RA group, while there was no significant difference in C II activity between the groups. This result highlights the central role of C I in mitochondrial dysfunction in RA, setting it as the main target of future RA therapies. C I is the first, largest, and most complicated component of the respiratory chain. Furthermore, as the major entry point for electrons to the respiratory chain, C I is considered as the rate-limiting factor in overall respiration. Additionally, it generates significant levels of ROS, especially during reverse electron transport. Compared to patients suffering from RA, members of the OA group displayed a milder, but still notable decrease in C I activity compared to healthy individuals in the control group. Again, a significant deficit in C II activity did not occur.

5.2. Beneficial effects of EP delivery of diclofenac into the knee joint

This study has demonstrated the added value of EP to the transdermal delivery of diclofenac into the knee joint in experimental arthritis. The analgesic effect of EP-enhanced delivery was comparable to that of the oral administration and manifested in decreased nociceptive sensitivity, reduced joint swelling and lower cytokine concentration in the synovial fluid and lower inflammatory enzyme activities in the synovial tissue. The EP treatment also influenced the number of PMN–endothelial interactions at the level of the synovial microcirculation.

In accordance with literature data, our results revealed that EP causes higher concentration values in the serum as compared with the topical administration. The diclofenac concentration in the synovia was equal to the serum concentrations; however, conventional topical treatment did not result in a detectable amount of diclofenac sodium in the synovial fluid.

Microcirculatory changes were investigated with IVM, and like other non-selective cyclooxygenase inhibitors, diclofenac diminishes the number of PMN–endothelial interactions when. Sticking in the synovial vessels was considerably reduced with the oral administration of diclofenac. However, it was only moderately ameliorated by the EP-enhanced diclofenac hydrogel, and there were no changes in response to the simple topical application of the hydrogel.

Administration of C/K injection into the knee joint resulted in primary and secondary hyperalgesia in the inflammatory monoarthritis. Secondary hyperalgesia develops at the paw to heat and mechanical stimuli, and primary hyperalgesia is present over the inflamed knee joint. Secondary hyperalgesia reactions were investigated at the peak of the joint inflammation and both demonstrated a significant amelioration as a consequence of diclofenac treatment. Like the oral diclofenac treatment, EP completely restored thermal nociceptive sensitivity and increased mechanical touch sensitivity, but to a lesser extent. Knee swelling, which is an objective parameter of joint inflammation, was significantly reduced with both the oral and the EP-enhanced diclofenac treatment. Again, this increased volume was significantly reduced by both oral and EP-enhanced diclofenac treatments 48 h after arthritis induction.

Free radical formation has been demonstrated in the synovial fluid and synovial membrane under clinical conditions and proposed as a causative factor in joint disorders. Our present findings, demonstrated increases in XOR activity in response to arthritis attenuated by diclofenac treatment applied either in oral or simple topical routes of administration the latter being more accentuated in the case of EP. Data regarding the final step of PMN–endothelial interactions (i.e. sticking) were well correlated with the tissue accumulation of PMNs, as determined by MPO activity. Diclofenac treatment attenuated the MPO activity in the inflamed synovial tissue, applied either in oral or EP-enhanced topical routes of administration.

5.3. HA implants provide better fixation in TKA

This study reviews the current evidence and updates knowledge on the use of HA-coated tibial stem for primary TKA. The treatment groups were homogenous in terms of

characteristics of patients, thus the prediction of primary and secondary outcomes (i.e. MTPM and KSS and KFS) was likely independent from individual risk variables, patient selection or the overall severity of osteoporosis at prosthesis implantation. Direct meta-analysis comparison was made and the sample size of included trials was large enough to provide good evidence that HA-coating yields better stability than other, uncemented prostheses; However, cement fixation of prostheses stems still performs greater anchorage against migration. More importantly, the HA-coating is not outperformed by cemented prosthesis in providing good functional outcome with regards to pain intensity, range of motion and walking distance.

As a primary outcome of our study, the migration pattern of the prosthesis stems was determined as the maximum total point motion (MTPM) of the tibial stem measured by RSA. The MTPM value is the unit of measurement for the largest 3D migration of any point on the prosthesis surface. The migration pattern was defined as at least 2 postoperative follow-up moments within the first 2 years of follow-up. MTPM mainly depends on mechanical factors such as the bone-implant interface or different biological reactions at the implant-bone interface therefore is a reliable parameter to assess the added value of HA-coating in implant surface.

RCTs in our meta-analysis have demonstrated lower incidence of MTPM with HA–coated implants when compared other non-cemented stems, except one trial. As for the comparison of HA-coated cementless and cemented group, the overall rates of MTPM were very low in the cemented group and displayed lower incidence than HA-coated cementless prosthesis. Clinical outcomes, the KSS and KFS in our meta-analysis demonstrated equal functionality of HA-coated cementless and cemented implants. These scoring systems are validated and responsive methods for assessing objective and subjective outcomes after TKA. KSS is a weighted score which regards to pain intensity, range of motion, stability and flexion deformities, contractures and poor alignment. The KFS considers mobility parameters of the patient such as the walking distance and stair climbing with deduction for walking aids. In spite of the predictive value of radiological stability, a recent meta-analysis has revealed the differences between postoperative radiological and clinical performance of TKAs at the same time.

6. SUMMARY OF NEW FINDINGS

Our findings provided supportive data for the mitochondrial involvement in the pathomechanism of OA and RA. We have shown, for the first time that despite of similarities

in the clinical picture, the mitochondrial dysfunction substantially different in OA and RA. We have demonstrated the decisive role of Complex I and disruption of the ETC integrity in the inner mitochondrial membrane.

We have demonstrated improved drug delivery with EP in the conservative treatment of arthritis. We have confirmed the direct action of EP-enhanced transdermal diclofenac sodium delivery on the synovial microcirculation as proven by the decreased rolling and the reduced sticking of leukocytes. The biochemical measurements also demonstrated that diclofenac achieved an efficient tissue concentration in the synovium since inflammatory enzyme activities decreased. In summary, EP-enhanced transdermal diclofenac sodium delivery attenuated the microcirculatory deterioration and the consecutive stages of tissue inflammation; therefore, this type of mechanism might be an interesting focus for therapeutic strategies in arthritis.

Our review on implants outcomes provided the best available evidence that HA-coated cementless prosthesis outperforms other cementless prostheses both in respect to stability and functionality. Cemented fixation of prostheses provides the best stability in a 2-year follow up, however, functional results are not superior to HA-coated cementless fixation. Based on these results, HA-coated cementless TKA is a recommended option for treating end-stage arthritis of the knee, and clinicians consider together with patients the factors associated with the risk of revision when choosing the most appropriate procedure.

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