LITERATURE REVIEW

The analgesic mechanisms of EA and diclofenac

The acupuncture treatment in OA of knee gains its popularity recently although the scientific support for its efficacy is rare [28,43]. The study comparing the therapeutic outcome of acupuncture in OA of knee in treated versus no treatment control group demonstrated that acupuncture may have an analgesic effect and reduce analgesic drug consumption in patients waiting for anthroplastic surgery. In addition, 7 of 42 patients in acupuncture treated group respond so well that they do not want the operation [44]. However, other studies [45,46] showed that both real and sham acupuncture significantly reduce pain, stiffness, and physical disability in the OA of knee, but there are no significant differences between groups. The overall failure to find significant differences between true and sham acupuncture can be interpreted in a number of ways [43]. Three hypotheses have been proposed. Firstly, both treatments exert their effects through placebo factors. Secondly, sham acupuncture (wherever point locations) is as effective as real acupuncture. Lastly, differences exist between true and sham acupuncture but these can not be detected by the investigators. Even the first hypothesis cannot be entirely ruled out, but it appears unlikely because the pain relief rate from placebo often occur in approximately 30-35 % of pain patients while that from acupuncture is considerably higher (e.g., 55-85%) [47]. Distinguishing between the last two hypotheses is not easy. However, the fact that the results from different studies consistently show a trend in favour of true acupuncture may lend support to the third hypothesis.

Low frequency (2 Hz) EA is believed to exert its analgesic effect by stimulation of nerve fibers in acupuncture points, which send impulses to simultaneously affect centers in spinal cord, midbrain and hypothalamus-pituitary axis [47]. Low frequency EA acts on the spinal sites to increase enkephalin release and thereby blocks the incoming pain signals. The EA's stimulation of enkephalin release in midbrain leads to inhibition of spinal cord pain transmission via serotonin release from raphe descending system. Finally, EA acts on the hypothalamus-pituitary axis to release beta-endorphin into the

blood and CSF, the released beta-endorphin from pituitary gland is believed to affect the midbrain via pituitary-portal venous system.

The role of opioids concerning low frequency EA analgesia has been investigated in various experimental models. The acupuncture analgesia in human [40] and animal models [48] is reversible by naloxone, a specific opioid antagonist, indicating the participation of endogenous opioids in acupuncture analgesia. In animal models the role of spinal Methionine-enkephalin is believed to be involved in acupuncture analgesia at spinal level [24]. An increase in total endorphin content after EA is found in several brain regions. However, a positive correlation between the degree of analgesia induced and endorphin content is observed only in the midbrain area (e.g., periaqueductal gray) and septum accumbens. Microinjections of naloxone into the periaqueductal gray, habenula, septum, nucleus accumbens, raphe magnus nucleus, or amygdala show significant attenuation of acupuncture analgesia [27,40], whereas blockade of endogenous opioid peptide degradation by administration of bacitracin or D-amino acid enhances the acupuncture analgesia in animals [49]. These data suggest the role of opioid analgesic mechanism of acupuncture.

The central antinociception mediated through endogenous opioid mechanism is not specific to only acupuncture analgesia. Some centrally acting analgesics (e.g., morphine) is known to inhibit pain signals by stimulating opioid receptors [50]. Nevertheless, in addition to peripheral prostaglandin inhibition, some NSAIDs (e.g., diclofenac) exert their analgesic effect through central mechanisms [41]. The recently postulated mechanisms for central antinociception of NSAIDs are partly mediated via opioid mechanism [41,42], similar to the analgesic mechanisms of acupuncture mentioned above. In animal studies, systemic pretreatment with naloxone significantly reverse the antinociceptive effect of diclofenac [42] and ketorolac [51]. Moreover, the antinociceptive effect after local injections of diclofenac into the periaqueductal gray, raphe magnus nucleus can also be partially reversed by local injectioins of naloxone given at the same site [42]. Nonetheless, it is important to emphasize that naloxone does not reverse the antinociceptive effect of all NSAIDs, but it only reverses the effect of diclofenac, indomethacin, sodium salicylate and ketorolac [42,51]. In human experiment, naloxone can also inhibit the early phase of diclofenac induced analgesia in chronic pain patients [52]. An increase in plasma beta-endorphin concentrations is

demonstrated after administration of diclofenac in patients carrying a ventricular shunt [53]. It is not known whether the interaction of diclofenac with opioid mechanisms is of direct or indirect nature [42]. However, the ability of diclofenac to reduce the heroin withdrawal syndrome in human seems to implicate a direct pharmacological interaction [54,55].

Besides the antinociception mediated via opioid mechanism of both EA and diclofenac, each treatment may possess its own additional different analgesic mechanisms. EA may exert its additional analgesic effects through gate theory of pain control [56] or through changes in other central neurochemical substances (e.g., norepinephrine, acetylcholine, dopamine, glutamate, GABA, etc.)[40]. On the other hand, additional analgesic effect of diclofenac may be exerted via inhibition of both peripheral and central prostaglandin synthesis, inhibition of lipoxygenase pathway, central NMDA/excitatory amino acid mechanisms, serotonin mechanism, or interference with G-protein-mediated signal transduction [41,42]. These differences in additional mechanisms of action possibly contribute to the differences in the analgesic outcome between EA and diclofenac, and may contribute to some degree of additive effects when both treatments are combined.

The serum markers of cartilage metabolism in OA

Cartilage contains a relatively small number of cells which elaborate an abundant extracellular matrix. This matrix consists of two predominant components: collagen and proteoglycans, whereas collagen gives cartilage its strength and tensile properties, proteoglycans entrapped within the insoluble collagen network give the tissue its ability to undergo reversible deformation. The major proteoglycans present in mature articular cartilage consists of a core protein to which the glycosaminoglycans (GAGs), chondroitin sulphate (CS) and keratan sulphate (KS) are covalently attached [57]. Most of the KS and CS-containing proteoglycans in the mammalian body are found in cartilage [58,59]. The newly synthesized proteoglycan monomers interact extracellularly with hyaluronic acid (HA) and link protein to form proteoglycan aggregates which are firmly immobilized in the matrix and may reach molecular sizes in excess of 200,000 kDa. Proteoglycans are turned over within the tissue. Extracellular proteases degrade the macromolecules by fragmenting the core protein. These fragments diffuse out of

cartilage into synovial fluid and/or other body fluids, where further degradation may occur before they are eliminated from the circulation in the liver or through the kidney [58]. In addition, HA can also diffuse from the tissues and joint fluid to enter the circulation via lymphatic system and rapidly cleared from the blood mainly as the result of its uptake by receptors on hepatic endothelial cells [60].

In the early stages of OA, the turnover of proteoglycans may be enhanced [61]. Eventhough the biosynthesis of proteoglycans in OA cartilage may be increased, there is a net depletion of these molecules from the extracellular matrix due to excessive catabolism. The decline in proteoglycan levels in cartilage reduce its resilience [62] and ability to absorb imposed mechanical stress. With time, these mechanical stresses may disrupt the subchondral and cartilaginous matrix further leading to the pathological manifestation of OA. In general, the radiological evidence of loss of cartilage from OA joints, and clinical symptoms, generally do not appear until the disease is well advanced. Thus, a method allowing early detection of the loss of proteoglycans from articular cartilage would therefore be of considerable diagnostic value to identify those individuals at risk and could also be useful in monitoring patient response to various treatments [63]. Since the damage of cartilaginous matrix results in the release of its components (GAGs and HA) into the synovial fluid and subsequently into the blood, the levels of GAGs and HA in synovial fluid and serum may therefore be used as the markers of cartilage metabolism [64]. The quantitative measurements of cartilage proteoglycans and their fragments, can be performed by using monoclonal antibodies that recognize structural or functional domains of proteoglycan monomers in serum and other biological fluids by using the technics of radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA).

The HA content of OA cartilage is reported to be lower than in normal cartilage [65]. In a canine model of OA induced by medial meniscectomy, a reduced level of HA is found in femoral head cartilages eventhough proteoglycans in these tissues are normal [66]. The loss of HA from cartilage may therefore represent an early event in the pathogenesis of OA. In addition, moderately increased serum levels of HA have also been observed in patients with OA, especially in the patients with OA of knee or OA of a generalized type [67]. However, the elevated serum levels of HA can also be found in malignancies (e.g., sarcomas, lymphomas, breast cancer, brain tumors,

bronchial carcinomas, etc.), other inflammatory diseases (e.g., rheumatoid arthritis and systemic scleroderma), liver diseases (e.g., cirrhosis, alcoholic liver disease, viral hepatitis, etc.) and chronic renal failure [68]. Serum HA concentrations are also affected by physical activity and circadian variations. Thus, the serum levels of HA may be the valid marker of cartilage metabolism if determined in the OA patients who are free from malignancies and other inflammatory, liver and kidney diseases. If the serum HA is used in order to monitor response to OA treatment, the time for blood sample collection and the patient's activity state between before and after treatment should be the same.

Changes in chondrocyte metabolism in OA produce compositional changes in the newly synthesized proteoglycans that occur in an attempt to remodel or repair the tissue in response to its altered mechanical environment. The altered synthetic activity can result in the expression of epitopes that are unexpressed or minimally expressed in normal adult articular cartilage but highly expressed in growth cartilage of young animals [61]. The monoclonal antibody 3-B-3 [64,69] recognizes an epitope denoted as 3-B-3(+) that consisting of a nonreducing terminal unsaturated glucuronic acid residue adjacent to N-acetylgalactosamine-6-sulphate after prior treatment of the CS-containing proteoglycans with chondroitinase ABC. However, this antibody also recognizes a native epitope denoted as 3-B-3(-) that containing a saturated glucuronic acid residue at the non-reducing terminal that occurs in CS chains of proteoglycans isolated from osteoarthritic cartilage. The expression of the 3-B-3(-) epitope in normal human cartilage and synovial fluid of normal knee is very low, but is greatly increased in those of osteoarthritic knee [69]. These data suggest that the 3-B-3(-) epitope may be a marker for the altered CS. However, previous study reported that the serum concentrations of 3-B-3(+) epitope in patients with OA of knee are significantly higher than those in healthy subjects [70], suggesting a greater turnover of joint tissue chondroitin 6-sulphate proteoglycans in OA. Similar to 3-B-3(+) epitope, the serum concentrations of W-F-6 epitope, a native epitope associated with 6-sulphated isomers of CS, in patients with OA of knee are significantly higher than those in healthy subjects [70], This may reflect the increased expression of the W-F-6 epitope in OA, or its preferential release from osteoarthritic joint(s) into serum, or both.

It is hypothesized that if the joint recovers well following injury and successfully adapts to resume good function, then the chondrocytes response would cease and the expression of epitopes would return to normal [69]. Thus, if any treatments benefit the chondrocyte metabolism, the concentrations of cartilage markers (e.g., HA as well as 3-B-3(+) and WF6 epitopes) in synovial fluid and serum would be normalized towards the value reported in normal subjects.