Nonsteroidal anti-inflammatory agents and musculoskeletal injuries in Thoroughbred racehorses in Kentucky

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Injuries sustained by horses during racing have been considered as an unavoidable part of horse racing. Many factors may be associated with the musculoskeletal injuries of Thoroughbred race horses. This study surveyed the amounts of nonsteroidal anti-inflammatory agents (NSAIDs) in injured horse's biological system (plasma) at Kentucky racetracks from January 1, 1995 through December 31, 1996. During that period, there were 84 catastrophic cases (euthanized horses) and 126 noncatastrophic cases. Plasma concentrations of NSAIDs were determined by High Performance Liquid Chromatography in injured and control horses. The possible role of anti-inflammatory agents in musculoskeletal injuries of Thoroughbred race horses was investigated by comparing the apparent concentrations of NSAIDs in injured horses to concentrations in control horses. The plasma concentrations of phenylbutazone and flunixin were higher in injured horses than in control horses. Most injured and control horses did not have a detectable level of naproxen in their plasma samples. Further studies must be carried out to determine whether horses with higher plasma concentrations of NSAIDs have an altered risk of musculoskeletal injuries compared with other horses.

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INTRODUCTION

Injuries sustained by horses during racing have been considered an unavoidable part of horse racing for many years. More recently, awareness and concern about race-related injuries have increased within the racing industry and among the general public. These injuries exact a significant, but largely unmeasured, economic toll from the racing industry worldwide 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 preexisting injuries (Cohen *et al.*, 1999; Axelsson *et al.*, 2001; 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 (Peters, 1940; Pratt & O'Connor, 1978). One report favoring this idea concluded that horses raced on 'muddy-dirt' track surface had a significantly lower risk of breakdown in comparison with horses raced on 'normal-dirt tracks' (Mohammed *et al.*, 1991). Other investigators did not find an association between the racetrack condition and the risk of injury (Hill *et al.*, 1986). An alternative idea is that preexisting pathologic conditions could increase the risk of musculoskeletal injuries during racing or training (Stover *et al.*, 1992). Therefore, a prerace physical examination by track veterinarians may determine possible pathologic conditions thereby, reducing possible injury cases 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 & O'Connor, 1976).

Other investigators (Mohammed *et al.*, 1991) reported that most racing horse injuries occurred in the first or second season

of racing (36%, 34%, respectively) compared with later seasons (5% after the fourth season). It was also revealed that most injuries (55%) occurred in summer (July through October). Horses raced in the summer had a three- to four-fold increased risk of breakdown compared with those raced in the winter time (Mohammed *et al.*, 1991). There was also a positive association between the age of the horse and the risk of injury. Similar results have also been reported by other investigators (Estberg *et al.*, 1996).

There are many other factors associated with musculoskeletal injuries of Thoroughbred racehorses that have not been reported previously. Most importantly, it was reported that musculoskeletal injuries account for most (approximately 93%) of the racing- and training-related racehorse deaths (Estberg *et al.*, 1996). Awareness of the factors that contribute to injuries of horses would enable trainers, owners, veterinarians, and racing officials to control better and, therefore, prevent financial and emotional losses caused by such injuries.

In the present investigation, the possible role of one intrinsic factor, that of nonsteroidal anti-inflammatory agents on musculoskeletal injuries was studied in Thoroughbred racehorses at Kentucky racetracks. Anti-inflammatory agents are commonly used in veterinary medicine to treat musculoskeletal problems, and the available products are generally classified as steroidal and nonsteroidal agents.

The main objective of this study was to evaluate the role of nonsteroidal anti-inflammatory agents in musculoskeletal injuries of racing Thoroughbred horses and include the following: a) develop methods for the determination and quantification of nonsteroidal anti-inflammatory drugs (NSAIDs) in plasma of horses, and b) determine whether a correlation exists between NSAIDs presence 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 included in these studies 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 January 1, 1995 and December 31, 1996. A complete study record was generated for each horse by assembling information from the racing injury report generated by the commission veterinarian, race summaries published in the Daily Racing Form, the Livestock Disease Diagnostic Center (LDDC) University of Kentucky, Lexington, KY necropsy report and the data generated in this study.

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 categorized as catastrophic or noncatastrophic, based on a postrace examination by the commission veterinarian. A racing injury was categorized as 'catastrophic' if the horse was euthanatized because of severity of injuries. If the horse was not euthanatized, the racing injury was categorized as 'noncatastrophic'.

From January 1, 1995 through December 31, 1996 there were 210 injury cases on Kentucky racetracks meeting the above criteria. Among these 210 injury cases, 84 (40%) cases were catastrophic and 126 (60%) cases were noncatastrophic. From these 210 injury cases, 161 (both catastrophic and noncatastrophic cases) were included in this NSAIDs study. Cases were excluded by careful examination of the study record. Six cases were excluded from the NSAID portion of the study because the injuries were unrelated to the musculoskeletal system. Twenty-two cases were excluded from the NSAID study because the causes of injuries were unrecognized and twenty-one cases were excluded due to the inability to complete the data sets. Among the remaining 161 cases, 70 cases were catastrophic and 91 cases were noncatastrophic.

Among the injury cases on Kentucky racetracks, three of the injuries occurred at Dueling Grounds in 1995 and one occurred at Blue Grass Downs racetrack in 1995. At Ellis Park track, there were 38 injury cases in 1995 and 42 injury cases in 1996. At Turfway Park, there were 39 injury cases in 1995 and 31 injury cases in 1996. At Churchill Downs, 22 injury cases were observed during 1995 and 19 injury cases occurred during 1996. At Keeneland, there were nine injury cases in 1995 and five injury cases in 1996. Among the 161 cases that were included in our study, 32 injury cases occurred in 1995 and 25 injury cases occurred in 1996 at Turfway Park track. There were 30 injury cases in 1995 and 33 injury cases in 1996 at Ellis Park track. Two of these injuries occurred at Dueling Grounds in 1995. There were six and 17 injury cases at Keeneland and Churchill Downs in 1995, respectively. Three and 13 of the cases that were included in study occurred in 1996 at Keeneland and Churchill Downs, respectively (Fig. 1).

Blood samples were taken from the injured horses in heparin tubes by a commission veterinarian, along with two control horses from the same race, the winner and a special horse picked randomly by the stewards of the track. The blood samples were centrifuged at 224 g and 4 °C for 15 min to separate the plasma, which was frozen until analyzed for NSAIDs by High Pressure Liquid Chromatography (HPLC). The total number of catastrophic and noncatastrophic cases from which plasma samples were available was 161 cases.

To test the hypothesis that the injured horses have a higher concentration of NSAIDs than the special and winner horses, a 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, Richmond, CA, USA). The odds ratio (StatXact, Version 8, Cytel, Cambridge, MA, USA) was used to compare the proportion of injured horses that had phenylbutazone concentrations greater than 7 μg/mL (proposed pharmacologically effective level in horses) and flunixin concentrations greater than 0.1 μg/mL (proposed pharmacologically effective level in horses)
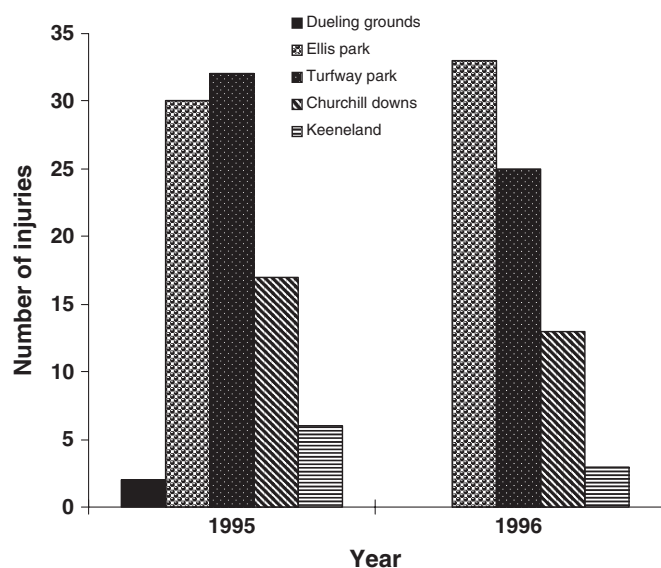
Fig. 1. Frequency distribution of injured horses included in MI study at Kentucky Race tracks.

with the proportions of the winner and special horses that had phenylbutazone concentrations greater than 7 μg/mL and flunixin concentrations greater than 0.1 μg/mL. The level for significance was set at $P < 0.05$.

Analysis by HPLC

Standard solutions of phenylbutazone, flunixin, and naproxen (Sigma Chemical, Saint Louis, MO) were prepared in the HPLC mobile phase 60% solvent A (Acetonitrile) (Fisher Scientific, Fair Lawn, NJ, USA) 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 0.1 μg/mL to 4 μg/mL. Mefenamic acid (Sigma Chemical) in mobile phase (20 μL of 50 μg/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. These tubes were rinsed with dichloromethane (DCM) (Sigma Chemical) before placing the samples in them. 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, 224 *g* for 90 min on a Beckman centrifuge to reduce the emulsions (Beckman Coulter, Inc., Fullerton, CA, USA). The upper (aqueous) layer was discarded by aspiration, and the DCM phase (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 resuspended 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 analysis.

The instrument employed was a Beckman System Gold HPLC system with two 110B solvent delivery Pumps, a 168 Photodiode array Detector and 502 Autosampler (Beckman Coulter, Inc., Fullerton, CA). The column was a Varian Bondesil C18, 5 *u* particle size, 4.6 mm × 25 cm column size (Varian, Inc., Lake Forest, CA, USA). 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 m*u*. type HV Millipore) (Millipore Corp, Bedford, MA, USA). The UV detector wavelength was set at 240 nm for naproxen, 263 nm for phenylbutazone, 280 nm for flunixin, and 300 nm for internal standard optimized for compound detection. Twenty μL injections were prepared with a 20 μL loop.

The area of the peaks corresponding to phenylbutazone, flunixin, naproxen, and mefenamic acid (internal standard) was recorded. The internal standard areas were used to normalize the phenylbutazone, naproxen, and flunixin areas. Integrated peak values were entered into Quattropro for statistical analysis of standards and also for calculation of unknown amounts of phenylbutazone, flunixin, and naproxen. Standard curves were generated with SigmaPlot (Version 3.03, Jandel Scientific, San Rafael, CA, USA). Samples including higher amount of drugs than the highest standard sample were diluted with blank plasma and re-analyzed.

RESULTS

Among 210 injury cases, 161 cases (70 cases catastrophic, 91 cases noncatastrophic) were included in this NSAIDs study. Because blood samples were available from all injured horses and also from the winner and a randomly selected horse, the data sets included samples from these three populations of horses.

Two horses in the injured group and two horses in the special group had plasma concentrations of phenylbutazone measured between 110 μg/mL and 380 μg/mL. This is a very unusual range for phenylbutazone in racehorses; these horses were not included in results and data analysis. It is possible that these samples might have been collected from the sites where the medication was administered. The average apparent concentration of phenylbutazone in plasma from injured horses was 5.84 μg/mL ± 0.563 (Standard Error of Mean, SEM), from winning horses was 4.268 μg/mL ± 0.458 (SEM), and from special horses plasma samples, which presumably forms a standard baseline of plasma concentrations of phenylbutazone in horse racing in Kentucky was 4.337 μg/mL ± 0.454 (SEM) (Fig. 2). Average apparent plasma concentration of phenylbutazone in catastrophic cases was 6.05 μg/mL ± 0.944 (SEM) and in noncatastrophic cases was 5.677 μg/mL ± 0.683 (SEM).
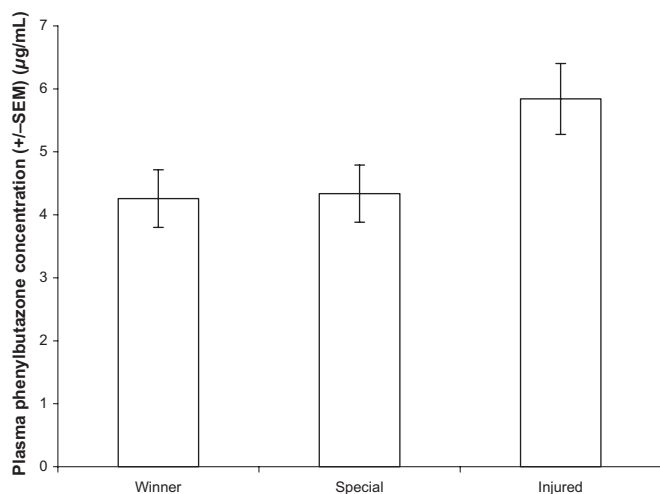
Fig. 2. Mean plasma concentrations (μg/mL, $\pm$SEM) of phenylbutazone in winner, special, and injured horses.

In injured horse cases (catastrophic and noncatastrophic), ninety-five injured horse cases had apparent plasma concentrations of phenylbutazone less than 7 μg/mL (proposed minimal effective plasma concentration of phenylbutazone), 15 horses had no detectable concentrations of phenylbutazone, 12 horses had apparent plasma concentration of phenylbutazone greater than 15 μg/mL, while 35 horses had apparent plasma concentration of phenylbutazone between 7 and 15 μg/mL (Fig. 3a). As shown by this data, about 70% of the injured horses running in Kentucky had apparent plasma concentrations of phenylbutazone between 0 μg/mL and 7.0 μg/mL. Forty-two and 53 horses in the catastrophic and noncatastrophic groups, respectively, had apparent phenylbutazone concentrations of less than 7 μg/mL.

The average apparent plasma concentration of phenylbutazone in winning horse cases was 4.268 μg/mL $\pm$ 0.458 (SEM). Thirty-one 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, 21 winning horses had apparent plasma concentration of phenylbutazone between 7 and 15 μg/mL, while 13 horses had greater than 15 μg/mL (Fig. 3b). As shown in these data, about 78% of the winning horses running in Kentucky had plasma concentrations of phenylbutazone between 0 μg/mL and 7 μg/mL.

The average apparent plasma concentration of phenylbutazone in special horse cases (these horses were randomly chosen by the stewards of the track in which injuries cases were observed) was 4.337 μg/mL $\pm$ 0.454 (SEM). Thirty-six 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. Ten horses had apparent plasma concentrations of phenylbutazone greater than 15 μg/mL, while 29 horses had plasma apparent concentration between 7 μg/mL and 15 μg/mL (Fig. 3c). Approximately, 75% of special horses running in Kentucky had apparent plasma concentrations of phenylbutazone between 0 μg/mL and 7 μg/mL.
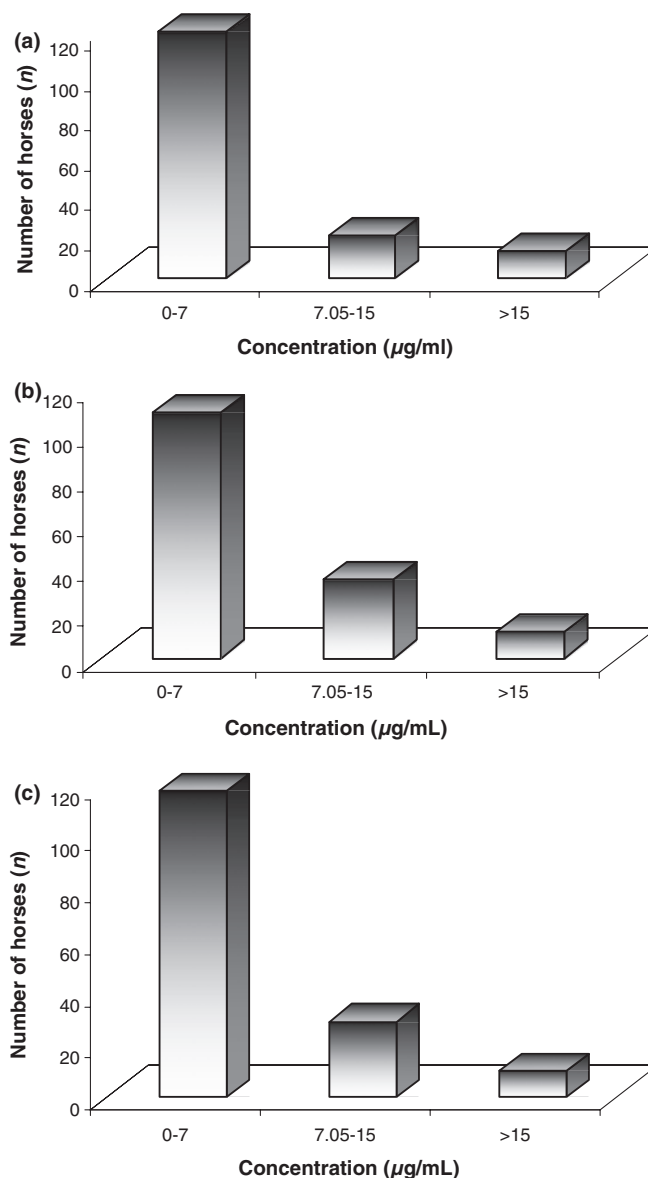

Fig. 3. Frequency distribution of apparent phenylbutazone concentrations in plasma samples from injured horses (a), winning horses (b), and special horses (c).

A sign test showed that the apparent plasma concentrations of phenylbutazone in injured horses were significantly greater than in special (93/157, $P = 0.02$) and winner (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 >7 μg/mL was not significant for all pair-wise combinations ($P > 0.3$).

The average apparent concentration of flunixin in plasma samples of injured horse cases was found to be 1.632 μg/mL $\pm$ 0.158 (SEM), of winning horse cases was 1.067 μg/mL $\pm$ 0.078 (SEM), and of special horse cases was 0.695 μg/mL $\pm$ 0.069 (SEM) (Fig. 4). The average apparent plasma concentration of

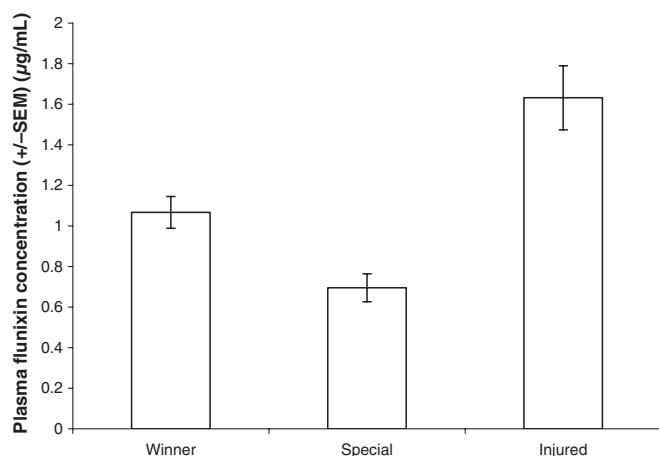**Fig. 4.** Mean plasma concentrations (μg/mL, ±SEM) of flunixin in winner, special, and injured horses.

flunixin in catastrophic cases was 1.923 μg/mL ± 0.289 (SEM) and in noncatastrophic cases was 1.403 μg/mL ± 0.167 (SEM).

Among 157 injured horses (catastrophic and noncatastrophic), 30 (about 19%) horses did not have flunixin in their plasma samples. Of 30 injured horses, 14 were in the catastrophic group and 16 were in the noncatastrophic group. One hundred and twenty seven injured horses had apparent plasma concentrations of flunixin of greater than 0.1 μg/mL, which is likely a pharmacologically effective plasma concentration of flunixin (Fig. 5a). Ten of these injured horses had apparent plasma concentration of flunixin greater than 3.55 μg/mL. Figure 5b shows a frequency distribution of plasma concentrations of apparent flunixin in plasma samples of winning horses. Of 157 horses, 45 (about 29%) cases did not have flunixin in their plasma samples. One hundred and eleven horses had apparent plasma concentrations of flunixin between 0.1 and 3.55 μg/mL, while one horse had between 3.56 and 7 μg/mL. Figure 5c shows a frequency distribution of apparent plasma concentrations of flunixin in plasma samples of special horses. Seventy horses (about 45%) did not have any detectable level of flunixin in their plasma samples, while the remaining 87 horses had apparent plasma concentrations of flunixin between 0.1 μg/mL and 3.55 μg/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 0.1 μg/mL was significantly greater than 1 ($P < 0.01$) for both winner and injured horses. The odds ratio was not significant for injured compared to winner horses ($P = 0.063$).

Most horses did not have a detectable level of naproxen in their plasma samples. The average apparent plasma
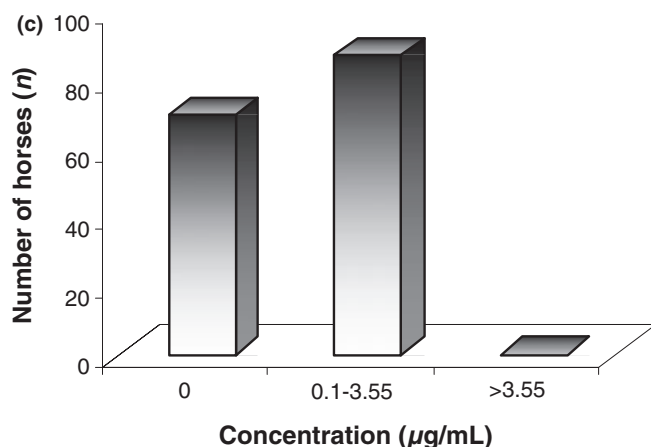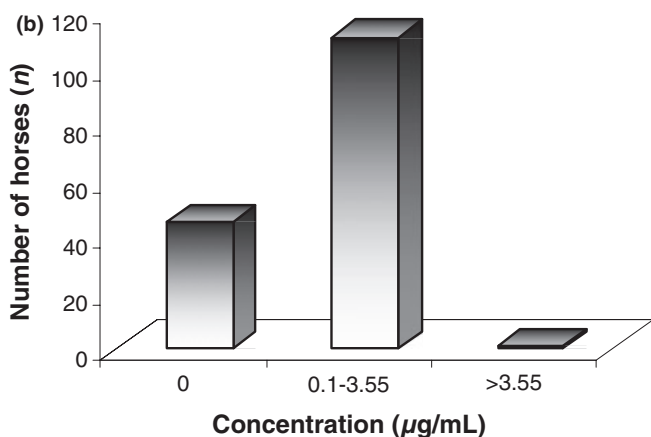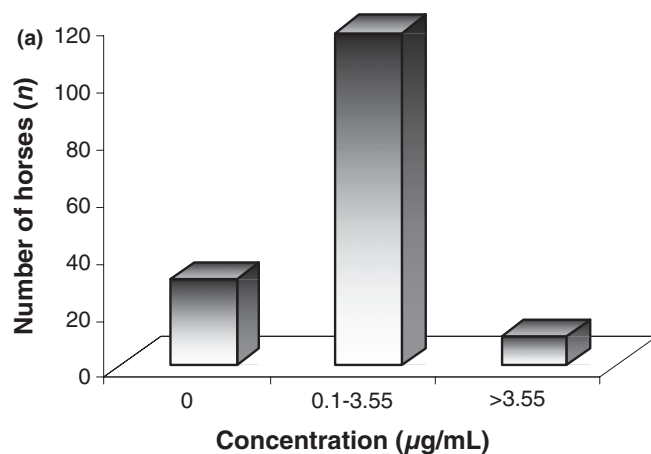

Fig. 5. Frequency distribution of apparent flunixin concentrations in plasma samples from injured horses (a), winning horses (b), and special horses (c).

concentration of naproxen in injured horse cases was 0.358 μg/mL ± 0.229 (SEM), in winning horse cases was 0.075 μg/mL ± 0.04 (SEM), and in special horse cases was 0.127 μg/mL ± 0.104 (SEM) (Fig. 6). The average apparent plasma concentration of naproxen in catastrophic cases was 0.026 μg/mL ± 0.014 (SEM) and in noncatastrophic cases was 0.618 μg/mL ± 0.407 (SEM).
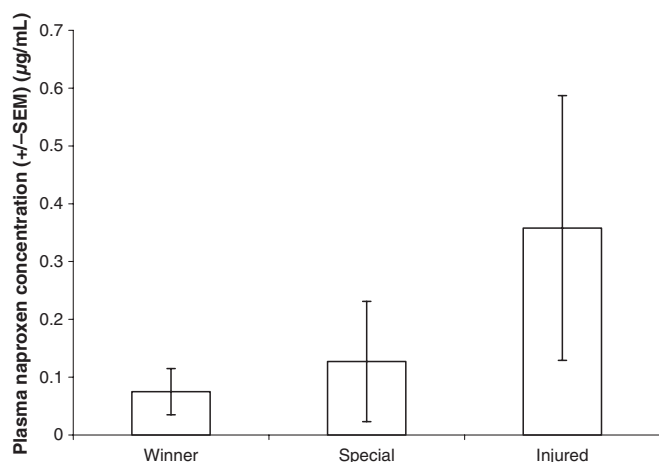
Fig. 6. Mean plasma concentrations (μg/mL, ±SEM) of naproxen in winner, special, and injured horses.

Of injured horse cases (catastrophic and noncatastrophic cases), 145 (~92%) cases did not have naproxen in their plasma samples. Eight horses had between 0.1 and 0.350 μg/mL, one horse had 0.852 μg/mL, and three horses had over 8 μg/mL (8.9, 11.3, 33.1 μg/mL) of naproxen concentrations in their plasma samples (Fig. 7a). In winning horse cases, 147 (~94%) cases did not have naproxen in their plasma samples. Six cases had between 0.1 and 0.35 μg/mL, one case had 0.550 μg/mL, and three cases had over 1 μg/mL (1.53, 3.83, 4.77 μg/mL) of naproxen in their plasma samples (Fig. 7b). In special cases, 146 (~93%) cases did not have any detectable level of naproxen in their plasma samples. Eight cases had between 0.1 and 0.350 μg/mL, one case had 0.406 μg/mL and two cases had over 1 μg/mL (1.96, 16.2 μg/mL) of naproxen in their plasma samples (Fig. 7c). As majority of the horses did not have detectable level of naproxen in their plasma samples, the statistical analysis was not performed for apparent naproxen concentrations in study animals.

DISCUSSION AND CONCLUSIONS

Even though the awareness and concern about race-related injuries have increased within the racing industry and general public, there are few studies on the potential causes of racing and training injuries. Identification of the alterable risk factors could provide methods for preventing or controlling racing injuries. Even though studies have attempted to evaluate the risk factors associated with musculoskeletal injuries in racing horses, it is believed that the overall number of injuries has increased rather than decreased in the past decade despite the modern treatment and diagnosis methods in equine medicine.

Nonsteroidal anti-inflammatory agents belong to various chemical classes, although most of them are organic acids and have in common anti-pyretic, 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
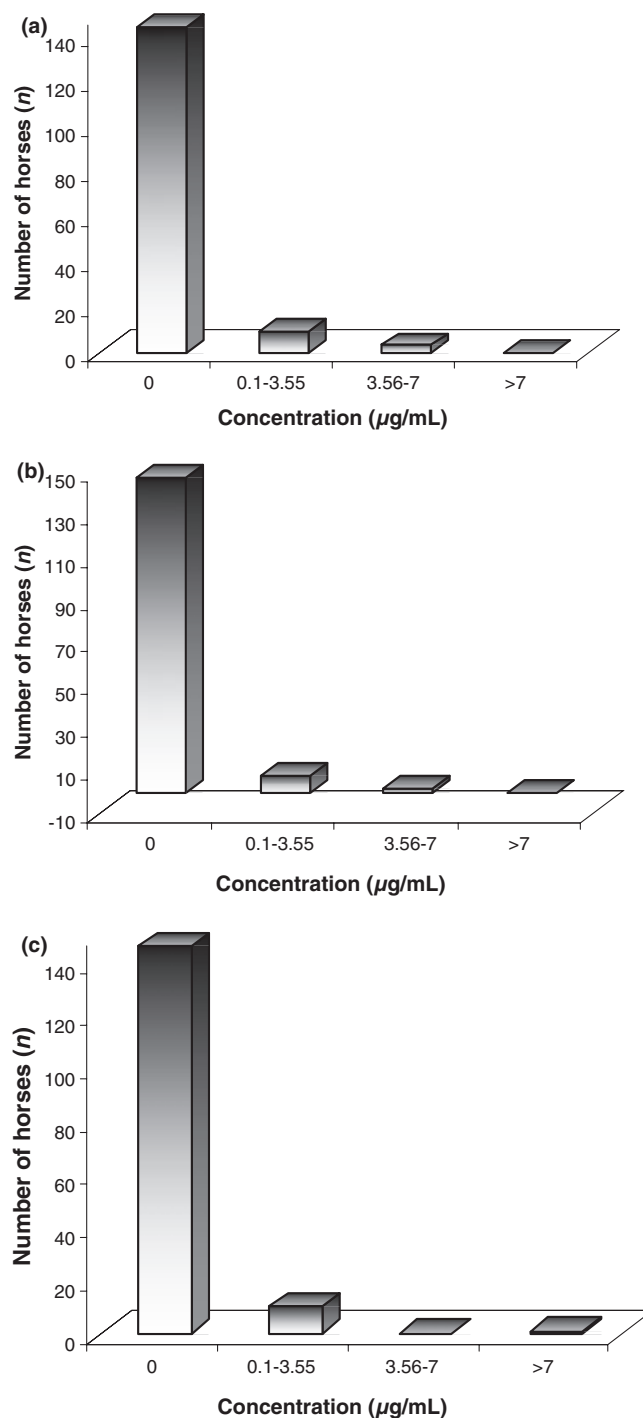

Fig. 7. Frequency distribution of apparent naproxen concentrations in plasma samples from injured horses (a), winning horses (b), and special horses (c).

commonly used NSAID in the USA; it is generally used during the training program of horses, but horses generally are not allowed to race on pharmacologically effective levels of this drug.

Nonsteroidal anti-inflammatory agents simply block the synthesis of prostaglandin and reduce the hypersensitivity of the inflamed tissue to pain, eventually normalizing inflamed tissue. Analgesic effects of this group of drug are different from

local anesthetics and narcotic analgesics. 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 1970's, statistical results from California showed that the incidence of breakdowns among Thoroughbred racehorses stayed constant, and during that period the use of phenylbutazone was legalized 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 prostaglandins 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 is 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. It has been reported that daily dose of 5 g naproxen during training to 25 yearling Quarter horse colts reduced the overall frequency of musculoskeletal injuries dramatically by four-fold during the training phase and by thirty-fold during the racing phase compared with 25 untreated control horses (Hamm, 1978). Therefore, treatment with naproxen is not considered as a possible risk factor in musculoskeletal injuries of racehorses.

In this 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 winning and special horse categories. But the majority of the horses in injured, winning, and special group (70%, 78%, 75%, respectively) had apparent plasma concentrations of phenylbutazone less than 7 μg/mL. On the other hand, majority of the horses in injured group (81%) had apparent plasma concentration of flunixin at or greater than 0.1 μg/mL, which is likely the minimal effective concentration of flunixin (Dr. Thomas Tobin, personal communication, Lexington, KY). Seventy-one percent and 55% of the horses in the winning and special groups had apparent plasma concentrations of flunixin at or greater than 0.1 μg/mL, respectively.

There is little information on the effects of NSAIDs on articular cartilage metabolism. Most of the research has only concentrated on anti-inflammatory actions of this group of agents in the equine cartilage model. The adverse effects of this group of agents in equine articular cartilage are therefore not known. Studies conducted on isolated chondrocytes and cartilage explants have shown that in the presence of NSAIDs, there was a reduction in the rate of glycosaminoglycan synthesis

(Muir *et al.*, 1988), and the secretion of completed proteoglycan (Adolphe, 1986). It is known that the proteoglycan content of joint cartilage accounts for its elasticity and ability to resist compression. It has also been reported that indomethacin at relatively high doses inhibits chondrocyte division in rabbit cell monolayer cultures (Hunter *et al.*, 1984) and salicylate suppressed the augmented proteoglycan synthesis in canine osteo-arthritic cartilage *in vitro* (Palmoski *et al.*, 1980). Based on an *in vitro* human chondrocytes study, it has been suggested that naproxen does not have deleterious effects on articular chondrocytes (Bassleer *et al.*, 1992). A recent report examined the effects of continuous oral administration of phenylbutazone on serum and synovial fluid biomarkers of skeletal matrix metabolism in horses (Fradette *et al.*, 2007). It was shown that phenylbutazone significantly increased the concentration of osteocalcin in synovial fluid compared to a control group. The results of this study suggested an undetermined anabolic effect of phenylbutazone administration on periarticular bone or a transient induction of osteogenesis in articular chondrocytes or a mesenchymal subpopulation of synoviocytes. It has also been shown that phenylbutazone decreases the mineral apposition rate in cortical bone and appeared to decrease the healing rate of cortical defects in horses (Rohde *et al.*, 2000). In a related study, it has been shown that oral administration of phenylbutazone for 14 days significantly reduces proteoglycan synthesis in articular culture explants from healthy horses to a degree similar to that induced by *in vitro* exposure to interleukin-1ß (IL-1ß) (Beluche *et al.*, 2001). This study suggested that phenylbutazone should be used judiciously in athletic horses with osteoarthritis, because chronic administration may suppress proteoglycan synthesis and potentiate cartilage damage.

Most of the studies conducted on the adverse effects of NSAIDs on articular cartilage commonly used normal articular cartilage explants, and these studies were conducted *in vitro*. Further research must be conducted by using the diseased articular cartilage and *in vivo* studies to determine the possible effects of NSAIDs in an equine cartilage model. It has been reported that NSAID effects on cartilage metabolism could be different, related to whether cartilage was taken from weight-bearing or non-weight-bearing locations and also related to the disease state of the cartilage (Palmoski & Brandt, 1983). It could be possible that NSAID's effects on articular cartilage could be detrimental because of inhibition of proteoglycan synthesis especially in the presence of degenerative joint disease as is the case with the known effects of corticosteroids.

As mentioned, because little information is available on the adverse effects of NSAIDs on articular cartilage and the higher amounts of NSAIDs (especially flunixin and phenylbutazone in injured horses plasma samples), it is possible that this group of drugs (if administered repeatedly) 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 indicate a possible role of this group of drug (especially for flunixin and phenylbutazone) in musculoskeletal injuries of racehorses because the average concentrations of these two drugs were higher in injured horses. Phenylbutazone is

characterized by a narrow therapeutic index in horses. The proposed therapeutic concentration of phenylbutazone in horses is 7 μg/mL (Jenny *et al.*, 1979). On the other hand, a majority of injured horses had a plasma concentration of phenylbutazone less than 7 μg/mL, which is the proposed 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 could be possible that these horses had preexisting pathological conditions that were not severe before the race, that these changes could have been accentuated during the race because of application of additional force, and were then observed as musculoskeletal injuries by a commission veterinarian of the KRC. As mentioned before, horses with prerace pathologic conditions are at an increased risk of musculoskeletal injuries, and therefore, injuries observed in this study might be the results of these pathologic conditions and not the results of medication.

At the time of this study, we were not able to control the sample collection sites from the study animals. Therefore, we must add the caveat that some of these samples might have been collected from the sites where the medication was administered, which could significantly affect the results and conclusions of this study. However, it is our suspicion that any such samplings errors, if they exist, were distributed randomly throughout our sample population. 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. It is very clear that other possible risk factors (age, racetrack surface, length of race, gender, training program, preexisting pathologic conditions, etc) contribute to musculoskeletal injuries of horses, and these must be eliminated and/or must be similar for each individual horse in future studies to determine the role of NSAIDs as a possible risk factor in musculoskeletal injuries of racehorses.

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REFERENCES

Adolphe, M. (1986) Articular chondrocytes in culture: applications in pharmacology. *Advances in Cell Culture*, **5**, 19–37.

Axelsson, M., Bjornsdottir, S., Eksell, P., Haggstrom, J., Sigurdsson, H. & Carlsten, J. (2001) Risk factors associated with hind limb lameness and degenerative joint disease in the distal tarsus of Icelandic horses. *Equine Veterinary Journal*, **1**, 84–90.

Bassleer, C., Henrotin, Y. & Franchimont, P. (1992) Effects of sodium naproxen on differentiated human chondrocytes cultivated in clusters. *Clinical Rheumatology*, **11**, 60–65.

Beluche, L.A., Betone, A.L., Anderson, D.E. & Rohde, C. (2001) Effects of phenylbutazone on bone activity and formation in horses. *American Journal of Veterinary Research*, **62**, 1916–1921.

Cheney, J.A., Shen, C.K. & Wheat, J.D. (1973) Relationship of racetrack surface to lameness in Thoroughbred racehorses. *American Journal of Veterinary Research*, **39**, 1285–1289.

Ciofalo, V.B., Latranyi, M.B., Patel, J.B. & Taber, R.I. (1977) Flunixin: a non-narcotic analgesic. *The Journal of Pharmacology and Experimental Therapeutics*, **200**, 501–507.

Cohen, N.D., Peloso, J.G., Mundy, G.D., Fisher, M., Holland, R.E., Little, T.V., Misheff, M.M., Watkins, J.P., Honnas, C.M. & Moyer, W. (1997) Racing-related factors and results of pre race physical inspection and their association with musculoskeletal injuries incurred in Thoroughbreds during races. *Journal of American Veterinary Medical Association*, **211**, 454–463.

Cohen, N.D., Mundy, G.D., Peloso, J.G., Carey, V.J. & Amend, N.K. (1999) Results of physical inspection before races and race-related characteristics and their association with musculoskeletal injuries in Thoroughbreds during races. *Journal of American Medical Association*, **5**, 654–661.

Estberg, L., Stover, S.M., Gardner, I.A., Johnson, B.J., Case, J.T., Ardans, A., Read, D.H., Anderson, M.L., Barr, B.C., Daft, B.M., Kinde, H., Moore, J., Stoltz, J. & Woods, L.W. (1996) Fatal musculoskeletal injuries incurred during racing and training in Thoroughbreds. *Journal of American Veterinary Medical Association*, **208**, 92–96.

Fradette, M.E., Celeste, C., Richard, H., Beauchamp, G. & Laverty, S. (2007) Effects of continous oral administration of phenylbutazone on biomarkers of cartilage and bone metabolism in horses. *American Journal of Veterinary Research*, **68**, 128–133.

Fredricson, I.A., Berguist, A., Bjorne, K., Hjerten, G. & Alm, L.O. (1982) Racing on hardwood fiber tracks. *Thoroughbred Racing*, **215**, 501–510.

Hamm, D. (1978) Continuous administration of naproxen to the horse during training. *Journal of Equine Medicine and Surgery*, **2**, 125–128.

Hernandez, J., Hawkins, D.L. & Scollay, M.C. (2001) Race-start characteristics and risk of catastrophic musculoskeletal injury in Thoroughbred racehorses. *Journal of American Medical Association*, **1**, 83–86.

Hill, T.D., Carmicheal, D., Maylin, G. & Krook, M. (1986) Track condition and racing injuries in Thoroughbred horses. *Cornell Veterinarian*, **76**, 361–379.

Hill, A.E., Stover, S.M., Gardner, I.A., Kane, A.J., Whitcomb, M.B. & Emerson, A.G. (2001) Risk factors for and outcomes of nancatastrophic suspensory apparatus injury in Thoroughbred racehorses. *Journal of American Medical Association*, **7**, 1136–1144.

Hunter, T., Duncan, S., Dew, G. & Reynolds, J. (1984) The effects of antirheumatic drugs on the production of collagenase and tissue inhibitor of metalloproteinases (TIMP) by stimulated rabbit articular chondrocytes. *Journal of Rheumatology*, **11**, 9–13.

Jenny, E., Steinijans, V.W. & Seifert, P. (1979) Pharmacokinetic interaction of isopropylaminophenazone and phenylbutazone in the horse. *Journal of Veterinary Pharmacology and Therapeutics*, **2**, 101–108.

Mohammed, H.O., Hill, L. & Lowe, J. (1991) Risk factors associated with injuries in Thoroughbred horses. *Equine Veterinary Journal*, **23**, 445–448.

Muir, H., Carney, S.L. & Hall, L.G. (1988) Effects of tiaprofenic acid and other NSAIDs on proteoglycan metabolism in articular cartilage explants. *Drugs*, **35**, 15–23.

Mundy, G.D. (1997) Review of risk factors associated with racing injuries. *Proceedings of the 43rd Annual Convention of the Association of American Equine Practioners*, pp. 204–210, Phoenix, AZ.

Palmoski, M.J. & Brandt, K.D. (1983) Benoxaprofen stimulates proteoglycan synthesis in normal canine knee cartilage in vitro. *Arthritis Rheumatism*, **26**, 771–774.

Palmoski, M.J., Colyer, R.A. & Brandt, K.D. (1980) Marked suppression by salicylate of the augmented proteoglycan synthesis in osteoarthritic cartilage. *Arthritis and Rheumatism*, **23**, 83–91.

Peters, J.E. (1940) Lameness incident to training and racing of the Thoroughbred. *Journal of American Veterinary Medical Association*, **96**, 200–209.

Pratt, G.W. & O'Connor, J.T. Jr (1976) Force plate studies of equine biomechanics. *American Journal of Veterinary Research*, **37**, 1251–1255.

Pratt, G.W. & O'Connor, J.T. Jr (1978) A relationship between gait and breakdown in the horse. *American Journal of Veterinary Research*, **39**, 249–253.

Rohde, C., Anderson, D.E., Bertone, A.L. & Weisbrode, S. (2000) Effects of phenylbutazone on bone activity and formation in horses. *American Journal of Veterinary Research*, **61**, 537–543.

Stover, S.M., Johnson, B.J., Daft, B.M., Read, D.H., Anderson, M., Barr, B.C., Kinde, H., Moore, J., Stoltz, J. & Ardans, A.A. (1992) An association between complete and incomplete fractures of the humerus in race horses. *Equine Veterinary Journal*, **24**, 260–263.

Williams, R.B., Harkins, L.S., Hammond, C.J. & Wood, J.L. (2001) Racehorse injuries, clinical problems and fatalities recorded on British racecourses from flat racing and national Hunt racing during 1996, 1997 and 1998. *Equine Veterinary Journal*, **5**, 478–486.