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# NON-STEROIDAL ANTI-INFLAMMATORY AGENTS AND MUSCULOSKELETAL INJURIES IN THOROUGHBRED RACEHORSES IN KENTUCKY

L. Dirikolu, W. E. Woods*, J. Boyles*, A. F. Lehner†, J. D. Harkins*, M. Fisher**, D. J. Schaeffer and T. Tobin*

*Department of Veterinary Sciences, College of Veterinary Medicine, University of Illinois, Urbana, Illinois; *Maxwell H. Gluck Equine Research Centre and the Department of Veterinary Science, University of Kentucky, Lexington, Kentucky; †Diagnostic Centre for Population and Animal Health, Michigan State University, East Lansing, Michigan; **The Kentucky Racing Commission, Ironworks Pike, Lexington, Kentucky, USA*

## ABSTRACT

Injuries sustained by horses during racing have been considered an unavoidable part of horse racing. Many factors may be associated with the musculoskeletal injuries of Thoroughbred racehorses. This study surveyed the post race concentrations of nonsteroidal anti-inflammatory agents (NSAIDs), namely naproxen, phenylbutazone and flunixin (3 most commonly used NSAIDs in the mid 1990s in horses), in the plasma of injured horses collected at Kentucky racetracks between 1st January 1995 and 31st December 1996. During that 2-year period, there were 84 catastrophic cases, ie involving euthanasia and 126 non-catastrophic cases. Plasma concentrations of NSAIDs were determined by liquid/liquid extraction combined with High performance liquid chromatography (HPLC) both for injured and control horses. The possible role of anti-inflammatory agents in musculoskeletal injuries of Thoroughbred racehorses was investigated by comparing the apparent concentrations of NSAIDs in injured and control horses. It was not possible to unify and control the sites from which the plasma samples were drawn from these horses. The apparent plasma concentrations of phenylbutazone and flunixin were higher in injured horses than those of control horses. However, a majority of the injured and control horses did not have detectable levels of naproxen in their plasma samples. Further studies are needed to determine whether horses with higher plasma concentrations of NSAIDs have an altered risk of musculoskeletal injuries compared with other horses.

## INTRODUCTION

For many years, injuries sustained by horses during racing have been considered an unavoidable part of horse racing. More recently, however, awareness and concern about race-related injuries have increased within the racing industry and the general public. These injuries exact a significant, but largely unmeasured, economic toll and generate adverse publicity for Thoroughbred racing. Although horse racing has existed for centuries, few studies on the potential causes of racing and training injuries have been conducted.

Both intrinsic (related to horse) and extrinsic factors (surrounding environment) can predispose horses to breakdown during racing. Intrinsic factors include conformation, age, gender and pre-existing injuries (Cohen *et al.* 1999; Hernandez *et al.* 2001; Hill *et al.* 2001; Williams *et al.* 2001). Extrinsic factors include environmental and nutritional conditions, length of race, racetrack surface, frequency of starts and training method (Cheney *et al.* 1973; Fredricson *et al.* 1982; Mundy 1997; Hernandez *et al.* 2001; Williams *et al.* 2001). Since 1940, it has been believed that poor track conditions are the major cause of musculoskeletal injuries (MIs) in Thoroughbred racehorses (Pratt and O'Connor 1978). One report concluded that horses raced on 'muddy-dirt' track surfaces had a significantly lower risk of breakdown than horses raced on 'normal-dirt tracks' (Mohammed *et al.* 1991). However, other investigators did not find an association between racetrack condition and risk of injury (Hill *et al.* 1986). An alternative idea is that pre-existing pathological conditions could result in an increased risk of musculoskeletal

injuries during racing or training (Stover *et al.* 1992). Therefore, pre-race physical examination by track veterinarians may determine possible pathological conditions, thereby reducing injuries among Thoroughbred racehorses (Cohen *et al.* 1997). A related concern is that, because more force is exerted on the forelimbs at high speed, faster races might cause more racing injuries (Pratt and O'Connor 1976).

Awareness of the factors that contribute to injuries of horses would enable trainers, owners, veterinarians and racing officials to reduce financial and emotional losses due to such injuries. Even though studies have attempted to evaluate the risk factors associated with musculoskeletal injuries in racehorses, it is believed that the overall number of injuries has increased rather than decreased in the past decade, despite modern diagnostic and treatment methods.

In the present investigation the possible role of one intrinsic factor, nonsteroidal anti-inflammatory drugs (NSAIDs), on musculoskeletal injuries of Thoroughbred racehorses at Kentucky racetracks, was studied. The main objective of the study was to evaluate the role of NSAIDs in musculoskeletal injuries and aims included: a) establish the prevalence of racing injuries involving the musculoskeletal systems of horses at 6 racetracks in Kentucky; b) develop methods for determination and quantification of NSAIDs in plasma of horses; and c) determine whether a correlation exists between presence of NSAIDs and racing injuries by comparing the amounts of these agents in the biological systems (plasma samples) of the injured horses with amounts in control horses.

## MATERIALS AND METHODS

### *Selection of horses*

All horses were diagnosed by a commission-appointed veterinarian of the Kentucky Racing Commission (KRC) as having sustained a musculoskeletal injury while racing in an official KRC race between 1st January 1995 and 31st December 1996. Each horse having an obvious change in soundness in the opinion of the commission veterinarian during the race or immediately after the finish of the race was considered to have a racing injury. If the injury was related to the musculoskeletal system, the horse was included in this study. Racing injuries were categorised as catastrophic or non-catastrophic, based on a post race examination by the commission veterinarian. During the study period, there were 210 injury cases on Kentucky racetracks meeting the above criteria. Of these, 84 (40%) were catastrophic and 126 (60%) were non-catastrophic. From these, 161 cases (70 catastrophic and 91 non-catastrophic) were included in the study.

### *Sample collection and handling*

Blood samples were taken from the injured horses in heparin tubes by a commission veterinarian, along with 2 control horses from the same race, the winner and a horse picked randomly by the stewards. The blood samples were centrifuged at 2,000 rpm and 4°C for 15 min to separate the plasma, which was frozen until analysed for NSAIDs by high pressure liquid chromatography (HPLC).

### *Statistical analysis*

To test the hypothesis that the injured horses have a higher level of NSAIDs than the controls, Sign test was used because the concentrations of NSAIDs in horses included in our study were not normally distributed nor transformable to a normal distribution (Systat, Version 12, Systat co., California, USA). The odds ratio (StatXact, Version 8, Sytel co., Massachusetts, USA) was used to compare the proportion of injured horses that had phenylbutazone concentrations greater than 7,000 ng/mL (pharmacologically effective level in horses) and flunixin concentrations greater than 100 ng/mL (pharmacologically effective level in horses) with the proportions of the control horses that had phenylbutazone concentrations greater than 7,000 ng/mL and flunixin concentrations greater than 100 ng/mL. The level for significance was set at $P>0.05$.

### *Analysis by high performance liquid chromatography (HPLC)*

### *General background*

Standard solutions of phenylbutazone, flunixin and naproxen (Sigma Chemical, Missouri, USA) were prepared in the HPLC mobile phase (60% Solvent A (Acetonitrile) (Fisher Scientific) and 40% Solvent B [12.5% methanol, 43.75% of 1% acetic acid, and 43.75% of 0.1 M ammonium acetate] (Fisher Scientific). Adequate amounts of drugs were measured and dissolved in mobile phase to yield 1 mg/mL stock drug concentration. Extraction standards were prepared by the addition of known amounts of phenylbutazone, flunixin and naproxen in mobile phase solution to blank plasma samples at a range of 100 to 4,000 ng/mL. Mefenamic acid (Sigma Chemical) in mobile phase (20 $\mu$L of 50 $\mu$g/mL mefenamic acid solution) was added to each sample, standard and blank as an internal standard.

The plasma samples (1 mL/sample) were placed in screw-top culture tubes which were first rinsed with dichloromethane (DCM) (Sigma Chemical). One mL of 0.1 M HCl (Fisher Scientific) and 10 mL of DCM were added to each tube. The tubes were tightly capped and checked for possible leaks by gently inverting each tube. These samples were mixed on a rotorack for 10 min, and all tubes were centrifuged at 4°C, 2,000 rpm for 90 min on a Beckman centrifuge to reduce the emulsions (Beckman Coulter, Inc., California, USA). The upper (aqueous) layer was discarded by aspiration, and the DCM (organic) phase was evaporated to ≤ 20 µl under a stream of $N_2$ at 40°C. All tubes were watched carefully to prevent complete drying. The residue was re-suspended first in 250 µl acetonitrile and vortexed. After that, 750 µl of mobile phase was added to each tube and the tubes were capped and vortexed. Each sample was then transferred to nitrogen gas rinsed amber autosampler vials; the surface of the vials was rinsed with nitrogen again and the vials capped. The HPLC vials were placed in an autosampler for HPLC analysis.

### *Instrumentation*

The instrument employed was a Beckman System Gold HPLC system with 2 110B solvent delivery Pumps, a 168 Photodiode array Detector and 502 Autosampler (Beckman Coulter, Inc., California, USA). The column was a Varian Bondesil C18, 5 *u* particle size, 4.6 mm x 25 cm column size (Varian, Inc., California). The mobile phase consisted of 60% Solvent A (acetonitrile), and 40% Solvent B [methanol (12.5%), 1% of acetic acid (43.75%), and 0.1 M ammonium acetate (43.75%) at a flow rate of 1 mL/min. Solvents and chemicals used in this assay were all HPLC grade, and the solvents were degassed and filtered (0.45 um. type HV Millipore) (Millipore Corp, Massachusetts). The UV detector wavelength was set at 240 nm, 263 nm, 280 nm and 300 nm optimised for compound detection. Twenty µL injections were made with a 20 µL loop.

## RESULTS

Two horses in the injured group and 2 horses in the special group had plasma concentrations of phenylbutazone between 110 and 380 µg/mL. This is a very unusual range for phenylbutazone in racehorses and these horses were not included in results or data analysis. The average apparent concentration of phenylbutazone plasma was 5,841 ng/mL ± 563 (SEM) for injured horses, 4,268 ng/mL ± 458 (SEM) for winning horses, and 4,337 ng/mL ± 454 (SEM) in special horses which presumably forms a standard baseline of plasma concentrations of phenylbutazone in horse racing in Kentucky (Table 1).

In injured horse cases (catastrophic and non-catastrophic), 15 horses did not have phenylbutazone in their plasma samples; 95 injured horse cases had apparent plasma concentrations of phenylbutazone less than 7 µg/mL (minimal effective plasma concentration of phenylbutazone). As shown by this data, about 70% of the injured horses running in Kentucky had apparent plasma concentrations of phenylbutazone of 0–7.0 µg/mL. The average apparent plasma concentration of phenylbutazone in winning horses was 4,268 ng/mL ± 458 (SEM); 31 winning horses did not have phenylbutazone in their plasma samples. Ninety-two winning horses had apparent plasma concentrations of phenylbutazone of less than 7 µg/mL. These data shows that, about 78% of the winning horses running in Kentucky had plasma concentrations of phenylbutazone of 0–7 µg/mL. The average apparent plasma concentration of phenylbutazone in special horses was 4,337 ng/mL ±454 (SEM); 36 cases did not have any phenylbutazone in their plasma samples and 82 cases had apparent plasma concentrations of phenylbutazone of less than 7 µg/mL. Approximately 75% of special horses running in Kentucky had apparent plasma concentrations of phenylbutazone of 0–7 µg/mL.

Statistical analysis showed that the apparent plasma concentrations of phenylbutazone in injured horses were significantly greater than in special (93/157, P=0.02) and winning (95/157, P=0.006) horses. The proportion of races in which the concentrations of phenylbutazone in special horses exceeded the concentrations in the winner horses did not differ significantly from 50% (69/157, P=0.5094). The odds ratio for the number of horses that had apparent phenylbutazone plasma concentrations greater than 7,000 ng/mL was not significant for all pairwise combinations (P>0.3).

The average apparent concentration of flunixin in plasma samples of injured horses was 1,632 ng/mL ± 158 (SEM), of winning horses was 1,067 ng/mL ± 78 (SEM), and of special horses was 695 ng/mL ± 69 (SEM) (Table 2). Among the

**TABLE 1: Mean apparent plasma concentrations of phenlybutazone (± SEM) in injured, special and winning horses**

| Horse | Mean ± SEM phenylbutazone concentration |
|---|---|
| Injured | 5,841 ng/mL ± 563 |
| Special | 4,337 ng/mL ± 454 |
| Winner | 4,268 ng/mL ± 458 |

**TABLE 2: Mean apparent plasma concentrations of flunixin (± SEM) in injured, special and winning horses**

| Horse | Mean ± SEM flunixin concentration |
|---|---|
| Injured | 1,632 ng/mL ± 158 |
| Special | 695 ng/mL ± 69 |
| Winner | 1,067 ng/mL ± 78 |

**TABLE 3: Mean apparent plasma concentrations of naproxen (± SEM) in injured, special and winning horses**

| Horse | Mean ± SEM naproxen concentration |
|---|---|
| Injured | 358 ng/mL ± 229 |
| Special | 127 ng/mL ± 104 |
| Winner | 75 ng/mL +- 40 |

injured horses, 127 had apparent plasma concentrations of flunixin greater than 0.1 $\mu$g/mL which is probably a pharmacologically effective plasma concentration of flunixin. Among winning horses, 112 had apparent plasma concentrations of flunixin greater than 0.1 $\mu$g/mL. In special horses, 87 had apparent plasma concentrations of flunixin greater than 0.1 $\mu$g/mL.

Apparent plasma concentrations of flunixin in injured (101/157, P=<0.0001) and winner horses (84/157, P=0.010) were significantly greater than in special horses. The proportion of races in which the concentrations of flunixin in injured horses exceeded the concentrations in winner horses did not differ significantly from 50% (81/157, P>0.2). Compared to special horses, the odds ratio for flunixin to be greater than 100 ng/mL was significantly greater than 1 (P<0.01) for both winning and injured horses. The odds ratio was not significant for injured compared to winning horses (P=0.063).

Most horses did not have detectable levels of naproxen in their plasma samples. The average apparent plasma concentration of naproxen in injured horses was 358 ng/mL ± 229 (SEM), in winning horses was 75 ng/mL +- 40 (SEM), and in special horses was 127 ng/mL ± 104 (SEM) (Table 3). Among injured, winning and special horses, 145 (~92%), 147 (~94%) and 146 (~93%), respectively, did not have any detectable level of naproxen in their plasma samples. Since most horses did not have any detectable level of naproxen in their plasma samples, the statistical analysis was not performed for apparent naproxen concentrations in study animals.

## DISCUSSION AND CONCLUSION

NSAIDs belong to various chemical classes, although most of them are organic acids and have in common antipyretic, analgesic and anti-inflammatory activity. Because of potent anti-inflammatory actions, NSAIDs have been used widely for the treatment of musculoskeletal disease in performance horses. Phenylbutazone is the most commonly used NSAID; it is generally used during the training programme of horses, but horses generally are not allowed to race on pharmacologically effective levels of this drug.

NSAIDs simply block the synthesis of prostaglandin and reduce the hypersensitivity of the inflammed tissue to pain, eventually normalising inflammed tissue. Unlike local and narcotic analgesics, NSAIDs do not block the pain perception, and because of this reason, it is believed that NSAID treatment, especially with phenylbutazone, should not be considered as a risk factor in musculoskeletal injuries of racing Thoroughbred horses. It has also been reported that during the 1970s, statistical results from California showed that the incidence of breakdowns among Thoroughbred racehorses stayed constant and during that period the use of phenylbutazone was legalised and the percentage of horses running on phenylbutazone was increased. On the other hand, it should be kept in mind that some prostaglandins play important roles in the early phase of bone healing (Rohde *et al.* 2000). It has been suggested that a local increase of prostaglandin concentrations is a response of bone to trauma and prostaglandins may stimulate differentiation and proliferation of osteoprogenitor cells during early bone healing.

There are no reports about flunixin-induced musculoskeletal injuries in Thoroughbred racehorses. Flunixin however, has a potent analgesic component that is reportedly stronger than other NSAIDs, such as phenylbutazone, and even more potent than some narcotic agents, such as pentazocine, meperidine or codeine (Ciofalo *et al.* 1977). These potent analgesic effects could be a possible risk factor in musculoskeletal injury of racing horses. Currently, treatment with naproxen is not considered as a possible risk factor in musculoskeletal injuries of racehorses.

In the present study, most of the horses in the injured, winning and special groups did not have any detectable concentrations of naproxen in their plasma samples. On the other hand, average apparent plasma concentrations of flunixin and phenylbutazone in injured horses were higher than that in both the winning and special horse categories. But the majority of the horses in the injured, winning and special groups (70%, 78%, 75%, respectively) had apparent plasma

concentrations of phenylbutazone less than 7,000 ng/mL (minimal effective plasma concentrations of phenylbutazone). On the other hand, most of the injured horses (81%) had apparent plasma concentration of flunixin at or greater than 100 ng/mL which is probably the minimal effective concentration of flunixin. Of the horses in the winning and special groups 71% and 55% had apparent plasma concentrations of flunixin at or greater than 100 ng/mL, respectively.

There is little information on the effects of NSAIDs on articular cartilage metabolism. The adverse effects of this group of agents in equine articular cartilage are therefore not known. Because little information is available on the adverse effects of NSAIDs on articular cartilage and the higher amounts of NSAIDs (especially given flunixin and phenylbutazone in injured horses plasma samples), it is possible that this group of drugs could change the articular cartilage metabolism resulting in instability of the treated joints. It is also possible that the amount of drugs in plasma could determine the possible role of this group of drugs, especially for flunixin and phenylbutazone in musculoskeletal injuries of racehorses. On the other hand, most injured horses had plasma concentrations of phenylbutazone below 7,000 ng/mL, which is the minimal effective blood concentration of phenylbutazone. Additionally, it is known that NSAIDs are mainly used in the treatment of a variety of musculoskeletal problems in performance horses. It is possible that these horses had pre-existing pathological conditions which were not severe before the race, that these changes could have been accentuated during the race due to application of additional force, and were then observed as musculoskeletal injuries. As mentioned earlier, horses with pre-race pathological conditions are at an increased risk of musculoskeletal injuries, and therefore, injuries observed in the present study might be the result of these pathological conditions rather than medication. In conclusion, further studies must be designed to determine whether higher plasma concentrations of NSAIDs can truly be associated with an increased risk of musculoskeletal injuries.

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