Relationship between Disease Activity and Circulating Level of Collagen II C-Telopeptide Fragments in Papain Induced Osteoarthritis Rat Model

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ARTICLE HISTORY

Received: December 09, 2012
Revised: September 05, 2013
Accepted: September 11, 2013

ABSTRACT

Osteoarthritis (OA) is a progressive degeneration of articular cartilage leading to failure in functional mobility of joints. It is characterized by morphological, biochemical and molecular changes in histology of cartilage. Different biological markers are used as indicators to precisely predict the stage of cartilage destruction of joints in OA patients and to evaluate the therapeutic efficacy of drugs used for OA. The present research was chalked out to establish relationship between disease activity and serum level of C-terminal telopeptide of type II collagen (CTX-II) in experimentally induced OA rat model. Out of 30 male Wistar rats, 25 were used to induce OA by injecting papain (10mg/0.5mL of 0.05M sodium acetate) in right knee joints whereas five (control) were injected with sterile normal saline solution on day 0. Blood samples (5mL each) were collected on weekly basis up to 28th days of post papain injection. Sera were separated and subjected to perform ELISA for estimating CTX-II fragments as cartilage biomarker (CartiLaps ® ELISA kit) in experimental groups. Maximum level of CTX-II (pg/mL) (40.44±3.07) was observed in sera samples of day 14 post papain injection followed by days 21 (40.22±2.01), 28 (36.82±3.81), 7 (34.48±4.17), 1 (15.08 ±4.22) and day 0 (2.55±0.10). The early changes in serum CTX-II from day 0 to 14 showed significant association with cartilage damage. Later on, no significant difference was observed in CTX-II level on day 14, 21 and 28 post papain injection. It is concluded that elevation in serum CTX-II level was concomitant with the onset of disease and degradation of cartilage. Moreover, CTX-II is a sensitive diagnostic biomarker to monitor joint disorder severity in papain induced OA rat experimental model on different days. These findings may be used as base line for early diagnosis of disease and initiation of therapy for successful outcome.

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Key words: Articular cartilage
CTX-II
ELISA
Osteoarthritis
Papain

INTRODUCTION

Osteoarthritis is a chronic disorder of joints characterized by degenerative changes of articular cartilage consequently disturbing biological metabolism of cartilage and underlying bone (Goldring and Goldring, 2010; Pinna et al., 2013). Major moiety of cartilage is aggrecan composed of glycosaminoglycan, chondroitin sulphate and keratin sulphate (Sharma et al., 2013; Khan et al., 2013). Due to wear and tear of cartilage in OA, the metabolic fragments first come in blood and then in urine as degradation products thus may serve as biomarkers under pathological conditions (Bay-Jensen et al., 2011; Takahashi et al., 2012). Cartilage degradation biomarkers including types I, II and III collagen and aggrecan synthesis (CPII and CS 846) can be used to check the extent of destruction (Svoboda et al., 2013). Biomarkers of inflammation including interleukins, tumor necrosis factor, growth transforming factor and eosinophilic proteins are used to check the level of OA (Otterness et al., 2000).

Early diagnosis of cartilage destruction play key role in efficient therapy and favorable prognosis. The surrogate biological markers may also be used to evaluate the ability of agents involved in structure modification (Felson and Lohmander, 2009). Costly techniques like arthroscopy, magnetic resonance imaging (MRI) and computational topography (CT) require anesthesia prior to
examination of affected joints. At early stages of OA, biomarker CTX-II is considered most reliable for accurate joint destruction (Gamero et al., 2003).

Blood and urine levels of biomarkers are directly related with the severity of cartilage destruction (Goode et al., 2012). In OA cartilage destruction bone turnover markers decrease and serum COMP and urine CTX-II increase significantly. Increase in bio-molecules of synovial destruction is directly related with the extent of cartilaginous tissue damage. The concentration of bio-molecules can efficiently be determined using ELISA both in serum (Bay-Jensen et al., 2011) and urine of different animals (Takahashi et al., 2012). So, bio-molecules of joint tissues destruction may be used to determine severity of OA (Naito et al., 2010). In focus research was carried out to observe the relationship of CTX-II levels with extent of OA induced by injecting papain in experimental Wistar rats. CTX-II level can be estimated to determine the disease progression and efficacy of anti-osteoarthritis therapy.

**MATERIALS AND METHODS**

Male Wistar rats (n=30) weighing 150-200g were selected. Feed and water were provided *ad-libitum*. Guidelines provided by International Association for the Study of Pain (IASP) was followed during experimental trials. To develop OA, 10mg papain in buffered solution (0.05M sodium acetate, pH 4.5) was injected intrarticularly (Murat et al., 2007). Five rats (n=5) were injected with 0.5 mL of normal saline (0.9%) in right knee joint which served as control group.

A volume of 5 mL blood was collected without anticoagulant on each sampling day, i.e., 0, 1, 7, 14, 21 and 28. Sera was collected and stored at -18°C till further use. CTX-II was estimated using serum preclinical CartiLaps® ELISA kit (Immunodiagnostic system, UK. cat # AC-081) as described by Garvican et al. (2010) using 1.2 pg/mL detection limit. To evaluate the accuracy of ELISA kit, concentration of standards was determined from their respective absorbance values (0.122, 0.296, 0.329, 0.403, 0.542 and 0.847) at wavelength of A450/650.

Quantitative data for CTX-II were analyzed statistically and expressed as mean±SD. Differences between groups were tabulated using one way ANOVA applying DMR using SPSS software, version 13.0 at probability level of 0.05.

**RESULTS AND DISCUSSION**

Level of C-telopeptides terminal of type II collagen (CTX-II) in sera samples of normal (day zero) and OA (days 1, 7, 14, 21 and 28) groups was measured from pre calibrated kit. Highest concentration of CTX-II was recorded on 14th day post papain injection followed by 21st, 28th, 7th and 1st. The lowest value was observed in control group. Differences in CTX-II concentrations on days 14, 21 and 28 were not significant statistically. Concentration of CTX-II in sera samples of 7th day was close to the 28th day. Concentrations in sera samples on 1st day and of normal group were lowest as compared with other days. All OA groups differ significantly from control group (Table 1).

![Fig. 1: Standard curve of CTX II by ELISA (Absorbance Vs Concentration)](image)

Rise in concentration of CTX-II with progression of OA indicated that this biomarker may be related to the extent of OA.

Many types of cleaved CTX-II fragments are considered to be the potential biomarkers of cartilage biosynthesis or breakdown. These included pyridinoline (PYD), triple helix fragments, CTX-II, core protein fragments, N and C propeptides (Gamero et al., 2002). Relationship of biological markers with the destruction of cartilaginous tissues played key role in the development of highly sensitive assays (Christgau et al., 2001). Bleasel et al. (1999) observed and reported the relationship of degradation molecules with the severity of joint disorders.

Relation between degradation of cartilage with raised levels of CTX-II in sera of OA patients has been used as
biomarker by a number of researchers. Garnero et al. (2001) determined relationship between bone, cartilage and synovial markers with severity of joint destruction. It was concluded that all bone turnover markers decreased in OA patients compared with healthy controls whereas markers of cartilage turnover were significantly increased. Measurement of CTX-II concentration in OA rat sera carried out in present study was in accord with the biomarker of collagen degradation used by Garnero et al. (2003). Svoboda et al. (2013) used collagen types I and II and aggrecan synthesis molecules to detect early cartilage degenerative changes in sera of young athletes.

In another study, urinary CTX-II and free deoxyypyridinoline (DPD) levels in patients with rapidly destructive and slowly progressive hip OA were investigated. Increased CTX-II levels were associated with rapid destructive OA (Garnero et al., 2003). Raised levels of biomarker in serum indicated subsequent structural changes in knee joint in present study. These findings are in corroboration with clinical investigations reported by Garnero et al. (2002). Garnero et al. (2001) related CTX-II levels with radiological assessment of damage to knee joint cartilage. Urinary CTX-II has been used as biomarker in diagnosis and quantitative degradation of cartilage by Mazieres et al. (2006) and Meulenberg et al. (2007). This biomarker has been included to evaluate histological grading of localized affected joints and hypertrophic OA (Lorenz et al., 2005). Conrozier et al. (2012) used CTX-II as indicator biomarker in serum and urine to evaluate the effects of hyaluronic acid in knee OA patients.

In agreement with present study, circulating levels of CTX-II determined were consistent with previous investigations of urinary CTX-II levels in rats (Ishikawa et al., 2004). Raised CTX-II concentration in sera of rats was significant at early stages of joint inflammation (Oestergaard et al., 2006). The diagnostic value of serum CTX-II at early stage of cartilage degradation due to joint inflammation was evaluated by Oestergaard et al. (2006). The predictive importance of CTX-II molecular marker for cartilage destruction has been undertaken in other models of destructive arthritis (Hoegh-Andersen, 2004) and arthritis induced by adjuvant (De Cueninck et al., 2003). CTX-II was used as an early indication of joint inflammation in collagen induced arthritis and was a reflection of progressive cartilage degradation (Oestergaard et al., 2006). Significantly higher levels of CTX-II were observed by Matyas et al. (2004) in sera of OA dogs than normal on surgical induction. Elevated levels of CTX-II persisted in sera of rats for long period in present study which is in agreement with the findings of Goranov (2007) in human OA.

In contrast results has been reported in cases under natural conditions (Hayashi et al., 2009). Higher concentrations of CTX-II were observed in serum of horses at early stage of OA (Frisbie et al., 2008). Similar studies were undertaken in sheep following injury to cartilage by Lu et al. (2006) and in guinea pigs by Huebner and Kraus (2006).

ELISA is a reliable and sensitive tool to measure the type II collagen molecules of cartilage destruction for the assessment of OA in various samples of animals (Takahashi et al., 2012). Bay-Jensen et al. (2011) used ELISA to determine the concentration of type II collagen in serum samples and related the levels with degenerative joint diseases. Similarly, type III collagen biomarkers were measured in urine samples by ELISA and related with the progression of cartilage degradation by Barascuk et al. (2011).

In view of present study along with previous research work, maximum CTX-II concentration was observed in sera of OA rats on 14th day post papain injection that has no significant difference with 21st and 28th CTX-II levels. So the treatments may be started from any of the three days post injection to assess the therapeutic efficacy of drugs in this rodent model, moreover if correlated with histological findings of Murat et al. (2007), the starting point of disease may be 28th day of post papain injection in rodent model.

In conclusion, it is demonstrated that measurement of serum CTX-II provides a useful estimate of disease progression in relation to time. Based on these findings, it seems reasonable to use CTX-II biomarker to monitor the effects of chondroprotective drugs in papain induced OA model.

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