Influence of Exercise on the Distribution of $^{99mTc}$-Technetium-Methylene Diphosphonate Following Intra-articular Injection in Horses

THESIS

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By

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Abstract

Objective: To determine the effects of exercise on the distribution and pharmacokinetic parameters of $^{99m}$Tc-Methylene diphosphonate ($^{99m}$Tc-MDP) following intra-articular (IA) injection in horses.

Animals: 5 horses.

Procedures: In this randomized, controlled, crossover study, one antebrachiocarpal joint (ACJ) per horse was assigned to the Exercised group (n=5), and the contralateral ACJ was evaluated in the Non-Exercised group (n=5) after a minimum of 7 days. Following IA injection of $^{99m}$Tc-MDP (148 MBq), blood and scintigraphic images of the carpus were obtained at 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 240, 360, 480, 600, 720, and 1440 minutes. Plasma and scintigraphic radioactivity were determined over time, and pharmacokinetic parameters were generated via non-compartmental and compartmental analyses. Each horse was monitored by physical and lameness examination and ACJ synovial fluid analysis before injection and at days 1, 2, 3, and 7.

Results: Lameness was not observed. Mean synovial fluid WBC count increased at day 1 (Exercised: 721 ± 234 cells/uL, p < 0.05; Non-Exercised: 948 ± 223 cells/uL, p < 0.01), but returned to baseline at days 3 and 7. Mean time to maximum plasma radioactivity was earlier in the Exercised group (16.00 ± 2.35 min) than the Non-Exercised group (43.75 ± 3.64 min) (p < 0.001). Linear regression of the scintigraphic radioactivity-time curves showed a greater negative slope in the Exercised group within the first 25 minutes.
(p = 0.03). There was no difference in absorption or elimination rate constants in a 2-compartment model.

Conclusions and Clinical Relevance: Intra-articular $^{99m}$Tc-MDP was safe and effective for evaluating synovial solute distribution. Exercise significantly increased early transfer of $^{99m}$Tc-MDP from the ACJ into plasma, although absorption and elimination rate constants were not affected. Exercise may affect synovial clearance and withdrawal times of IA medications.
Acknowledgments

The author would like to thank Dr. Akikazu Ishihara, Brian Johnson and Emily Falk for their technical assistance, Tim Vojt for helping with design and formatting of figures, and the Galbreath Equine Center faculty and staff for their assistance and use of facilities. The author would also like to sincerely thank Drs. Elizabeth Santschi, Wm. Tod Drost, and Mitch Phelps for their scientific insights and suggestions, and Dr. Alicia Bertone for her guidance and mentorship. Finally, the author thanks the United States Equestrian Federation and Pfizer Animal Health for partial funding of this study.
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characterization of 2 novel feline caliciviruses isolated from cats with idiopathic

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<tr>
<td>PK</td>
<td>Pharmacokinetics</td>
</tr>
<tr>
<td>$^{99m}$Tc</td>
<td>$^{99m}$Technetium</td>
</tr>
<tr>
<td>$^{99m}$Tc-MDP</td>
<td>$^{99m}$Technetium-methylene diphosphonate</td>
</tr>
<tr>
<td>IA</td>
<td>Intra-articular</td>
</tr>
<tr>
<td>ACJ</td>
<td>Antebrachiocarpal joint</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>CPM</td>
<td>Counts per minute</td>
</tr>
<tr>
<td>DPM</td>
<td>Disintegrations per minute</td>
</tr>
<tr>
<td>$C_{\text{max}}$</td>
<td>Maximum observed radioactivity</td>
</tr>
<tr>
<td>$T_{\text{max}}$</td>
<td>Time of maximum observed plasma radioactivity</td>
</tr>
<tr>
<td>$L_z$</td>
<td>Terminal phase elimination rate constant</td>
</tr>
<tr>
<td>Cl (Cl/F)</td>
<td>Observed apparent clearance</td>
</tr>
<tr>
<td>$V_z$ (V_z/F)</td>
<td>Observed apparent volume of distribution</td>
</tr>
<tr>
<td>AUC$_{\text{last}}$</td>
<td>Area under the quantifiable radioactivity-time curve</td>
</tr>
</tbody>
</table>
Chapter 1: Introduction

Intra-articular therapeutic agents such as glucocorticosteroids, hyaluronic acid, glycosaminoglycans and antibiotics are commonly used in equine athletes for the treatment of joint disease. The temporal relationship between IA medications and athletic performance is the subject of much debate and regulation (McKay, 1976; Owen, 1984; Trotter, 1991; Trotter, 1996). The distribution of IA therapeutics, including retention within the joint over time, require investigation to ensure the safety of the equine athlete, riders and drivers. The rate limiting step in elimination of clinically detectable levels of glucocorticosteroid within the joint is believed to be transfer from synovial fluid to plasma (Soma, 2006).

Previous studies (Simkin, 1974; Simkin, 1995b) have demonstrated that synovial solutes, in addition to several radioisotopes of various molecular sizes, are cleared from the human knee at constant rates. The clearance of these solutes, expressed as a clearance constant in min⁻¹, can be compared between individuals and longitudinally within an individual (Levick, 1988; Simkin, 1995a; Simkin, 1995a; Wallis, 1985a). These articular clearance values have not been investigated in the horse. Clearance rates of $^{99m}$Tc (as $^{99m}$Tc-diethylene triamine pentaacetic acid), a freely diffusible water soluble molecule,
have been used to represent other synovial molecules that equilibrate with plasma in human studies. This method provides a measurement of joint perfusion and drainage and subsequent transfer of solutes from the IA space to circulating blood. Clearance of IA $^{99m}$Tc-DTPA, measured by serial counts of emitted gamma-rays, was shown to be significantly increased by dynamic exercise in humans with knee effusion (James, 1994). It is thought that increased clearance of synovial fluid and solutes following exercise is directly related to increased blood flow to the synovium, as well as increased IA hydrostatic pressure leading to altered Starling forces across the synovium (Dyson, 2001; Hardy, 1995; Hardy 1996; Levick, 1979a; Levick, 1979b; Levick, 1984; Macoris, 2001; Nade, 1983; O’Driscoll, 1983; Simkin, 1990; Stolk, 1994). These studies of joint fluid dynamics support that exercise of a cyclical nature would lead to earlier synovial solute clearance.

Despite the various human, canine, and rabbit studies utilizing radiopharmaceuticals for investigating synovial fluid exchange, a similar technique for evaluating the distribution of IA gamma-emitting radioisotopes in horses has not been reported. The goals of the present study were to evaluate the safety of IA $^{99m}$Tc-MDP in normal horses, and to define the effect of exercise on the blood and joint distribution of IA $^{99m}$Tc-MDP using both non-compartmental analysis and compartmental modeling. The authors propose to test the hypotheses that IA $^{99m}$Tc-MDP is safe in normal horses, and that exercise will affect the distribution and pharmacokinetics of the radiopharmaceutical $^{99m}$Tc-MDP following IA injection in horses.
Chapter 2: Materials and Methods

Experimental Design

The study was performed as a randomized, controlled, crossover trial. One randomly selected ACJ per horse was assigned to the Exercised group protocol (n=5), and the contralateral ACJ was evaluated in the Non-Exercised group protocol (n=5) after a washout period of a minimum of 7 days. The experimental protocol was approved by The Ohio State University Institutional Animal Care and Use Committee.

Horses

Five female Thoroughbred horses (age [mean ± SD]: 3.2 ± 1.6 years; body weight: 478.2 ± 20.7 kg) were included in the study. Prior to enrollment, all horses were considered healthy and free from lameness or ACJ inflammation based on inspection by an experienced examiner (ALB). Each carpus was examined by an ACVR Diplomate (WTD), and determined to be free of visible radiographic abnormalities based on a single flexed lateral-medial projection. The horses were exercised for at least 2 weeks using a high speed equine treadmill\(^1\) prior to initiation of the study. Horses were exercised three times per week with the following regimen to simulate the exercise of race training: walking (9 km/h) for 5 min, followed by trotting (16 km/h) for 5 min, galloping (32 km/h) for 5 min and walking (9 km/h) for 5 min. Throughout the experiment, the horses...
were housed individually in stalls in a temperature-controlled environment, and fed a commercial grain mixture twice daily with access to hay and water \textit{ad libitum}.

\textit{IA Administration of} $^{99m}$Tc-MDP

On day 0 of each protocol, a 14G, 5.25” peripheral venous catheter$^2$ was placed using aseptic technique in either jugular vein. Prior to IA injection, the skin over the dorsolateral pouch of the appropriate ACJ was clipped free of hair and aseptically prepared. Approximately 1ml of 3% mepivacaine hydrochloride was infiltrated subcutaneously over the joint pouch. A 20G 1.5” needle was steriley inserted into the dorsolateral ACJ pouch, and 0.5-1ml synovial fluid was aspirated and placed in an EDTA tube$^3$ for analysis of WBC count and total protein concentration. 148 MBq (4 mCi; 5ml total volume) of $^{99m}$Tc-MDP were administered IA by the author (JAD), who was included in the academic nuclear medicine permit designated by Environmental Health and Safety – Radiation Safety. All personnel wore adequate personal protective equipment during injection as outlined in the academic nuclear medicine permit. The total dose of radiopharmaceutical was determined to be appropriate for imaging based on unpublished pilot data obtained by the author, and a small volume was utilized to avoid increased IA volume and elevated IA hydrostatic pressure.

\textit{Blood and Image Collection}

Following injection, horses in the Exercised group protocol were immediately trotted on a high speed equine treadmill$^1$ for 5 minutes at 4.4 m/s. Blood collection began immediately at termination of treadmill exercise. In both groups, 2ml of blood were obtained from the indwelling jugular vein catheter at 5, 10, 15, 20, 25, 30, 45, 60, 90,
120, 240, 360, 480, 600, 720, and 1440 minutes following injection. The blood was placed directly into individual sodium heparin blood collection tubes and stored at room temperature for processing within 6 hours of collection. Prior to initiating image collection, each horse was sedated with detomidine hydrochloride (0.01 mg/kg IV) and butorphanol tartrate (0.01 mg/kg IV). Additional sedation was administered throughout image collection and consisted of 0.002-0.004 mg/kg detomidine and butorphanol IV as needed to acquire the images. Static, 90-second, dorsal scintigraphic images of both forelimbs, from mid-radius to proximal metacarpus (Figure 1), began 5 minutes after injection in the Non-Exercised group protocol, and 10 minutes post-injection in the Exercised group protocol to permit travel between the treadmill and nuclear medicine suite. The gamma-camera was placed at a similar distance dorsal to the carpi during each image acquisition. Scintigraphic imaging occurred at (5, Non-Exercised group only), 10, 15, 20, 25, 30, 45, 60, 90, 120, 240, 360, 480, 600, 720, and 1440 minutes following injection.

![Figure 1: Dorsal scintigraphic image of a left ACJ 60 minutes after IA 99mTc-MDP.](image)
**Evaluation of Joint Inflammation**

Within 2 hours prior to $^{99m}$Tc-MDP injection on day 0, and at days 1, 2, 3, and 7 post-injection for both protocols, each horse was assessed at the trot in a straight line on a hard ground surface for forelimb lameness and graded on a 0-5 scale as described by the American Association of Equine Practitioners (AAEP, n.d.) (see Appendix A). The tissues over the ACJ were palpated and visually inspected for swelling on days 0, 1, 2, 3, and 7. The swelling was scored as follows: 0 = no subcutaneous swelling; 1 = minor (≤1cm diameter) subcutaneous swelling at injection site; 2 = mild (1-3cm diameter) subcutaneous swelling at injection site; 3 = moderate (extending past the dorsolateral ACJ pouch or >3cm diameter) subcutaneous swelling; 4 = severe (entire dorsal surface of carpus) subcutaneous swelling. The circumference of the carpus at the ACJ was measured on days 0, 1, 2, 3, and 7 by placing a measuring tape around the ACJ over the widest part of the accessory carpal bone. All clinical assessments were performed by the same examiner (JAD). Synovial fluid WBC count and total protein concentration were evaluated with repeated arthrocentesis prior to injection on day 0, and at days 1, 3, and 7 post-injection, by means of an automated cell counter and refractometry, respectively. Normal WBC count was considered ≤ 500 cells/uL, and normal total protein concentration was considered ≤ 2.5 g/dL.

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**Sample Processing**

**Blood Sample Processing**
Heparinized blood samples were centrifuged for 5 minutes at 2800 rotations per minute. One half ml of the plasma supernatant was pipetted into a new plastic test tube for each sample, which was then placed into a liquid scintillation well counter. The well counter was calibrated daily using a known cesium source, and was set to record CPM within a range of 119-162 kiloelectron Volts. Technetium emits gamma ray energy at 140 kiloelectron Volts. All CPM measurements were converted to DPM based on a known 77.3% efficiency of the well counter for detecting technetium disintegrations. Finally, all plasma radioactivity data (DPM per ml plasma: DPM/ml) were corrected for radioactive decay using the following formula:

\[ A_0 = \frac{A}{e^{\lambda t}} \]

Where \( A_0 \) is the activity at the time of IA injection, time 0 (decay-corrected DPM); \( A \) is the activity at time \( t \) (uncorrected DPM); \( \lambda \) is the decay constant (0.693/half life); and \( t \) is the time post-injection when the blood was acquired, in minutes. The half life of \(^{99m}\text{Tc}\) is 363 minutes.

*Image Processing*

The static, 90-second, scintigraphic images were processed using Mirage software to determine radioactivity within the ACJ over time (decay-corrected CPM per ROI point: CPM/point). An ROI was drawn by the author (JAD) outlining the ACJ (Figure 2). The author was blinded to treatment group at the time the ROIs were drawn. The total number of counts within each 90-second ROI was determined using the Mirage software, and the counts were multiplied by 2/3 to produce an uncorrected CPM value. These CPM were then corrected for radioactive decay using Formula 1. In order to account for
unavoidable variability in hand-drawn ROIs, the decay-corrected CPM were divided by
the total number of points (pixels) per ROI, yielding a final CPM/point measurement of
radioactivity within the ACJ.

![Image of Region of Interest](image)

Figure 2: Region of interest drawn around the ACJ to quantify total radioactivity within
the joint.

In order to assess the distribution of the radiopharmaceutical in the joint and surrounding
bone and soft tissues, a separate set of ROIs were produced from the same images at the
20 minute and 360 minute time points (Figure 3). These time points were chosen to
represent early and delayed phases of radiopharmaceutical distribution. Four rectangular
ROIs of the following sizes were drawn over each static image: ROI 1 = 7 x 7mm; ROI 2
= 10.75 x 13.25mm; ROI 3 = 20.5 x 22.75 mm; ROI 4 = 36.5 x 39 mm. All size
measurements were made using a digital caliper\textsuperscript{10} with 0.01mm resolution and accuracy
to 0.02mm. The mean ± SE CPM were determined for each ROI; ROI 1 and ROI 2
represented the activity within the antebrachiocarpal joint, and ROI 3 and ROI 4 represented the activity within the surrounding bone and soft tissues.

Figure 3: ROI of specified dimensions drawn over the scintigraphic images to determine the distribution of radioactivity within the ACJ vs. the surrounding tissues. ROI 1: solid line; ROI 2: short dashes; ROI 3: long dashes; ROI 4: dashes & dots.

Data Analysis

Non-Compartmental PK Analysis and Compartmental Modeling

Non-compartmental and compartmental PK parameters were generated using WinNonlin\textsuperscript{11} for plasma radioactivity as well as activity within the scintigraphic ROI in the Exercised and Non-Exercised groups. Uniform weighting was used throughout the analysis. Compartmental model selection was guided by the Akaike Information Criterion (AIC) and parameter standard errors of estimate.

Statistical Analysis

Statistical analyses were performed using commercially available software.\textsuperscript{12} PK parameter estimates, as well as ROI data, were compared between groups using paired t-
test. Serial quantitative data (percent change in joint circumference, synovial fluid WBC counts and total protein concentrations) were analyzed using 2-way repeated measures ANOVA with Bonferroni post-tests. Scored data (edema and lameness scores) were analyzed using Kruskal-Wallis test for non-parametric data with Dunn’s Multiple Comparison post-test. Significance was set at \( p \leq 0.05 \) for all tests.
Chapter 3: Results

Evaluation of Joint Inflammation

All horses had normal pre-injection physical, radiographic, and lameness examinations and synovial fluid analyses, and all completed both study protocols with no lameness or physical examination abnormalities. There was no significant difference between Exercised and Non-Exercised groups for swelling score, percentage change in ACJ circumference, or synovial fluid WBC count and total protein concentration at any examination time. Joint circumference at day 7 was slightly increased (1.79 ± 1.11%) compared with pre-injection (0 ± 0%) and day 1 (-0.13 ± 0.30%) values in the Exercised group (p < 0.05). Pre-injection synovial fluid WBC counts were within the reference range\textsuperscript{7} in both the Exercised (130 ± 61 cells/uL) and Non-Exercised groups (113 ± 32 cells/uL). In both groups, WBC count increased, with a peak at 24 hours post-injection (Exercised: 721 ± 234 cells/uL, p < 0.05; Non-Exercised: 948 ± 223 cells/uL, p < 0.01) when compared to pre-injection values (Figure 4). WBC values returned to pre-injection levels in both groups at 3 and 7 days post-injection. Synovial fluid total protein concentration did not increase over the examination period in either group.
Figure 4: Synovial fluid WBC concentrations in exercised and non-exercised horses following IA $^{99m}$Tc-MDP.

Scintigraphic Analysis

Radioactivity remaining within the ACJ was higher in the Exercised group at 360 minutes ($p = 0.04$), 600 minutes ($p = 0.02$), 720 minutes ($p = 0.02$), and 1440 minutes ($p = 0.03$). There was no difference in CPM/point between the groups at any other time. However, linear regression of the curve from 5 through 25 minutes revealed a greater negative slope in the Exercised group ($p = 0.03$). The line of best fit for the Exercised group was $y = -7.94x + 824; r^2 = 0.93$. The line of best fit for the Non-Exercised group was $y = -3.20x + 752; r^2 = 0.83$. Both slopes were considered non-zero, with 95% CI of -14.55 to -1.33 (Exercised) and -5.82 to -0.58 (Non-Exercised) (Figure 5).
Figure 5: Scintigraphic radioactivity-time curve following ACJ injection of $^{99m}$Tc-MDP in exercised and non-exercised horses. Linear regression of the curve from 5 to 25 minutes (inset). Asterisks (*) represent a difference between Exercised and Non-Exercised Groups ($p \leq 0.05$).

The distribution of radioactivity within the joint and surrounding tissues was analyzed at 20 minutes and 360 minutes in both groups. At 20 minutes post-injection, there were no differences between groups in radioactivity remaining within the joint (ROI 1, $p = 0.50$; ROI 2, $p = 0.71$), or the surrounding tissues (ROI 3, $p = 0.30$; ROI 4, $p = 0.34$) (Figure 6A). At 6 hours post-injection, more radioactivity remained in both the joint (ROI 1, $p = 0.03$; ROI 2, $p = 0.04$) and the surrounding tissues (ROI 3, $p < 0.01$; ROI 4, $p < 0.001$) in the Exercised group (Figure 6B).
Figure 6: Mean radioactivity within four ROI representing the ACJ and the surrounding tissues at 20 minutes (A) and 360 minutes (B) after IA injection of 99mTc-MDP. Asterisks (*) represent a difference (p ≤ 0.05) between groups.
Region of Interest Non-Compartmental Analysis of $^{99m}$Tc-MDP

Non-compartmental pharmacokinetic parameters of technetium activity within the scintigraphic region of interest are summarized in Table 1. The area under the quantifiable radioactivity-time curve, $AUC_{\text{last}}$, was higher in the Exercised group than the Non-Exercised group ($p = 0.05$). The observed apparent clearance, $Cl$, determined by dose divided by $AUC$, was lower in the Exercised group ($p < 0.01$). The observed apparent volume of distribution, $V_z$, was not different between groups. Furthermore, the terminal phase elimination rate constant, calculated from 8 to 24 hours post-injection, $L_z$, was not different between groups.

Table 1: Non-compartmental pharmacokinetic parameters of $^{99m}$Tc-MDP activity in an ACJ scintigraphic region of interest in exercised and non-exercised horses.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Exercised</th>
<th>Non-Exercised</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (CPM/point)</td>
<td>665.6</td>
<td>726.6</td>
</tr>
<tr>
<td>$L_z$ (1/min)</td>
<td>0.00050</td>
<td>0.00048</td>
</tr>
<tr>
<td>* $Cl$ (ml/min)</td>
<td>1.256</td>
<td>1.676</td>
</tr>
<tr>
<td>$V_z$ (ml)</td>
<td>2615</td>
<td>3940</td>
</tr>
<tr>
<td>* $AUC_{\text{last}}$ (min*CPM/point)</td>
<td>447,871</td>
<td>321,800</td>
</tr>
</tbody>
</table>

* Exercised and Non-Exercised are different ($p<0.05$)

Plasma Non-Compartmental Analysis and Compartmental Modeling of $^{99m}$Tc-MDP

Non-compartmental pharmacokinetic parameters of technetium activity in plasma following IA injection are summarized in Table 2. Similar maximum observed plasma
radioactivity, $C_{\text{max}}$, was noted between the Exercised and Non-Exercised groups. However, $C_{\text{max}}$ occurred earlier in the Exercised group ($p < 0.001$). There were no detectable differences in $\text{AUC}_{\text{last}}$, observed apparent clearance, observed apparent volume of distribution, or terminal phase elimination rate constants between groups for plasma PK parameters. More radioactivity per ml of plasma was present in the Exercised group at 360, 480, 600, and 720 minutes post-injection ($p < 0.05$) (Figure 7).

Table 2: Non-compartmental pharmacokinetic parameters of $^{99m}\text{Tc-MDP}$ activity in plasma after ACJ injection in exercised and non-exercised horses.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Exercised</th>
<th>Non-Exercised</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (DPM/ml)</td>
<td>22,485 3405</td>
<td>22,706 2743</td>
</tr>
<tr>
<td>* $T_{\text{max}}$ (min)</td>
<td><strong>16.00</strong> 2.35</td>
<td>43.75 3.64</td>
</tr>
<tr>
<td>$L_z$ (1/min)</td>
<td>0.00084 0.00021</td>
<td>0.00060 0.00016</td>
</tr>
<tr>
<td>$C_l/F$ (ml/min)</td>
<td>0.06874 0.00867</td>
<td>0.06126 0.00791</td>
</tr>
<tr>
<td>$V_{z}/F$ (ml)</td>
<td>91.54 11.83</td>
<td>139.80 30.77</td>
</tr>
<tr>
<td>$\text{AUC}_{\text{last}}$ (min*DPM/ml)</td>
<td>9,654,907 823,243</td>
<td>8,891,620 559,163</td>
</tr>
</tbody>
</table>

* Exercised and Non-Exercised are different ($p<0.05$)
Figure 7: Plasma radioactivity-time curve following ACJ injection of $^{99m}$Tc-MDP in exercised and non-exercised horses. Asterisks (*) represent a difference ($p \leq 0.05$) between groups.

A two-compartment pharmacokinetic model of $^{99m}$Tc-MDP activity following intra-articular injection was produced utilizing plasma data, describing absorption from the ACJ to plasma, distribution within peripheral tissues, and renal elimination (Figure 8). Compartmental parameter estimates of $^{99m}$Tc-MDP in plasma are summarized in Table 3. There was no difference between groups for rate constants of absorption of $^{99m}$Tc-MDP from joint to plasma ($K_A$), transfer of $^{99m}$Tc-MDP between plasma and peripheral tissues ($K_{1,2}; K_{2,1}$), or elimination of the technetium from plasma ($K_E$).
Figure 8: A two compartment model describing $^{99m}$Tc-MDP activity following intra-articular injection.

Table 3: Compartmental parameter estimates of $^{99m}$Tc-MDP in plasma after ACJ injection in exercised and non-exercised horses.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Exercised</th>
<th>Non-Exercised</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_A$ (1/min)</td>
<td>0.15280 0.04655</td>
<td>0.06314 0.02269</td>
</tr>
<tr>
<td>$K_E$ (1/min)</td>
<td>0.00209 0.00076</td>
<td>0.00120 0.00037</td>
</tr>
<tr>
<td>$K_{1,2}$ (1/min)</td>
<td>0.01466 0.00829</td>
<td>0.00521 0.00154</td>
</tr>
<tr>
<td>$K_{2,1}$ (1/min)</td>
<td>0.01236 0.00556</td>
<td>0.00140 0.00040</td>
</tr>
<tr>
<td>$V_1/F$ (ml)</td>
<td>41.59 9.36</td>
<td>37.12 8.09</td>
</tr>
<tr>
<td>$V_2/F$ (ml)</td>
<td>58.13 16.21</td>
<td>143.60 34.69</td>
</tr>
</tbody>
</table>

* Exercised and Non-Exercised are different (p<0.05)
Human and canine studies have described the use of intra-articular radioactive compounds, including $^{99m}$Tc-DTPA, to delineate the effects of various pathologic and physiologic conditions on synovial fluid and solute dynamics (James, 1994; Wallis, 1985a; Wallis, 1985b; Wisham, 1960). Specifically, studies by Wisham (1960) and James (1995) utilizing radioactive sodium and $^{99m}$Tc-DTPA, respectively, showed increased clearance of the markers in the immediate post-exercise period in human knees; these findings are similar to the results of the current study. Though intra-articular radiopharmaceuticals have been used in other species, the effect of exercise on their distribution has only been defined in humans. This study has demonstrated the safety and usefulness of $^{99m}$Tc-MDP for examining the effects of exercise on synovial solute distribution in horses, and for potential future application in equine joint disease. Intra-articular $^{99m}$Tc-MDP, with or without exercise immediately post-injection, was well tolerated in our horses. A mild, transient local inflammatory response occurred in both groups, evidenced by a small increase in joint circumference and a mild increase in synovial fluid nucleated cell count. The synovial fluid changes were consistent with those reported for repeated arthrocentesis, and were markedly less than those reported for normal saline injection. Arthrocentesis alone results in a mild increase in synovial nucleated cell count (2380 WBC/uL) without significant increase in protein content.
Intra-articular injection of saline has been shown to elicit moderately increased synovial leukocyte (31,388 WBC/uL) and protein (3.9 g/dL) concentrations at 24 hours post-injection, both of which returned to baseline values within 7 days (Wagner, 1982). The minor changes in clinical parameters, along with a lack of lameness during the study period, suggest that intra-articular $^{99m}$Tc-MDP is safe in normal horses.

Analysis of the scintigraphic radioactivity-time curve revealed what appeared to be biphasic elimination of $^{99m}$Tc-MDP from the scintigraphic region of interest in the Exercised group. A greater negative linear regression slope from 5 to 25 minutes post-injection was evident in the Exercised group, suggesting faster decline of radioactivity within the region of interest during early image collection. Furthermore, non-compartmental analysis of plasma data revealed a faster time to maximum plasma radioactivity ($T_{max}$) in the Exercised group. These findings suggested that the effects of exercise on $^{99m}$Tc-MDP clearance from the ACJ occurred early, resulting in faster transfer from synovial fluid to plasma and interstitium within the first 25 minutes following exercise. These findings are consistent with a study by Wisham (1960), which showed that the clearance constant of radioactive Na$^{24}$ from normal human knees increased and then returned to baseline within 8 minutes following short walking exercise. Also, clearance of IA $^{99m}$Tc-DTPA was shown to be significantly increased following dynamic exercise in human knees with effusion, over a total period of approximately 1 hour (James, 1994). Though human studies utilizing radiopharmaceuticals to determine distribution in the period immediately following
exercise have shown similar results to the current equine study, none have evaluated the
distribution of the radiopharmaceutical up to 24 hours post-exercise. Following the
immediate post-exercise period in the current study, $^{99m}$Tc-MDP appeared to have greater
retention within the joint in the Exercised group. Also, the observed apparent joint
clearance, calculated from the area under the curve for the joint scintigraphic data, was
lower in the Exercised group. Since the dose administered was not different between
groups, the apparent difference in joint Cl was attributed to the larger AUC$_{last}$ in the
Exercised group horses. This suggests greater exposure of the joint (scintigraphic region
of interest) to $^{99m}$Tc-MDP in the Exercised group over time, which correlates with the
greater retention of radioactivity within the ROI at later time points. A lack of specificity
in scintigraphic imaging results in the inclusion of the ACJ as well as surrounding
interstitium, vasculature, and bone in the scintigraphic region of interest, which may
account for this finding. The non-specific imaging modality contributed to the
appearance of greater joint retention. Greater delivery of the $^{99m}$Tc-MDP to bone and soft
tissue in the Exercised group due to higher cardiac output during exercise may also
account for greater joint exposure and the appearance of higher retention of radioactivity
in the region of interest in later time points in exercised horses. Though the scintigraphic
data indicated increased joint retention in the Exercised group, the plasma radioactivity
analysis, which indicated greater early transfer of $^{99m}$Tc-MDP from joint to plasma, is
more specific and likely more accurately represents the post-exercise distribution of
$^{99m}$Tc-MDP. The pharmacokinetic parameters calculated from both scintigraphic and
plasma data indicate greater transfer of $^{99m}$Tc-MDP in the immediate post-exercise period, with return to baseline from 25 minutes to 24 hours post-exercise.

Regional scintigraphic analysis indicated that exercise resulted in redistribution of $^{99m}$Tc-MDP from the joint into the surrounding tissues post-exercise. These findings suggest that intra-articular $^{99m}$Tc-MDP may be driven by exercise from the ACJ into the surrounding tissues and interstitium, followed closely by absorption into lymphatics and the venous system. Exercise is thought to increase synovial clearance through increased synovial blood flow, resulting in greater transport of solutes from the joint into circulation. A study evaluating articular blood flow in exercising and resting canines has demonstrated increased blood flow to all articular soft tissues with exercise (Simkin, 1990). Also, exercise has been shown to increase distal limb perfusion and subsequent radiopharmaceutical uptake into bone in horses undergoing nuclear scintigraphy (Dyson, 2001). In addition to increased cardiac output and peripheral perfusion during exercise, changes in IA pressure during cyclical flexion and extension of the joint are hypothesized to stimulate joint clearance (Macoris, 2001). The pumping action of the joint during motion is thought to drive synovial fluid from the joint (Levick, 1979a; Levick, 1979b; O’Driscoll, 1983), resulting in the creation of subatmospheric IA pressure while the joint is in a neutral position at rest. Elevation of IA pressure by infusion of saline leads to fluid transport out of the joint, and it is thought that elevation of IA pressure by joint flexion most likely does the same (Levick, 1979b; Nade, 1983). It has been shown that flexion of the metatarsophalangeal joint in anesthetized ponies leads to a reliable increase in IA pressure (Stolk, 1994). Furthermore, a study by Macoris and Bertone (2001)
showed that cyclical motion of effused joints (baseline IA pressure > 30mmHg) results in peak pressures greater than 100mmHg. These changes in IA pressure alter Starling forces across the synovium, increasing intra-articular hydrostatic pressure, which ultimately leads to fluid exchange between the synovial fluid and plasma (Hardy, 1995; Hardy, 1996; Levick, 1984). Intra-articular volume can affect IA pressure and synovial clearance. Because of this, a relatively small and uniform volume, 4ml net, was utilized for radiopharmaceutical injection. The mean synovial fluid volume in normal, healthy radiocarpal joints was estimated to be 11.67 ± 3.28ml by Smith in 1979. In unpublished pilot data by the author, the addition of 4 ml to the ACJ did not result in increased IA pressure by manometer measurement in 2 horses with joint angles of approximately 180 degrees. Furthermore, addition of 4ml to the midcarpal joint in anesthetized horses with joint angles of 135 degrees (mild flexion) resulted in elevation of IA pressure to about 14mmHg (Hardy, 1995). Even without a large change in intra-articular pressure following addition of 4ml fluid, the extra fluid may have altered synovial fluid flow following injection. Synovial membrane has a low osmotic reflection coefficient and relatively high permeability, and injection of fluid into the joint may have altered the initial clearance of the marker (Bertone, 1998). However, the effect would have been uniform between groups; therefore, any differences in early radiopharmaceutical distribution can be attributed to the effect of exercise. Though the approximately 4ml net volume gain in the current study may seem like a large volume with respect to the normal IA volume, the effects of the volume gain were uniform between groups and should have been minimal. A combination of increased synovial perfusion and increased IA pressure
leading to altered Starling forces likely led to the distribution of $^{99m}$Tc-MDP from the joint to surrounding tissues and vasculature following exercise.

$^{99m}$Technetium is a readily available radionuclide commonly used in both clinical and research settings for nuclear scintigraphy. The radionuclide can be bound to various pharmaceuticals, which target specific tissues or organ systems to enhance imaging studies. One of the most common applications is $^{99m}$Tc-MDP, in which $^{99m}$Tc, a metastable isomer of $^{99}$Tc, is bound to the bone-seeking pharmaceutical, methylene-diphosphonate. The resulting radiopharmaceutical is water soluble, with a molecular weight of 485.9423 [g/mol]. In the present study, the $^{99m}$Tc-MDP radiopharmaceutical was chosen because of its commonality, as well as its similarity with physical characteristics of some intra-articular medications. For instance, the molecular weights of two commonly used intra-articular corticosteroids, methylprednisolone acetate (416.51 g/mol) and triamcinolone acetonide (434.50 g/mol) are similar, and triamcinolone is also water soluble. Furthermore, the bone-seeking pharmaceutical was chosen in order to best assess the distribution of the compound to surrounding tissues. Other radiopharmaceuticals, such as $^{99m}$Tc-DTPA, undergo very little distribution to tissues and exhibit more rapid elimination via glomerular filtration. Such compounds may have allowed for a more simplified pharmacokinetic profile, and the use of $^{99m}$Tc-MDP may limit the conclusions of this study for this reason. The short half-life, accessibility, and physical characteristics of $^{99m}$Tc-MDP lend it well to applications such as pharmacokinetics research, in addition to its clinical uses.
A two-compartment model, characterized by distinct absorption/distribution and elimination phases, best described the plasma radioactivity data in the present study as determined by standard pharmacokinetic analyses. The left-shift of the plasma radioactivity-time curve of the Exercised group in the present study may be explained by an earlier absorption of technetium from the joint into plasma (Shargel, 1999). Likely due to our inability to fully separate and accurately calculate the rate constants for the absorption and distribution phases in plasma, no significant difference was found between groups for $K_A$. However, the non-compartmental analysis and linear regression analysis both showed earlier technetium appearance in plasma. It could be possible to detect $^{99m}$Tc-MDP earlier in plasma without a change in the absorption rate constant (min$^{-1}$), if the IA pressure changes associated with joint motion result in earlier movement of the pharmaceutical through the interstitial space and into the vasculature. Increased IA pressure is known to increase interstitial fluid transport, as described above. This earlier passage through the interstitium would also be expected to be associated with changes in the microvascular pressure profiles and fluid transport compared to static, normal joints as described in the isolated joint model (Bertone, 1998; Bragdon, 2001). The joint arterial, venous and capillary pressures and flows, microvascular resistance, net filtration pressure, transitional microvascular pressure, vascular and tissue compliances, and osmotic reflection coefficient could all potentially be altered by exercise, although they were not evaluated in the current study. Any of the changes associated with joint motion, particularly increased intra-articular pressure, could lead to earlier transfer of synovial fluids and solutes without a change in $K_A$. Therefore, the left-shift in the
radioactivity-time curve can best be explained by earlier absorption of $^{99m}$Tc-MDP from the ACJ to plasma in the early post-exercise period. $^{99m}$Tc-MDP exists in plasma as free and protein-bound forms. The free form is eliminated via glomerular filtration, and is therefore dependent on kidney function and glomerular filtration rate (Castronovo, 1977). Non-compartmental analysis of plasma data showed no difference in $L_z$, the terminal phase elimination rate constant from 8 to 24 hours post-injection. Furthermore, compartmental analysis revealed no difference between groups for $K_E$, the rate constant for elimination of technetium from plasma. $K_E$ describes the elimination from plasma; therefore, it represents the rate of renal elimination of $^{99m}$Tc-MDP. Exercise has been shown to decrease renal arterial blood flow via sympathetic nervous system stimulation, which could potentially result in a decreased glomerular filtration rate if the autoregulatory function is overcome (Endo, 2008; Gleadhill, 2000). Consequently, elimination of renally-cleared compounds, such as $^{99m}$Tc-MDP, could be expected to decrease in exercised animals. However, the lack of difference between groups in $L_z$ and $K_E$ does not support decreased renal elimination in our exercised horses. In fact, the short exercise period (5 minutes) may have had no effect on renal blood flow or glomerular filtration of $^{99m}$Tc-MDP. Future studies incorporating additional data collection and PK analysis, such as urine radioactivity measurements or intravenous dosing, would enable further interpretation of these results by providing additional distribution and elimination data. Also, studies involving a more intense or prolonged exercise protocol may be necessary to further examine the effects of exercise on renal elimination.
The use of intra-articular $^{99m}$Tc-MDP was shown to be a safe and effective method for evaluating the effects of exercise on synovial transport. Clearance of the radiopharmaceutical from the ACJ was increased immediately post-exercise, but this effect appeared to wane after approximately 25 minutes. The resulting plasma radioactivity-time curve exhibited a left-shift in the Exercised group as compared to the Non-Exercised group, and there was no apparent difference between groups for absorption, inter-compartmental transfer, and elimination rate constants in the 2-compartment model. In concert, our data suggested this was a result of earlier appearance of $^{99m}$Tc-MDP in plasma post-exercise. Additional studies to evaluate the effect of more intense exercise on elimination rate constants would be warranted. Further work may be necessary to define the effects of exercise on pharmacokinetics of intra-articular solutes, including joint medications, which may have an effect on treatment of clinical disease states and competition withdrawal times.


Levick JR. An investigation into the validation of subatmospheric synovial pressure recordings and their dependence on joint angle. *J Physiol* 1979a; 289:56-68.


Endnotes

2. BD Angiocath, BD, Franklin Lakes, NJ.
3. BD Vacutainer, BD, Franklin Lakes, NJ.
4. Omega 500s, Technicare, Solon, OH.
6. TS Meter, American Optical Corp., Southbridge, MA.
7. The Ohio State University Hematology Laboratory reference range.
8. Atomlab Dose Calibrator (scintillation counter), Biodex Medical Systems, Shirley, NY.
9. Mirage, v5.4, Segami Corporation, Columbia, MD.
10. Kobalt 6” Electronic Caliper with Digital Display, Kobalt, North Wilkesboro, NC.
12. GraphPad Prism, v5.02 for Windows, GraphPad Software, San Diego, CA.
Appendix A: American Association of Equine Practitioners Lameness Grading Scale

“Because each horse has unique performance characteristics, evaluating lameness can be challenging. Experienced riders may detect minor alterations in gait before they are apparent to an observer. Lameness may appear as a subtle shortening of the stride, or the condition may be so severe that the horse will not bear weight on the affected limb.

With such extremes of lameness possible, a lameness grading system has been developed by the AAEP to aid both communication and record-keeping. The scale ranges from zero to five, with zero being no perceptible lameness, and five being most extreme. The AAEP guidelines explain the grading system this way:

0: Lameness not perceptible under any circumstances.

1: Lameness is difficult to observe and is not consistently apparent, regardless of circumstances (e.g. under saddle, circling, inclines, hard surface, etc.).

2: Lameness is difficult to observe at a walk or when trotting in a straight line but consistently apparent under certain circumstances (e.g. weight-carrying, circling, inclines, hard surface, etc.).

3: Lameness is consistently observable at a trot under all circumstances.

4: Lameness is obvious at a walk.

5: Lameness produces minimal weight bearing in motion and/or at rest or a complete inability to move.” (AAEP, n.d.)