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EFFECTS OF ACUTE SYNOVITIS EXPERIMENTALLY INDUCED BY AMPHOTERICIN-B ON THE BIOMARKERS OF CAMEL JOINT STRUCTURES

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ABSTRACT

The major objective of this study was to evaluate the effects of acute synovitis on the joint structures (bone, articular cartilage and synovial membrane) through the evaluation of biomarkers of bone, cartilage and synovial membrane in dromedary camels. Acute synovitis was experimentally induced by injecting amphotericin-B (20 mg in 4 ml sterilised distilled water) in the intercarpal joints of eight dromedary camels (treatment group) and other eight camels were used as a control group. Synovial fluid samples were collected prior to injection and 7, 14, 21 and 28 days post injection. Inflammatory biomarkers (Prostaglandin E_2) and biomarkers of bone (bone alkaline phosphatase, osteocalcin and pyridinum cross-links) and cartilage (Chondroitin sulfate 846 epitope, C-terminal propeptide of type II procollagen, sulfated glycosaminoglycans and hyaluronan) were determined in the synovial fluid samples. The results showed significant elevations in the inflammatory, articular cartilage and bone biomarkers in the synovial fluid from the treatment group compared to the controls. Elevations of bone resorption and formation biomarkers were late in compare to cartilage biomarkers. This study proved that amphotericin B is an appropriate drug to induce synovitis in the camel joints when injected intra-articular. According to the results of biomarker analysis, it can be concluded that cartilage degradation proceeds bone degradation in camel joint with synovitis. Within articular cartilage tissue, proteoglycan synthesis proceeds type II collagen formation.

Key words: Amphotericin-B, biomarkers, camel, joint, synovitis

Several biomarkers of bone formation and resorption have been assessed with various conditions in animals (Al-Sobayil, 2008; Tharwat *et al,* 2014; Tharwat and Al-Sobayil, 2015; Tharwat and Al-Sobayil, 2018a,b; Tharwat, 2020; Tharwat and Al-Sobayil, 2020a,b). Osteocalcin, bone alkaline phosphatase (BAP) are common examples of bone formation biomarkers. Total alkaline phosphatase (TAP) is a membrane-bound protein with enzymatic activity in hydrolysing phosphate esters. BAP is a bone formation biomarker that represents approximately 18% of total alkaline phosphatase in adult horses (Hank *et al,* 1993). With arthritic horses, BAP significantly increased with a positive correlation between the degree of articular cartilage degradation and its level in synovial fluid (Fuller *et al,* 2001). Osteocalcin is a noncollagenous protein found in bone and secreted by osteoblasts (Lian *et al,* 1985). Therefore, it is considered a specific osteoblastic biomarker produced during bone formation. With arthritis, osteocalcin levels in synovial fluid may increase (Gevers *et al,* 1988) or decrease (Garnero *et al,* 2001). The circadian rhythm of bone formation biomarkers including osteocalcin and BAP has been determined in serum of dromedary camels. Although BAP and osteocalcin are considered bone formation biomarkers, the correlation between them in camels is weak (Al-Sobayil, 2010).

In cartilage and bone, pyridinium cross-links including PYD and DPD are derived from hydroxylysine residues within the mature collagen molecule; they are considered the most promising bone resorption biomarkers (Tanimoto *et al,* 2004). DPD is found in high concentrations in collagen of bone, making it a potentially specific biomarker for bone resorption. However, patients with severe or end-stage of osteoarthritis had higher excretion of PYD compared to those with early osteoarthritis (Hellio le Graverand *et al,* 1996).

The most newly synthesised aggrecan molecules contain terminal chondroitin sulfate chains that have an epitope called CS846. CPII refers to the

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C-terminal propeptide of type II procollagen. Several studies have reported significant increase in the levels of CS486 and CPII in joints with arthritis compared to controls (Rizkalla *et al,* 1992; Nelson *et al,* 1998; Frisbie *et al,* 1999 and 2008). Sulfated glycosaminoglycans (SGAG) and hyaluronan (HA) are major biomarkers that reflect the degree of articular cartilage degradation. In addition, HA is considered a valuable biomarker for synovial membrane. It has been reported that the concentration of SGAG in synovial fluid from osteoarthritic horses was generally higher than its level compared to the controls (Frisbie *et al,* 2008). With osteoarthritis, HA levels significantly increase in human and animal joints (Bruyere *et al,* 2003; Taylor *et al,* 2006). In dromedary camels, the concentrations of SGAG and HA have been determined in normal synovial fluid (Al-Sobayil, 1997).

Prostaglandin E_2 (PGE_2) is considered one of the inflammatory mediators and causes proteoglycan depletion in the cartilage. May *et al* (1994) have assessed the concentration of PGE_2 in synovial fluid from normal and diseased joints of horses. The concentration of PGE_2 is significantly higher in arthritic joints with compared with controls (May *et al,* 1994; Spiers *et al,* 1994). The total concentrations of PGE2 in healthy dromedary camels have been recorded (Al-Sobayil, 1997).

Recently, various advanced techniques have been used to diagnose arthritis in early stages by assessing levels of joint tissue biomarkers. The aim of this study was to determine the effects of induced synovitis (by intra-articular injection of amphotericin-B) in the intercarpal joints of dromedary camels on the joint components measured by changes in levels of bone, cartilage and synovium biomarkers.

Materials and Methods

Camels

Sixteen apparently healthy adult female dromedary camels (aged 6-7 years) with normal haematobiochemistry, were used in this study. The carpal joints were ensured clinically and radiographically normal camels. The camels were randomly assigned into either treatment (n=8 camels) or control (n=8 camels) groups. The camels were fed alfalfa hay and water was given *ad lib*.

Clinical experiment

Each camel was secured in a sitting position and sedated with xylazine (0.2 mg/kg IV, Rompun 2%, Bayer Health Care, Monheim, Germany). The left carpus was aseptically prepared. The left intercarpal joints (8 joints) in the treatment group were aseptically injected with amphotericin-B (20 mg in 4 ml of sterile distal water). The left intercarpal joints of the animals in the control group (8 joints) were injected with only sterile distal water (4 ml) following the same manner as in the treatment group. Four mL of synovial fluid samples were collected from the left intercarpal joint of each camel on days 0 (prior to injection), 7, 14, 21 and 28 (i.e. 0, 1, 2, 3, 4 weeks). The synovial fluid samples were placed in plain vacutainer tube and then centrifuged at 3000 rpm for 10 minutes and the supernatant fluid was then aliquotted in a tube and immediately stored (for biomarker analysis) at -70°C.

Biomarker assays

Concentrations of osteocalcin and BAP were assessed using EIA Kit (Quidel Corporation, San Diego, CA, USA), which has been validated as biomarkers of bone formation (Lepage *et al,* 1990; Gomez *et al,* 1995). Concentrations of PYD and DPD were estimated using EIA Kit (Quidel Corporation, San Diego, CA, USA), which has been validated as markers of bone resorption (Visor *et al,* 1996; Weitz *et al,* 1999). Synovial fluid concentrations of the epitope CS846 and CPII were measured by a commercial ELISA kit (IBEX Diagnostics, Montreal, Quebec, Canada) as biomarkers of aggrecan and type II collagen synthesis, respectively (Rizkalla *et al,* 1992; Poole *et al,* 1994 and 2001; Nelson *et al,* 1998). A modified 1, 9-dimethylmethylene blue dye-binding assay was used on papain digested samples to determine SGAG concentration as a biomarker of cartilage matrix degradation (Farndale *et al,* 1986). Concentration of HA was estimated using ELISA kit (TECO medical Group, Sissach, Switzerland) and has been validated as a biomarker of cartilage matrix degradation. Concentration of PGE2 was extracted from synovial fluid and then assessed by using a commercially available high-sensitivity enzyme immunoassay kit (PGE2 ELISA, Assay Design, Ann Arbor, MI, USA) (May *et al,* 1994).

Statistical analysis

The data were statistically analysed using linear mixed model for repeated measures to evaluate the dependent variables that pertained to synovial fluid samples. The Duncan test was used to calculate multiple comparisons. The significance level was set at $P<0.05$. SPSS statistical package (2009) was used to perform all statistical calculations and statistical analysis.

Results

As expected, amphotericin-B injection resulted in a marked lameness and distention in the carpal joint. The lameness started 12 h after injection of amphotericin-B and continued through 5 days, resolving in all camels by day 10. Lameness was not seen in camels of control group. Fig 1 A and B shows the concentrations of BAP and osteocalcin in synovial fluid samples obtained from camels of both groups. A significant increase was seen in the levels of BAP at week 4 in treatment group compared to control group. The concentration of osteocalcin did not change in both groups during the time of study. Fig 1 C and D shows the levels of PYD and DPD biomarkers in the synovial samples of both the control and treatment camels. The levels of PYD significantly increased in treatment group at weeks 3 and 4 in the treatment group compared to the control group (Fig 1 C). The significant elevation of DPD was seen at week 4 in treatment group compared to the control (Fig 1 D).

Fig 2 A and B shows the levels of CS846 and CPII in synovial fluid from treatment and control groups. The concentrations of CS846 significantly increased in treatment group starting from week 2 until the end of the study. The levels of CPII significantly increased in treatment group compared to control group in the last two weeks of this study. The concentrations of SGAG and HA in synovial fluid of both groups are shown in Fig 2 (C and D). The levels of SGAG and HA were higher in treatment group compared to control group starting from week 1 until the end of the study. The levels of PGE2 significantly increased in the treatment group at weeks 1, 2 and 3 (Fig 2 E).

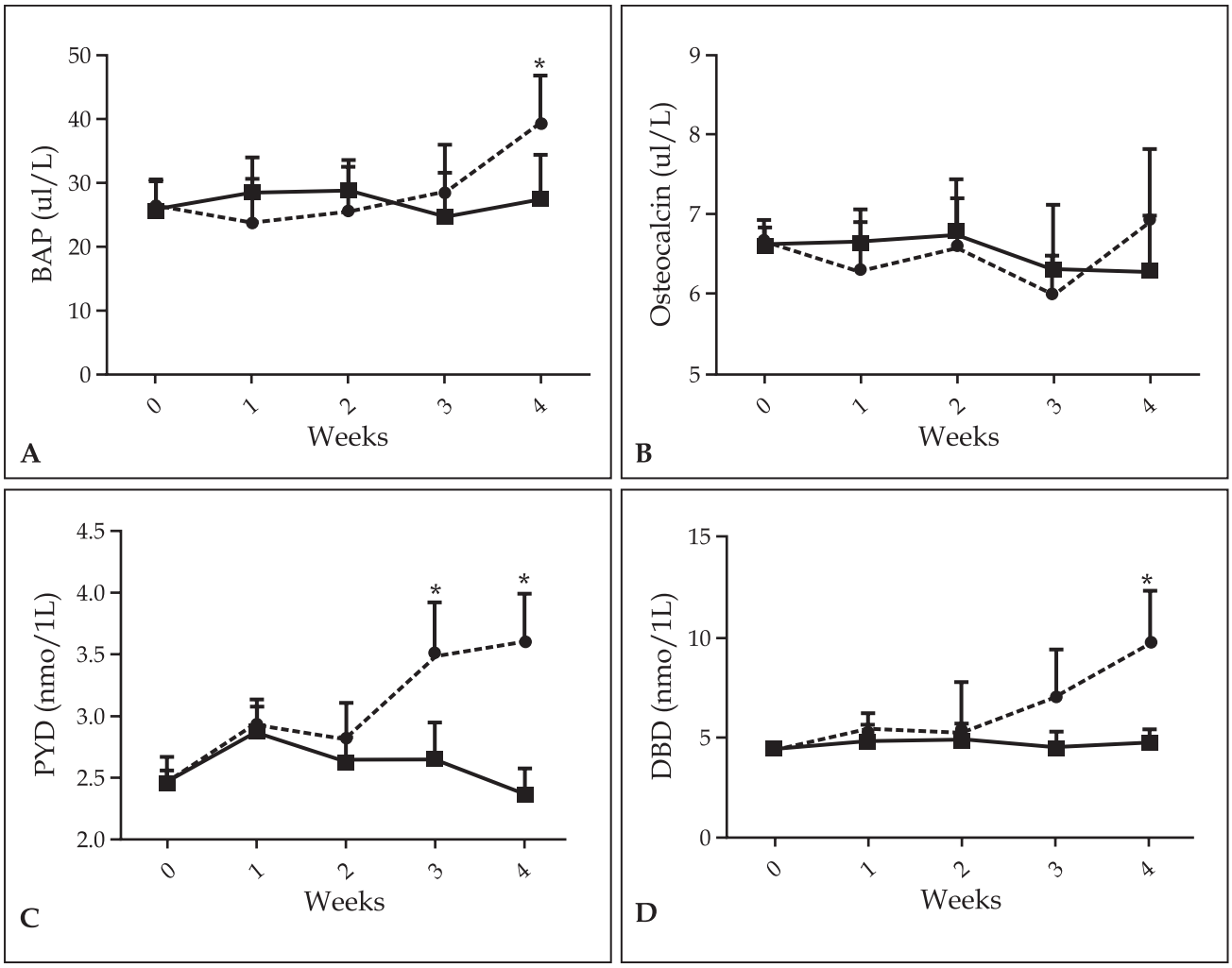

Fig 1. Concentrations of BAP (**A**), Osteocalcin (**B**), PYD (**C**) and DBD (**D**) in the synovial fluid of camels in both treatment (dotted) and control groups during weeks 0, 1, 2, 3 and 4. *Values in the treatment group significantly different from those in the control group at same time. BAP=bone alkaline phosphatase; PYD=pyridinoline; DPD=deoxypyridinoline.

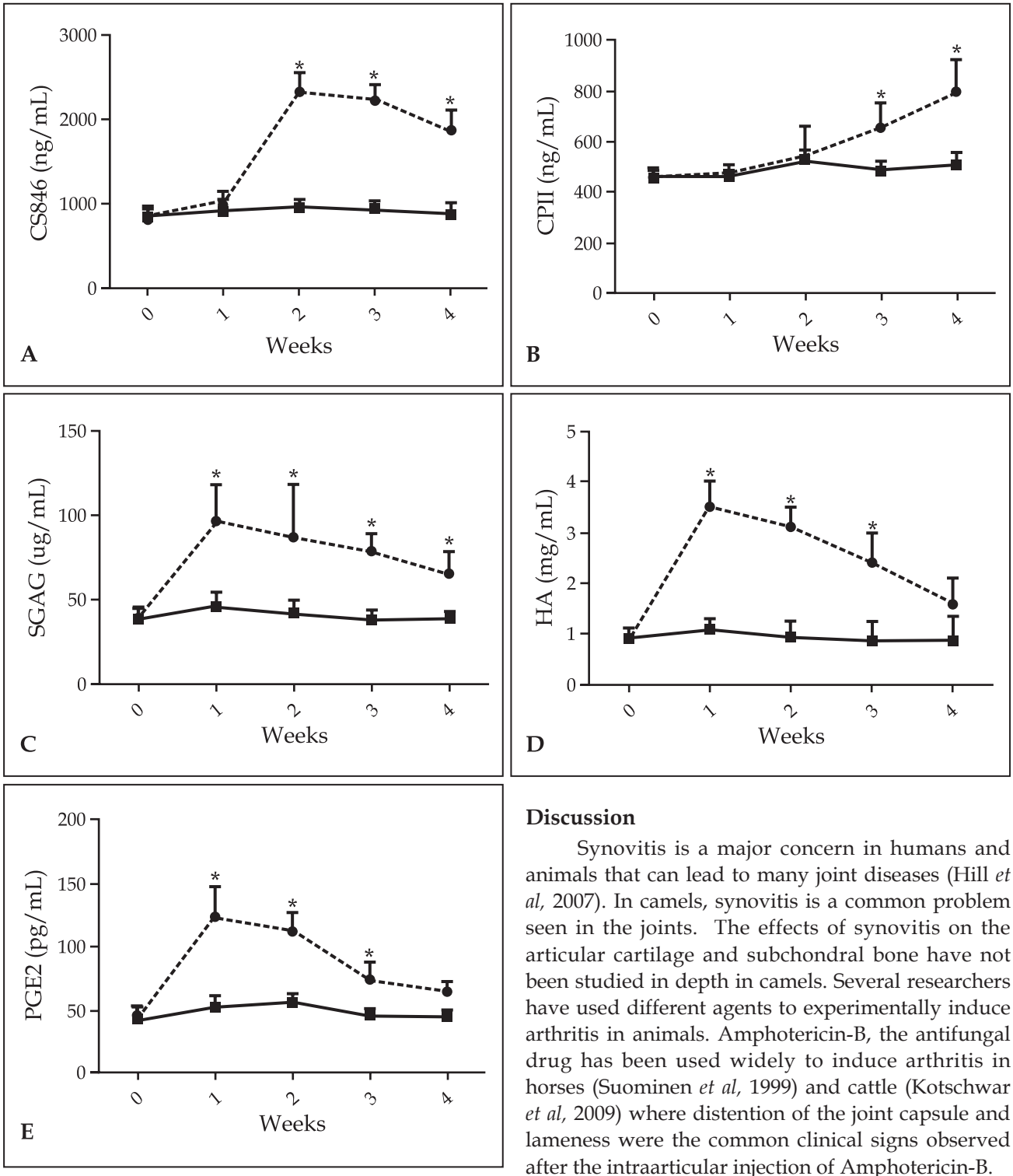

Fig 2. Concentrations of CS846 (**A**), CPII (**B**), GAG (**C**), HA (**D**) and PGE2 (**E**) in the synovial fluid of camels in both treatment (dotted) and control groups during weeks 0, 1, 2, 3 and 4. *Values in the treatment group significantly different from those in the control group at same time. CS846=chondroitin sulfate 846 epitope; CPII=C-terminal propeptide of type II procollagen; SGAG=sulfated glycosaminoglycans; HA=hyaluronan; PGE2=prostaglandin E2.

Discussion

Synovitis is a major concern in humans and animals that can lead to many joint diseases (Hill *et al,* 2007). In camels, synovitis is a common problem seen in the joints. The effects of synovitis on the articular cartilage and subchondral bone have not been studied in depth in camels. Several researchers have used different agents to experimentally induce arthritis in animals. Amphotericin-B, the antifungal drug has been used widely to induce arthritis in horses (Suominen *et al,* 1999) and cattle (Kotschwar *et al,* 2009) where distention of the joint capsule and lameness were the common clinical signs observed after the intraarticular injection of Amphotericin-B.

This study proved that the intraarticular injection of amphotericin B could be used as a model of synovitis in the camels. This study determined the effects of experimentally induced synovitis by intra-articular injection of amphotericin-B on the concentrations of bone, cartilage and inflammatory biomarkers. PGE2 is a common biomarker that has

been used as index of the level of synovitis (Frisbie *et al,* 2008). In the present study, the levels of PGE2 increased in the treatment group at weeks 1, 2 and 3 compared to the control group. This indicated that intra-articular injection of amphotericin-B induced synovitis in the intercarpal joint of camels that was significant during weeks 1, 2 and 3 post injection of amphotericin-B.

The levels of PYD significantly increased in treatment group at weeks 3 and 4. The significant elevation of DPD was seen at week 4. DPD is specific biomarkers for bone resorption because it is found in high concentrations in collagen of bone. PYD is the major cross-link found in collagens of all connective tissues including bone and cartilage. Therefore, the reason for early elevation of PYD compared to DPD might be due to the degradation occurred first in cartilage and other connective tissues (e.g. intraarticular ligaments) and then in the bone.

A significant increase was seen in the levels of BAP at week 4 in treatment group compared to control group. This indicated that bone degradation due to joint inflammation might enhance bone formation. On the other hand, the concentration of osteocalcin did not change significantly in both groups. Both osteocalcin and BAP are produced by osteoblast. However, the two biomarkers reflect different stages of osteoblast function (Bowles *et al,* 1996). BAP is secreted at early bone formation whereas osteocalcin is secreted later during bone mineralisation. Therefore, this might explain the unchanged osteocalcin concentration and at the same time a significant increase in BAP in the treatment group. Studies on camels showed that the correlation between osteocalcin and BAP is weak even though they are considered bone formation biomarkers (Al-Sobayil, 2010).

The levels of CPII significantly increased in treatment group compared to control group in the last two weeks of this study. The concentrations of CS846 significantly increased in treatment group starting from week 2 until the end of the study. The extracellular matrix of hyaline articular cartilage tissue composites of proteoglycan (i.e. SGAG and HA) and type II collagen. It has been reported that proteoglycan content of the cartilage matrix decreases in inflammed joints (Takafuji *et al,* 2002). According to this result, it might be expected that synthesis of proteoglycan in the degraded extracellular matrix of articular cartilage precedes the formation of type II collagen. The levels of SGAG and HA were higher in treatment group compared to control group starting from week 1 until the end of the study. This indicated that joint inflammation causes articular cartilage degradation.

From this study, it can be concluded that amphotericin B is an appropriate drug to induce synovitis in the camel joints when injected intra-articular. Cartilage degradation proceeds bone degradation in camel joint with synovitis. Within articular cartilage tissue, proteoglycan degradation proceeds collagen turnover. Similarly, it is expected that proteoglycan synthesis proceeds type II collagen formation during repairing of degraded articular cartilage repairing.

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