The effect of Intra-articular injection of amphotericin B on inducing arthritis in Equines

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Abstract: This study was intended to verify the clinical effect of intra-articular (IA) amphotericin B injection in the carpal joints of equines to induce arthritic changes and to correlate it with the histopathological findings. Materials and methods: Two ml Amphotericin B (10mg) were injected IA in the radio-carpal joints of the equines. Animals were grouped into three groups based on the observation period (3, 6 and 9 weeks) after injection to achieve different degrees of arthritis. Clinical assessment, synovial fluid analysis, radiologically and histopathological examinations were done. Results: Arthritic changes were observed in the 3 groups. Acute inflammatory reaction developed 3 hours post injection characterized by lameness and swelling of the joint, significant increase in the total protein, LDH, ALP and AST with increase in monocytes, neutrophiles and mucin precipitation. The macroscopic findings were limited to the discoloration of the articular cartilage, some hemorrhagic areas in the joint capsule and slight roughness of the articular surface in the sever cases. Radiographic arthritic evidences were synovial capsule swelling in acute cases and narrowing of the joint space in chronic ones. Histopathological changes included degenerative changes in the cartilage and bone in addition to synovitis and capsulitis in the form of loss of cartilage matrix architecture, fibrous replacement of cartilage, osteophyte formation, cartilage loss and thinning. Conclusion: Different degrees of persistent arthritis could be achieved with a single IA injection of amphotericin Bin Equines which could be used as experimental animal model for testing recent treatment techniques.

Keywords: amphotericin B, arthritis, equine, synovial analysis, histopathological examination, Mankin score

Introduction

Arthritis is a self-perpetuating, progressive joint disease (Johnson, et al., 1994 and Holderbaum et al., 2005) and is one of the most commonly orthopedic problems, both in human and animals. It is a complex series of events associated with number of risk factors including mechanical, biochemical and genetic factors (Boileau et al., 2004 and Mandelbaum et al., 2005).

Difficulties in studying osteoarthritis (OA) arose from the fact that this disease occurs naturally with fairly slow progression and hence the period for treatment testing is long. However, the pathology and pathogenesis are similar to those occurring in the most common forms of human and animal (Bendele, et al., 1999). Also, the low sensitivity of diagnostic tools and the low availability of diseased tissues explain why research on
animal models remains highly dynamic (Ameye et al., 2006) and have had contributed to the understanding of basic mechanisms of joint disease (van den Berg, 2009).

Animal models of OA commonly used in testing are generally either naturally occurring in aging animals or surgically induced models (Bendele et al., 1999 and van den Berg, 2001). Other animal models of OA include genetically modified mice, as well as enzymatically or chemically induced models (van den Berg, 2001).

Over the past century, several methods for chemical induction of OA have been described. Axhausen (1910) and Burch-Hardt (1924) used an application of tincture of iodine or ammonium hydroxide. Seeliger (1926) and Key (1932) used IA injection of HCl. Weissman et al. (1965) induced acute and chronic arthritis by streptolysine. Recently, Edwards et al. (1977), Bowman et al. (1983) and Fahmy et al. (1994) used amphotericin-B for induction of OA. Lameness was induced in horses by injecting the navicular bursa with 5 mg of amphotericin-B (Pleasant et al., 1997). Kotschwar et al. (2009) used amphotericin-B to induce transient and effective model for lameness with synovitis-arthritis in Cattles and which Crawford et al. (1991) adopted as a model to study the effect of pulsed radio frequency therapy (PRFT) on arthritis.

Amphotericin B is an antifungal polyene antibiotic obtained from a strain of Streptomyces nodosus. The drug acts by binding to sterols in the cell membrane of susceptible fungi with a resultant change in membrane permeability allowing leakage of intracellular components. Mammalian cell membranes also contain sterols and it has been suggested that the damage to human cells and fungal cells may share common mechanisms (John, 2000).

The aim of this study is in two folds: 1) to assess the effect of amphotericin B in inducing different degrees of arthritis and to correlate the clinical findings with the radiological and histopathological ones; 2) establishment of animal models of arthritis to be widely used as tools to evaluate and improve various diagnostic and therapeutic techniques.

**Materials and methods**

All experimental procedures in this study were approved by Faculty of veterinary medicine, Cairo University, under the supervision of the Faculty Veterinarian.

**Animals and groups:**

This work was done on 18 carpal joint (ninedonkeys), aging between 2–4 years and weighing between 150–200 kg. Animals were obtained, housed and supervised at the Department of Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine, Cairo University. Animals were fed a standard diet and had water and libitum. Animals were numbered and randomly assigned equally into one of three groups according to the follow up period after the IA injection of amphotericin-B; Group one (3 weeks), Group two (6 weeks) and Group three (9 weeks). The right joint of each animal was used as the experimental joint for induction of arthritis, whereas the left was the control.
The work procedures included a) Examination and Preparation of animals b) Induction of OA and c) Follow-up and assessment for 3, 6, and 9 weeks.

A- Preparation:
All animals in this work were subjected to complete visual inspection and clinical examination. Only sound animals, without any motion abnormalities, were included in this study. Animals were prepared with Ivermectin preparation. X-ray filming of the carpal joints prior to any interference was done. The settings of the x-ray machine were 50Kv and 2mA/sec exposure time and FFD 90cm. Four different views of the carpal joint (a- Lateral view; b- anterior-posterior view; c-cranio-palmer view and d- lateral flexed). The degree of arthritic changes was scored using a 0 to 4 scale adapted from Crawford, et al. (1991, Table 1). Radiographs were made of each carpus for each animal, on experimental days 0, and weekly afterwards.

Table 1: Crawford Radiographic scoring system

<table>
<thead>
<tr>
<th>Radiographic evaluation</th>
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<tbody>
<tr>
<td>0 = normal</td>
</tr>
<tr>
<td>1 = synovial effusion and/ or mild joint distension</td>
</tr>
<tr>
<td>2 = marked joint distension with no bone or cartilage change</td>
</tr>
<tr>
<td>3 = minimal bone changes, osteophytes &lt; 2 mm, with joint distension, no evidence of bone lysis or cartilage loss</td>
</tr>
<tr>
<td>4 = severe bone changes, osteophytes &gt; 2 mm, with or without evidence of cartilage loss and/or bone lysis</td>
</tr>
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</table>

B- Induction of Osteoarthritis:
Once animal was approved for the study, the appropriate forelimb carpal region was prepared with shaving of hair and aseptic skin preparation by using a povidone iodine scrub and 70% isopropyl alcohol swabs. Two ml of synovial fluid were aspirated into (EDTA) tubes for analysis from the right and left carpal joints by introducing a sterile 18-gauge needle at the radio-carpal space. One ml of each sample was used for determinations of total white cellcount, total erythrocyte count, total protein and mucin clot characteristics. The remainder of the sample was stored at -20°C for subsequent determination of alkaline phosphatase (ALP), Lactic acid dehydrogenase (LDH), Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. At the same procedure, the right carpal joint received a single shot of IA injection of 2 ml amphotericin B (10mg) while the left carpal joint received 2 ml saline as a control. The degree of lameness was scored using a 1 to 5 scale adapted from Crawford, et al. (1991; Table 2). To score the severity of lameness, each animal was evaluated for lameness twice weekly and assigned a lameness score. To eliminate interobserver variation, all lameness scores were assigned by one veterinarian blinded to treatment with training and expertise in equine lameness assessment.
### Table (2): Crawford lameness scoring system

<table>
<thead>
<tr>
<th>Lameness Evaluation</th>
<th>Description</th>
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<tbody>
<tr>
<td>I</td>
<td>no evidence of lameness</td>
</tr>
<tr>
<td>2</td>
<td>lameness barely detectable by expert observer</td>
</tr>
<tr>
<td>3</td>
<td>lameness easily detectable, with noticeable head nod</td>
</tr>
<tr>
<td>4</td>
<td>severe lameness, with marked head nod, and bearingweight on affected limb</td>
</tr>
<tr>
<td>5</td>
<td>severe lameness characterized by non-weight bearing on affected limb</td>
</tr>
</tbody>
</table>

Intraobserver variability was assessed periodically by randomly selecting animals for repeated assessment to ensure consistency of scoring. All lameness examinations were performed on even, non-sloped concrete floors free of obstructions and debris. Each lameness score was determined by watching the animal walk a minimum of 10 m in a straight line, turn, and walk back to the starting point. In order to have consistent time intervals between samplings or observations, these procedures were conducted on the same experimental day for each group.

### C-Synovial fluid analysis

**C.1-Physical characters:** The appearance and color of the synovial fluid were evaluated at the time of arthrocentesis. The tendency of a sample to clot was recorded. The mucinous precipitate quality was determined by adding 0.5 ml of synovial fluid to 2 ml of 2% acetic and mixing rapidly with glass rod as described by van Pelt et al., (1963). The mucine clot was graded as follows:

1. Normal (N) a light, ropey clump in a clear solution.
2. Fair (F) a light soft mass in slightly turbid solution.
3. Poor (P) a small friable mass in a turbid solution.
4. Very poor (VP) a few flakes in a very turbid solution.

For statistical purposes, a numerical value was assigned to each descriptive classification of mucinous precipitation, N= 4, F= 3, P= 2 and VP= 1. The relative viscosity of each synovial sample was determined with viscometer.

**C.2-Cytological examination:**
Red and white blood cell count in synovial fluid samples was performed using a Neubauer haemocytometer. Synovial smears were performed directly from the synovial fluid. The centrifugation was performed in case of synovial fluids with low cellular content and smears were made from the sediment. The smear was air dried, fixed with methyl alcohol and stained with May Grunwald Giemsa stains. Sample of synovial fluid in which gross evidence of blood observed were excluded (Knottenbelt, 2006).

**C.3-Biochemical analyses:**
The total protein was measured. The activities of enzymes (ALP, LDH, ALT and AST) in synovial fluid samples were estimated.
D-Macroscopical examination:
Necropsy was performed on each animal. The skin was removed from the carpi and transverse cuts were made with a band saw through the radius just above the distal epiphysis and through the metacarpal below the carpo-metacarpal joint. The carpal canal was removed to allow complete extension of the carpus. The carpus was then opened at radio-carpal joint, metacarpal joint. The synovial membrane and fibrous joint capsule could be examined. The cartilage surface of the bone, each cut, also, was carefully examined.

E-Microscopical examination:
All cases were fixed in 10% neutral buffer formalin. Decalcification of tissue cases were done by using 8% formic acid decalcifying solution in distilled water. The decalcifying solution was renewed every 48 hours until softening of the tissues. The decalcified cases were then trimmed, washed and dehydrated in ascending grades of alcohol, cleared in xylene, embedded in paraffin, sectioned at 4–6μm thickness and stained with haematoxylene and eosin (H&E) as well as Masson's trichrome (MT) stain for detection of collagen fibers. Sections were evaluated under light microscopy and morphologically classified in accordance with Table 3, based on the histological scoring system of Mankin et al. (1971).
In this study, grades of observed alterations were summed to graduate morphological lesions in each analyzed section. The sum of all characteristics, analyzed in each histological section, allowed for a better evaluation of cartilage morphology, as well as, to characterize the phase of the arthritis. The larger the sum of the score the more severe was the degenerative process in the articular cartilage.

Table (3): Parameters of qualitative morphological evaluation of articular cartilage in experimentally induced arthritis

<table>
<thead>
<tr>
<th>Feature</th>
<th>Grade</th>
<th>Feature</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I – Cartilage structure</strong></td>
<td></td>
<td><strong>II – Cellularity</strong></td>
<td></td>
</tr>
<tr>
<td>a) normal</td>
<td>0</td>
<td>a) normal</td>
<td>0</td>
</tr>
<tr>
<td>b) fibrillation in the superficial zone</td>
<td>1</td>
<td>b) diffuse hypercellularity</td>
<td>1</td>
</tr>
<tr>
<td>c) fibrillation in the middle zone</td>
<td>2</td>
<td>c) clones</td>
<td>2</td>
</tr>
<tr>
<td>d) irregularities in the superficial zone</td>
<td>3</td>
<td>d) hypocellularity</td>
<td>3</td>
</tr>
<tr>
<td>e) fissures</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>f) erosion</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>g) pannus</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>III – Metachromatic staining</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) normal</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b) slight reduction</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c) moderate reduction</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d) severe reduction</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>e) absent</td>
<td>4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Modified by Mankin et al., 1971
Results
Mild degree of OA was achieved 3-weeks post-injection with amphotericin B while the moderate and severe degrees were achieved after 6 and 9 weeks respectively. The different degrees of OA were assessed using the following parameters

1- Clinical lameness and swelling
The signs began within 3 hours post injection in the form of acute swelling as a result of joint capsule distention (Score 3) that persist and appear as enlargement of joint circumference with average increase $1\pm0.24$cm in comparison to the control joint. The lameness reach the top after 6 hours which persist for 3 days then decreased till the end of the experiment (score 2) and could detected by palpation and passive movement of carpal joint.

2. Radiological findings
The radiological examination revealed progressive changes in the carpal joint. There was marked soft tissue swelling (Score 2) for the first 3 days(Figure 3), which decreased gradually to reach (Score 1) at 9 weeks. In the meantime, osteoarthritic change in form of gradual narrowing of the joint space and thinning of the articular surface were progressively developing with time and could be noticed at 9 weeks (Figure 4).

Fig (3): Antero-posterior radiographic image (a) and lateral flexed (b) of the carpal joint, showing arthritic changes manifested by marked soft tissue swelling (Score 2) at 3 days post injection of amphotericin B (arrows).
Fig 4: Lateral radiographic image of the carpal joint, showing arthritic changes manifested by slight swelling (Score 1) and narrowing of joint space (Score 3) at 9 weeks post injection of amphotericin B (arrow).

3-Synovial fluid analysis:
Physical, cytological and biochemical characteristics of synovial fluid analysis of all animal models of OA are summarized in table (4) and figure (5).

Table (4): Summary of the physical, cytological and biochemical characteristics of synovial fluid analysis of the induction

<table>
<thead>
<tr>
<th></th>
<th>Viscosity</th>
<th>Appearance</th>
<th>Mucin ppt</th>
<th>Clot</th>
<th>pH</th>
<th>Total protein</th>
<th>AST</th>
<th>ALT</th>
<th>AlK</th>
<th>LDH</th>
<th>WBCs</th>
<th>RBCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.5±3.0</td>
<td>Colorless &amp; clear</td>
<td>4</td>
<td>-ve</td>
<td>7.3±0.04</td>
<td>1.56±0.06</td>
<td>15.33±2.76</td>
<td>6±2</td>
<td>12±2</td>
<td>36.5±11.5</td>
<td>33.3±17.3</td>
<td>10.66±10.66</td>
</tr>
<tr>
<td>3 weeks</td>
<td>10.9±11.09</td>
<td>pale yellow &amp; clear</td>
<td>3.3±0.33</td>
<td>+ve</td>
<td>6.9±0.06</td>
<td>3.56±0.04</td>
<td>38±4.0</td>
<td>42.6±16.6</td>
<td>57±5.0</td>
<td>136.6±18.6</td>
<td>248.3±19.3</td>
<td>60±189</td>
</tr>
<tr>
<td>6 weeks</td>
<td>7.8±11.3</td>
<td>Yellow &amp; clear</td>
<td>2.6±0.23</td>
<td>+ve</td>
<td>6.9±0.11</td>
<td>3.58±0.38</td>
<td>64.6±4.4</td>
<td>75.3±15.3</td>
<td>92.6±4.6</td>
<td>256.6±16.6</td>
<td>680±40.0</td>
<td>261.3±11.3</td>
</tr>
<tr>
<td>9 weeks</td>
<td>5.6±1.9</td>
<td>Yellow &amp; turbid</td>
<td>2.3±0.19</td>
<td>+ve</td>
<td>6.7±0.9</td>
<td>4.6±0.4</td>
<td>82.6±2.4</td>
<td>88±4.0</td>
<td>133.3±2.6</td>
<td>435.3±120.0</td>
<td>796.6±50.4</td>
<td>183.3±16.7</td>
</tr>
</tbody>
</table>

![Graph of AST, ALT, and ALP levels over time](chart1.png)

![Graph of LDH levels over time](chart2.png)
4- Macroscopical appearance:
Macroscopic appearances of the experimental induction groups showed slight yellowish discoloration of the articular surface with slight hemorrhagic areas at the joint capsule as shown in figure (6)

Fig.(6): Articular surface discoloration at 9 weeks post-injection of amphotericin B. Notice the yellowish discoloration of the joint (left) and the hemorrhagic areas (right).

5-Microscopical appearance:
In sections of all cases, the more frequent histological findings that reflected the degenerative effect of the drug were the presence of cartilage surface irregularities and fibrillation, edema, hypocellularity and reduction of metachromatic staining intensity.

The synovium showed focal papillary proliferation together with hyperaemic dilated vessels and mild lymphocytic infiltration in joints injected with amphotericin B of the 3 induction groups. It was observed that there is a cumulative effect of the drug with increase of the score through the 3 groups.

The 1\textsuperscript{st} group (3 weeks post induction): Superficial zone fibrillation & fissures with diffuse hypercellularity and slightly decreased metachromatic staining. Mankin score ranged from 3 – 6 (Fig. 7).

The 2\textsuperscript{nd} group (6 weeks post induction): Superficial zone fibrillation, irregularity with fissures in superficial & middle zone, hypocellularity and small erosions, and slightly decreased metachromatic staining. Mankin score ranged from 7 – 9 (Fig. 8).

The 3\textsuperscript{rd} group (9 weeks post induction): a layer of roughened granulation tissue, or pannus was noticed protruding over the surface of the cartilage. Under the pannus the cartilage was eroded and destroyed with penetration of subchondral bone by blood vessels. Marked reduced metachromatic staining was observed. Mankin score ranged from 9 – 12 (Figs. 9-11).

Fig (7): photomicrograph graph of articular cartilage 3 weeks post induction of OA showing: a) Superficial zone fibrillation (arrow) with diffuse hypercellularity(H&E 100x); b) Slightly decreased metachromatic staining with fissures (arrows) in superficial zone (MT, 100x).
Fig (8): photomicrograph graph of articular cartilage 6 weeks post induction of OA showing: a) Superficial zone fibrillation, irregularity, hypocellularity and small erosions (H&E, 100x); b) Slightly decreased metachromatic staining with fissures in superficial & middle zone (MT, 100x).

Fig (9): photomicrograph graph of articular cartilage 9 weeks post induction of OA showing: (a & b) a layer of roughened granulation tissue, or pannus (arrow), protrudes over the surface of the cartilage. Under the pannus the cartilage is eroded and destroyed (H&E, 100x).

Fig (10): photomicrograph graph of articular cartilage 9 weeks post induction of OA showing: a) Complete loss of cartilage, pannus formation with penetration of subchondral bone by blood vessels (H&E, 100x) and b) Markedly reduced metachromatic staining with loss of chondrocytes (MT, 100x).
Fig(1): The synovium showed focal papillary proliferation (arrows) together with hyperaemic dilated vessels (BV) and mild lymphocytic infiltration.

Discussion
The use of animal models is influential in understanding the etiology and the pathogenic mechanisms of arthritis as well as other medical conditions. Animal models have contributed considerably to the understanding of the arthritic processes and concomitant cartilage and bone destruction (van den Berg, 2009) and subsequently have been used in evaluating the clinical outcomes of potential anti-arthritis drugs (Bendele, et al., 1999).

Different techniques were used to create animal models of arthritis. Various animal species has been used as models of traumatic or surgically induced arthritis. Traumatic OA does occur in humans, and therefore these models may mimics aspects of pathogenesis and pathology. One important difference however is that humans with a traumatic injury generally discontinue use of the affected limb. Animals in the same situation generally do not. Therefore, the disease progression is usually much more rapid in the animal models (Bendele, et al., 1999).

Chemically induced models of OA in horses have been established through IA injection of chemicals such as polyeneantibiotics, filipin and amphotericin B (McIlwraith, et al., 1979, McIlwraith, et al., 1981 and Bowman, et al., 1983), Freund's adjuvant (Hamm, et al., 1984), and sodium monoiodoacetate (Trotter, et al., 1989). Though, none of these experimental models completely reproduced the clinical and pathological changes seen.

The dosage of amphotericin B required to induce arthritis varied. Suominen, et al., (1999) reported that IA injection of amphotericin B consistently resulted in aseptic arthritis in horses. Twenty mg of amphotericin B as a single IA injection in the right intercarpal joint was enough to induce aseptic arthritis in 24 Standard bred horses (Marttinen, et al., 2006). Kotschwar, et al., (2009) concluded that amphotericin B at 15 and 20 mg administered in a single IA injection is effective in establishing
synovitis/arthrosis model in cattle. These results are comparable with those of William et al., (1991) who reported that synovitis was induced in the middle carpal joints (MCJ) of ponies by injecting 10 mg of amphotericin B into that joint on day 1 of the experiment. In studies of evaluating lameness, pain, and the effectiveness of certain non-steroidal anti-inflammatory agents and other pain alleviating agents, Bowman, et al., (1983); Crawford, et al., (1991); Fahmy, et al., (1994); Hegazy, et al., (1994) used amphotericin B to induce arthritis in horses utilizing a single IA injection of 10 mg amphotericin B. The induced arthritis could be examined biochemically as early as few hours post injection and histopathologically after one week and progressively afterwards.

In order to provide a longer period, greater degree of lameness and for a more severe arthritis model, amphotericin B was used in three injections in a second group where multiple doses of 10 mg of amphotericin B in the right MCJs on days 1, 4, and 18 of the experiment were received William et al., (1991)

Amphotericin B was selected for this study to create an arthritis model that would exhibit lameness for several weeks following a single IA injection similar to that used by Crawford, et al., (1991) and that could be used for later evaluation of recent cell treatment technique.

Arthritis was experimentally induced in the radio-carpal joint of 9 donkeys by a single IA injection of 2ml of 10mg of amphotericin B. Symptoms of acute inflammatory reaction developed 3 hours post injection which was characterized by lameness and swelling of the joint. The severity of lameness and the degree of swelling varied. Suominen, et al., (1999) reported lameness of the 5th grade on lameness scoring system on day 0 after a single injection of 10mg amphotericin B. In our study, the lameness could be detected in normal gait during the first week (score 3) and only appeared on passive flexion of the carpal joint after that. Later on the lameness started to improve (score 2) till the end of the experiment which indicated that acute inflammation was turning on to the chronic degenerative form. However, lameness persisted longer which was possibly as a result of more delayed effect of amphotericin B as mentioned by Fahmy, et al., (1994).

The concomitant joint swelling that appeared as enlargement of joint circumference with average increase 1±0.24 cm in comparison to the control limb. could be explained by the fact that amphotericin B has an early toxic effect on cartilage matrix as well as synovial membrane leading to synovitis and capsulitis. In addition it has direct toxic effect on chondrocyte resulting in osteoarthritic changes in the injected joint(Fahmy et al., 1994) with increased synovial production and reduction of resorption (Fournier, et al., 1969) and thickening of joint capsule. These clinical arthritic symptoms declined gradually.

The macroscopic findings were limited to the discoloration of the articular cartilage from the normal bluish-white into a yellowish color and some hemorrhagic areas in the
joint capsule. Slight roughness of the articular surface could be felt in the sever cases. This could be a result of the somatic toxic effect by a material released from the ruptured lysosomes on the joint compartment. This observation resembles the tissue reaction in rabbit joint using streptolysine S (Weissman, et al., 1965) and in ponies using filipins (McIlwraith, et al., 1979 A&B and Bowman, et al., 1983).

Radiographic arthritic evidence in the form of synovial capsule swelling were found in all animals with amphotericin B treated joints after 3 weeks. Narrowing of the joint space was seen in the severe cases. All changes were increased in severity in relation to the experimental time due to the progression of joint destruction. These results run hand to hand with those reported by McIlwraith, et al., (1979 A&B) Bowman et al., (1983) and Fahmy, et al.,(1994). In the meantime the histopathological examination of the arthritic joints confirmed these results.

Synovial fluid analysisis of great value to the equine practitioner as a mean of determining the cause, type (inflammatory or non-inflammatory), duration and the stage of the disease (acute inflammatory phase or degenerative phase or destructive phase). It was found that there is a close correlation between the enzyme activity in the synovial fluid especially ALP, LDH, AST, ALT and severity of the changes in the joint as a result of disease. All these information are useful in therapeutic management and it’s follow up as reported in our experimental work and that described by ropes (1957) and van Pelt, et al., (1971).

Intra-articular injection of amphotericin B consistently resulted in significant increase in the immunoreactivity of matrix metalloproteinase MMP-8 and activity of both the latent and the active forms of MMP-2 and -9, MMP-9 levels declined to pre-induction levels within 2 weeks, whereas levels of MMP-2 remained still high after 5 weeks (Marttinen, et al., 2006). Their results proved that after acute arthritis induction by IA injection of amphotericin B; elevated MMP activity is present in the joint for several weeks to a degree that could promote cartilage degradation.

Relying on the present results, the model of chemically induced arthritis could be classified into three main stages: The first acute inflammatory stage is characterized by highly significant increase in the total protein,LDH,ALP and AST which act as indicator. An increase in monocytes, neutrophiles and mucin precipitation was recorded. These findings agree with those reported by Matthew, et al., (1962); Hunt (1965); Anon (1966) and McIlwraith, et al., (1979 A&B). These findings coincide with those stated by (Crawford, et al., 1991). The increase in total WBCs content in the synovial fluid is attributed to an increase in capillary permeability which permits the higher molecular weight protein fraction to enter the joint (Curtis, 1964). The permanent passage and retention of proteins in synovial fluid was occurred as reported by Cohen (1967) and Schubert et al., (1968).
The second moderate stage of chemically induced arthritis (6 weeks-second phase) was characterized by progressive changes of the synovial fluid levels (increase AST, ALT, ALP, LDH and WBCs, monocyte and neutrophil count with decrease in viscosity and mucinous precipitation) indicating progressive destruction of the articular cartilage. The third severe stage of chemically induced arthritis (9 weeks-third phase) was characterized by progressive changes of the synovial fluid levels till the end of the study. It was characterized at first by reduction in neutrophil count and an increase in ALP, AST and LDH levels, in addition to increase in monocyte. These changes in synovial parameters can be interpreted as a result of the synovial membrane inflammation as well as massive matrix destruction which were confirmed by the pathological examination. (McIlwraith, 1981 and Clyne, 1987).

Histopathologic changes included degenerative changes in the cartilage and bone in addition to synovitis and capsulitis in the form of loss of cartilage matrix architecture, fibrous replacement of cartilage, osteophyte formation, cartilage loss and thinning. These findings agree with that obtained by William et al., (1991). Articular cartilage degradation consistently began as early as 3 weeks after induction. Early in pathogenesis, the profile of cartilage degradation initially reflects the edema and delamination of the superficial layer, and development of fissures into the mid-zone that are commonly observed during early stages of human OA (Aigner et al., 2007). The model also exhibits features characteristic of late-stage human OA. At later time, loss of subchondral bone density and trabecular architecture were also present and are reminiscent of human OA as described by Felson et al., 2000 and Sarzi-Puttini, et al., 2005. Ultimately, these properties are likely to persist to end-stage OA, where joint failure occurs and invasive arthroplastic intervention is required.

Appleton et al., 2007 described a surgical method for induction of osteoarthritis in the knee joint of rats by surgery involving anterior cruciate ligament transection (ACL-T) and partial medial meniscectomy and reported similar histopathological results. However, we assume that chemical induction of OA is better as it is an easy, rapid and less invasive technique.

In conclusion, having a model to evaluate parameters after lameness and arthritis induction is very important for evaluation of pharmacological agents, establishment of dosing regimens and to assess different treatment methodologies. Amphotericin B at 20 mg by IA injection provides an effective (time/severity) model for lameness in equine. Moreover the histopathological degenerative changes obtained by chemical induction are comparable with the findings of surgical method for OA induction.
References


