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The use of phenylbutazone in the horse

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This review presents a brief historical prospective of the genesis of regulated medication in the US racing industry of which the nonsteroidal anti-inflammatory drug (NSAID) phenylbutazone (PBZ) is the focus. It presents some historical guideposts in the development of the current rules on the use of PBZ by racing jurisdictions in the US. Based on its prevalent use, PBZ remains a focus of attention. The review examines the information presented in a number of different models used to determine the effects and duration of PBZ in the horse. They include naturally occurring lameness and reversible-induced lameness models that directly examine the effects and duration of the administration of various doses of PBZ. The review also examines indirect plasma and tissue models studying the suppression of the release of arachidonic acid-derived mediators of inflammation. The majority of studies suggest an effect of PBZ at 24 h at 4.4 mg/kg. This reflects and substantiates the opinion of many clinical veterinarians, many of whom will not perform a prepurchase lameness examination unless the horse is free of NSAID. This remains the opinion of many regulatory veterinarians responsible for the prerace examination of race horses that they wish to examine a horse without the possibility of an NSAID interfering with the examination and masking possible musculoskeletal conditions. Based on scientific studies, residual effects of PBZ remain at 24 h. The impact of sustained effect on the health and welfare of the horse and its contribution to injuries during competition remains problematic.

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HISTORICAL PROSPECTIVE

Phenylbutazone (PBZ) is second only to aspirin as one of the oldest nonsteroidal anti-inflammatory drugs (NSAIDs). It was introduced into veterinary medical practice in the 1950s and still remains one of the more commonly used NSAIDs in the horse (Tobin *et al.*, 1986). In 1959 it was approved for use in racing by the State of Colorado and some attribute this ruling as the beginning of the era of controlled medication in racing (Tobin, 1981). PBZ became news worthy in 1968 when Dancers' Image won the Kentucky Derby and the postrace urine tested positive for PBZ. By the early 1970s it was legalized in most states and became well established by the mid 1970s (Gowen & Lengel, 1993). In some racing jurisdictions as long as the sum of the combined urine concentration of PBZ and its metabolite, oxyphenbutazone (OPBZ), did not exceed a prescribed concentration, the horse was not in violation of the medication rules. In 1977 the National Association of State Racing Commissioners Veterinary-Chemist Advisory Committee concluded that 'PBZ does not change a horse's innate ability to race, but by relieving inflammation it may enable the horse to race closer to maximum capabilities' (Gabel *et al.*, 1977). In the late 1979, the use of PBZ came under scrutiny which resulted in the publication of the book 'The Misuse of Drugs in Horse Racing: a Survey of Authoritative Information on Medication of Race Horses' by the Illinois Hooved Humane Society. This publication stirred controversy on the use of PBZ especially on race day and many jurisdictions revised their rules on race day use of PBZ. In 1982 a Committee appointed by the National Association of State Racing Commissions recommended 2 μg/mL as the decision or regulatory plasma/serum concentration of PBZ. Thin layer chromatography was the primary method of drug screening in urine at this time. This proposed concentration (2 μg/mL) was based on the concerns of racing chemists that high PBZ blood concentrations would produce urinary PBZ and metabolites that would interfere with or 'mask' detection of other drugs (Gabel *et al.*, 1977). Further studies indicated that the 'masking effect' was not a concern (Woods *et al.*, 1985a,b, 1986; Tobin *et al.*, 1986) and the upper plasma/serum threshold concentration was increased to 5 μg/mL. Complete uniformity does not exist among racing jurisdictions many have remained at 2 μg/mL and others are at 5 μg/mL, or at some concentration in between.

CLINICAL OPINIONS ON THE USE OF PBZ

Phenylbutazone is considered valuable drug in the training of sore horses to maintain fitness in those with early joint or ligament problems. The use of an NSAID such as PBZ enables a horse to continue training or return to training in a shorter period. On the other hand a major drawback to the use of PBZ is the veterinarians' inability to evaluate the degree of lameness with this medications present in the horse's system (Cannon, 1973). It was also the opinion of many veterinarians that PBZ would allow a horse to compete with mild chronic arthritic changes, but did not possess sufficient anti-inflammatory activity to allow a horse with a serious injury to compete. The NSAID can be used to restore normal performance in a horse debilitated by some injury to joints, tendons, or muscle achieved by its anti-inflammatory actions and relief of pain. The short-term effects are not in doubt, but the long-term merits of continuous administration of PBZ in many cases are problematic. The cynical remark that some therapies, such as corticosteroids and NSAID allow the patient to walk to the postmortem room is an overstatement but the veterinarian must consider the long-term effect of therapy and that resting the horse may be the best approach (Sanford, 1983). Many veterinarians consider the use of NSAID justified in show-horses, show-jumpers, and combined training and have presented opinions on the use of PBZ based on the activity of the horse (Dunn, 1972). The United States Equestrian Federation rules allow higher concentration during competition compared to racing industry rules. There is great therapeutic value in the use of PBZ in the treatment of acute inflammatory conditions or in older horses in a nonracing environment for the treatment of chronic osteo-arthritis where it can extend the useful life of the horse (Barragry, 1973).

A moral dilemma confronts the practicing veterinarian when prescribing PBZ or other medications for the treatment of the varied musculoskeletal condition in competition horses especially race horses; will the medication allow a horse to maintain a training schedule thereby allow the animal to function or is the medication contributing to further injury to the detriment of the horse? This is especially true in younger horses with a fresh injury and an unsuspecting owner administering an NSAID and inflicting further damage. Many veterinarians agree that the use of anti-inflammatory drugs could mask unsoundness in horses being examined in a prepurchase examination for soundness (Dunn, 1972). NSAIDs including PBZ, have masked clinical signs that have resulted in cecal perforation (Ross *et al.*, 1985). Masking of existing musculoskeletal condition is the concern of regulatory veterinarians who are examining horses on a daily basis knowing that the examination is not in a medication-free horse.

In a multicentre field study, PBZ and suxibuzone, a prodrug of PBZ, were equally effective in the treatment of a number of acute, chronic, nonspecific lameness in which all horses were consistently lame upon trotting. Approximately 50% of the horses showed an improvement within 3 days of treatment with 30% showing an additional improvement at 6 days (Sabate *et al.*, 2009). This study illustrated the concern of many veterinarians as to the duration of administration of PBZ; if significant improvement had not occurred within 4–5 days re-evaluation should be performed (Jeffcott & Colles, 1977; Reilly, 2000).

Toxicity of PBZ in the horse and ponies has been reviewed and several factors may predispose towards PBZ toxicity in the horse, including breed and age, but high dose is considered to be particularly important (Lees & Higgins, 1985). Clinical experience suggests that PBZ can be administered to horses in modest doses for a prolonged period of time without detectable side-effects.(Tobin *et al.*, 1986). Blood dyscrasias commonly described in man have not been reported in the horse and despite the lack of documented evidence, toxicity of PBZ in the horse is considered to be lower than that in human. PBZ should not be administered if there are signs of gastro-intestinal ulceration, clotting defects or any cardiac, renal or hepatic dysfunction (Jeffcott & Colles, 1977). Despite the apparent lack of toxicity, adverse effects on the gastrointestinal track have been reported when administered at high doses (Karcher *et al.*, 1990; Meschter *et al.*, 1990a,b). Possible toxic effects of NSAID are not limited to PBZ. Multiple daily administration of therapeutic doses of ketoprofen (2.2 mg/kg), flunixin meglumine (1.1 mg/kg), or PBZ (4.4 mg/kg) i.v., every 8 h, for 12 days produced changes in the glandular portion of the stomach; that was the area of the gastrointestinal tract most severely affected. Results of CBC, serum biochemical analyses, and fecal occult blood tests were not different from those of control horses with the exception of PBZ-treated horses that had a significant decrease in serum total protein and albumin concentrations.(MacAllister *et al.*, 1993). Moderate to severe ulcerative colitis was diagnosed during necropsy, exploratory celiotomy, and biopsy; it was concluded that the ulcerative lesions may have gone unreported due to the anti-inflammatory effects of NSAID (Karcher *et al.*, 1990). Renal papillary necrosis has been reported in horses to which PBZ was administered (Gunson, 1983) and medullary crest necrosis was reported in horses placed on maintenance doses of PBZ (Read, 1983). Renal crest necrosis has also been reported in horses to which flunixin and PBZ were administered (MacAllister *et al.*, 1993). In horses on daily doses of 8.8 mg/kg for 21 days plasma albumin concentrations decreased significantly from days 10 to 21, treatment also caused neutropenia. No other clinical or hematologic abnormalities were detected for PBZ or control horses (McConnico *et al.*, 2008). A retrospective study of 269 horses administered $\leq$ 8.8 mg/kg/day PBZ for 4 days or the lower dose of 2–4 mg/kg of body weight/day for up to 50 days remained clinically normal (Collins & Tyler, 1984).

The current lack of toxicity and observable side effects were based on the realization that the loading dose (4.4 mg/kg twice for 4 days) recommended by the manufacturer could be reduced. A revised schedule of 4.4 mg/kg twice daily for 1 day followed by 2.2 mg/kg twice daily for 4 days, then 2.2 mg/kg daily or as needed increased the margin of safety as no changes in clinical biochemistry or hematology were observed (Taylor *et al.*, 1983b). This modified dose regimen did not compromise clinical efficiency (Taylor *et al.*, 1983a). The American Association of Equine Practitioners recommends a dose of 2.2 mg/kg daily with the last dose not more than 24 h prior to post time (Harvey, 1983). Clinical use of PBZ for many years suggests that with

adequate care, hydration, and the use of the lower therapeutic doses of PBZ can be used safely without clinically detectable side effects. The exception is the administration of a combined treatment of PBZ with flunixin to horses as detrimental effects may outweigh any potential benefits. Gastroscopy of four horses revealed substantial gastric ulcers when administered the combination (Reed *et al.*, 2006).

In the equine, a dose-finding study for PBZ has not been reported, that is the evaluation of the improvement in clinical conditions at various doses. The current dose schedules are based on years of clinical use by many or administered doses to meet regulatory requirements of the industry. Based on the opinions and observations of veterinarians, investigators conducted a number of studies to determine the plasma PBZ concentrations 24 h following various dosing schedules, formulations, and dosages (Soma *et al.*, 1983; Chay *et al.*, 1984; Houston *et al.*, 1985; Soma *et al.*, 1985). Following completion of these studies, the recommended dosing schedule was as follows: oral administration of 4.4 mg/kg (2 g) for 3–4 days followed by a single i.v. dose of 4.4 mg/kg 24 h prior to racing. If these dosing recommendations were followed, plasma PBZ concentrations on race day should not exceed 5 μg/mL. However, these studies did not attempt to determine the pharmacological effect of PBZ at 24 h or the pharmacological effects of a plasma concentration of 5 μg/mL, and this was the major drawback in the studies.

A prime consideration in the continuous use of PBZ or other NSAIDs is the possible contribution to catastrophic and noncatastrophic injuries. In the human sport medicine, which also applies to the veterinary field, there is a lack of high-quality evidence to guide practitioners in their use and the possible adverse effects that have clinical relevance. Potential negative consequences on long-term use and the healing process are slowly growing (Fournier *et al.*, 2008). Specifically, NSAIDs are not recommended in the treatment of fractures, stress fractures or chronic muscle injury. The only exception may be very short-term use for analgesic purposes or as an adjunct to other analgesics. Judicious use of NSAID may be more appropriate in the management of acute muscle and ligament sprains, tendinitis, and muscle injury. However, length of treatment should always be kept as short as possible (Mehallo *et al.*, 2006).

Cyclooxygenase activity is involved in the healing of many skeletal tissues, either directly or indirectly through modulation of the inflammatory response. Consequently, pharmacological manipulation of cyclooxygenase using NSAID can profoundly affect skeletal health. All of the NSAIDs should not be painted with a broad brush as having negative effects on healing and recovery of all types of injuries. In particular, NSAID use does not appear to have a long-term negative effect on tendons and ligaments and NSAID therapy may inhibit adhesion formation during tendon healing, which leads to a better functional recovery (O'Connor *et al.*, 2008). There is limited information on the use of PBZ and other NSAID on healing and synovial membrane health in the horse and many of the studies were conducted *in vitro*.

Oral administration of PBZ for 14 days significantly decreased proteoglycan synthesis in articular culture explants from healthy horses; these authors suggested that PBZ should be used judiciously in equine athletes with osteoarthritis, because chronic administration may suppress proteoglycan synthesis and potentiate cartilage damage (Beluche *et al.*, 2001). It has been suggested that the use of PBZ early in the postoperative period may interfere with bone healing (Rohde *et al.*, 2000). In horses with experimentally–induced osteoarthritis the use of a COX-2 inhibitor, diclofenac, induced significantly less radial carpal bone sclerosis and overall gross cartilage erosion, compared with PBZ. Results obtained suggest that diclofenac had both clinical sign-modifying and disease-modifying effects. Only clinical sign-modifying effects were detected in association with PBZ administration (Frisbie *et al.*, 2009).

The effects of NSAIDs, including PBZ, were investigated on lipopolysaccharide challenged and unchallenged equine synovial membrane in terms of production of prostaglandins E_2 (PGE_2) and hyaluronan, viability, and histomorphologic characteristics. These investigators concluded that the commonly used NSAIDs suppress induced synovial membrane PGE_2 production without detrimental effects on synovial membrane viability and function (Moses *et al.*, 2001). Results of studies have also suggested that hyaluronan and carprofen might exert an anti-arthritic action through stimulation of PG synthesis and there is possible justification for therapeutic administration of enantiomeric rather than racemic carprofen (Frean *et al.*, 1999). Others have also suggested that use of carprofen in osteoarthritis horses may induce beneficial changes in articular cartilage matrix (Armstrong & Lees, 1999).

Catastrophic injuries remain an unavoidable but public relations nightmare in the racing industry, despite the fact that injuries in athletic competition are expected. The immediate perception in racing is that the injury is drug-related, when in fact there are many horse-related and external factors that contribute to musculoskeletal injuries. One study did conclude that higher concentrations of PBZ and other NSAID did contribute to a higher incidence of racetrack injury (Dirikolu *et al.*, 2009). The plasma concentrations of PBZ in this report were higher than are currently allowed in most racing jurisdictions. Racing jurisdictions allow plasma concentrations of PBZ or flunixin; therefore, many horses are competing at plasma concentrations near the allowable limits. About 20% of the plasma samples exceeded 5 μg/mL at time of prerace examination (R. Arthur, Personal communication). The question remaining is what are the long-term effects of the continuous use of a NSAID on the musculoskeletal health of the horse? Are the short-term benefits of allowing the horse to compete under the influence of an NSAID worth the long-term risks? The veterinarian does have a greater choice of NSAID than just PBZ for the treatment of osteoarthritis in horses (Goodrich & Nixon, 2006) and it is inevitable that a horse may have to compete on a residual concentration of drug used during training. It may be of benefit to the horse to expand the veterinarian's regimen of allowable residual concentration of a more diverse list of NSAID.

PHENYLBUTAZONE AND PERFORMANCE

Results from performance studies suggested that PBZ had no clear effect on the performance of normal, healthy horses (Sanford, 1974). Plasma concentration of prostaglandins were increased in human (Demers *et al.*, 1981) and equine during exercise (Birks *et al.*, 1991; Mitten *et al.*, 1995). These exercise-induced increases in cyclooxygenase activity were inhibited by the administration of PBZ, but PBZ did not produce detectable changes in systemic hemodynamic or acid–base variables in either standing or running horses (Hinchcliff *et al.*, 1994). In exercising horses, the effect of inhibition of cyclooxygenase activity on the hemodynamic responses were examined. Administration of PBZ abolished the exertion-induced increases in plasma 6-ketoprostaglandin F_1 alpha and TXB_2. PBZ treatment resulted in significantly higher heart rates and right atrial pressures than control. There was no effect of PBZ on carotid or pulmonary arterial pressures, oxygen consumption, carbon dioxide production, blood lactate concentrations, or plasma volume during exertion. These results suggest that cyclooxygenase products likely mediate or modulate some of the systemic hemodynamic responses to exertion in horses (Mitten *et al.*, 1996), but there is no evidence that the administration of PBZ and/or the suppression of cyclooxygenase products alters performance. In a similar study, the administration of PBZ (4.4 mg/kg) to the horse did not show significant differences from control horses in heart rate, right atrial, and pulmonary vascular pressures during high speed treadmill studies (Manohar *et al.*, 1996). Endurance-like exercise (12 km/h for 3 h) did not affect the kinetic disposition of PBZ and dexamethasone. The conclusion of these authors was that resting horses can be used for determination of pharmacokinetics as no differences were noted in the disposition kinetics and the plasma–concentration time curves for the horse when at rest or sampling during exercise (Authie *et al.*, 2010).

NOCICEPTION (PAIN PERCEPTION)

Pain experience and expression is difficult to determine in the horse as it is influenced by many factors such as species, breed, individual variations, and environmental characteristics. Equally difficult to assess is the alteration of pain by analgesic drugs. Latency to the onset of flexion of the limb in response to a noxious thermal stimulus and heat-evoked skin twitch have been reliable and reproducible measures of pain threshold and a nociceptive end-point for analgesic studies in the horse (Kamerling *et al.*, 1985). Thermal-evoked skin-twitch reflex and thermal-evoked hoof withdrawal reflex have been used to compare analgesic activity of procaine, mepivacaine and PBZ. Compared to procaine and mepivacaine, PBZ failed to alter pain thresholds over 36 hours postadministration (Kamerling *et al.*, 1984). This type of stimulation produces an acute pain response and can be objectively used to compare the duration of regionally administered anesthetic agents and other drugs used to reduce the perception of pain. In the horse, PBZ was indistinguishable from saline controls when using a thermal stimulus (Kamerling *et al.*, 1983). PBZ was not an effective drug when used to block thermal and specific nociceptive pain stimuli.

POSTOPERATIVE PAIN

Postoperative pain can be considered primarily a nociceptive pain produced by trauma to tissues due to direct intervention and disruption of these tissues. Inflammation due to surgical trauma is a part of the pain response and the use of NSAIDs has been promoted for this purpose postoperatively. Minimal differences were noted between PBZ and placebo administration in a group of horses undergoing arthroscopic surgery (Raekallio *et al.*, 1997). In a similar postoperative study, flunixin, PBZ or carprofen was administered intra-operatively just prior to the end of anesthesia. The time following surgery when additional analgesic drugs were required postoperatively were, 8.4, 11.7 and 12.8 h for PBZ, carprofen, and flunixin, respectively. Horses that were administered the opioids, butorphanol, during surgery needed significantly fewer analgesic agents postoperatively (Johnson *et al.*, 1993).

In a double-blind, randomized, prospective study of human patients undergoing arthroscopic surgery, those who were administered a prostaglandin inhibitor (naproxen sodium) had significantly less pain, less synovitis, less effusion, and faster recovery (Ogilvie-Harris *et al.*, 1985; Rasmussen *et al.*, 1993) than those without. In equally as large a prospective study, no advantages were observed over control group of patients when compared to physical therapy and administration of the NSAID, diclofenac (Birch *et al.*, 1993).

The use of NSAID in combination with more potent opioids for high-intensity pain and the weaker opioids for moderate- to low-intensity pain has been the topic of numerous publications discussing emerging trends in pain management (Schug *et al.*, 2007; Fischer *et al.*, 2008; Huang *et al.*, 2008; Layzell & Layzell, 2008). Despite the use of PBZ postoperatively on a routine basis, similar studies in the total management of postoperative pain using NSAID combined with the opioids are lacking in the equine. The role of NSAID in the management of postoperative pain was suggested in an early publication (Mather, 1992) and authors still suggest they may contribute to improved functional outcomes without significant adverse effects (Reuben & Reuben, 2007).

CENTRAL NERVOUS SYSTEM EFFECTS AND CROSSING OF THE 'BLOOD–BRAIN BARRIER'

Phenylbutazone has no known spinal or central nervous system (CNS) effects that are involved in the suppression of pain. The effects are primarily thought to be peripheral in action without CNS action or any noticeable sedation. To exert a central effect, NSAIDs have to cross the blood–brain barrier. Transfer across the blood-brain barrier is controlled by simple physico-chemical factors. OPBZ, indomethacin, and ketoprofen are characterized by high lipophilicity. At steady state, their free plasma

concentrations correspond to their cerebral spinal fluid concentrations (Bannwarth *et al.*, 1989). The presence of these NSAIDs in the brain may explain the antipyretic properties and some side effects of the NSAID. Concentrations of OPBZ in spinal fluid are similar to corresponding concentrations of unbound free OPBZ in plasma, which is approximately 5% of the total concentration of OPBZ in plasma (Gaucher *et al.*, 1983). Similarly, cerebral spinal fluid concentrations of ketoprofen reflect the unbound plasma ketoprofen concentrations and were in equilibrium with the plasma concentration from 2 to 13 h after administration (Netter *et al.*, 1985). Ibuprofen, flurbiprofen, and indomethacin rapidly cross the blood–brain barrier. Plasma protein binding limits the driving force for uptake of NSAID into the brain by reducing the free fraction of NSAID in plasma (Parepally *et al.*, 2006). The observation that long-term treatment of patients with ibuprofen results in a reduced risk and delayed onset of Alzheimer's disease suggests that it crosses the blood–brain barrier, has a central effect, and reduces inflammation in the Alzheimer's disease brain (Dokmeci & Dokmeci, 2004). Attempts to correlate the CSF concentrations of indomethacin with its regional inflammatory suppression and analgesic activity have not been successful (Bannwarth *et al.*, 1989). The assumption that all NSAID relieve pain only through an inhibition of prostaglandins synthesis, have no antinociceptive effects, central effects, and all actions are peripheral in nature have been challenged (McCormack & Brune, 1991).

PBZ IN SYNOVIAL FLUID

The efficacy of NSAIDs in joint diseases depends on their concentrations within the joint as the cells within the joint are the major site of action (Furst, 1985). There is no barrier to the diffusion of unbound NSAID into the joint cavity and their therapeutic effectiveness is determined by passage across the synovial membrane, which can depend on the degree of inflammation of the joint and on the pharmacokinetic properties of the drugs. Most NSAIDs are weak acids with a pK_a between 3 and 6 and the un-ionized forms are lipid soluble. The NSAID are primarily in the ionized form as the pK_a values are much lower than the pH of blood. The proportion changes to un-ionized as the environment becomes more acidic as in the stomach, kidneys and more importantly inflamed tissues (Day *et al.*, 1987). Inflamed joints concentrate NSAID because the pH of the synovial fluid is much lower than noninflamed joints. For example, the synovial concentration of OPBZ was higher in human patients with severe inflammation than in those with no or little inflammation (Gaucher *et al.*, 1983). Similar observations were made in an inflammatory carrageenan rat paw model where the concentrations of C^{14}PBZ was approximately 800-fold greater than plasma (Graf *et al.*, 1975).

NSAIDs are highly protein bound, but effect of protein binding on disposition into synovial fluid may not be a consideration, as bound drug will dissociate as fast as free drug diffuses. (Simkin, 1988). In inflammatory joint diseases, albumin-bound fraction diffuses better due to the increased capillary permeability to proteins; therefore, the concentration of the NSAID will be higher in inflamed tissues. (Netter *et al.*, 1989).

NSAIDs are classified in two categories based on their half-lives. Drugs with a short half-life, shortly after administration, the concentration in synovial fluid was lower than in plasma but reversed as the plasma concentration declines. In the horse, ketoprofen was no longer detectable in plasma after 5 h whereas synovial fluid concentrations were detected for 8 h. In the same study, carprofen with a half-life 10 times longer than ketoprofen, the concentrations in synovial fluid were significantly lower that plasma at all time points (Armstrong *et al.*, 1999). In rheumatoid patients on chronic therapy this may be a possible reason for the drug's extended duration of action of drugs with apparent short elimination half-lives in plasma (Fowler *et al.*, 1983). On the other hand, drugs with a longer half-life such as PBZ, the peak concentrations in synovial fluid were lower than plasma, remained lower and decreased in parallel with the plasma concentration (Netter *et al.*, 1983). This difference in the pharmacokinetics based on elimination half-life was observed in horses with no joint disease. Following the i.v. administration of naproxen, synovial concentrations peaked at ~8 h; were lower and followed a parallel decline in plasma and synovial fluid concentrations for up to 36 h. There were no differences in the secondary disposition rate constant for plasma and elimination rate constants for the synovial fluid indicating a parallel decline in both concentrations of naproxen (Soma *et al.*, 1995). Although this study was not done for PBZ in the horse similar relationships would be expected as the pharmacokinetics are similar (Soma *et al.*, 1983).

These differences based on the pharmacokinetic characteristics of the drug, delays in achieving synovial fluid concentrations and more importantly in assessing the effects of the administered drug to a diseased subject make it difficult to establish correlations between plasma concentrations and therapeutic response (Famaey, 1985). In human patients with osteoarthritis, the synovial fluid concentrations of PBZ were lower than plasma, with a good correlation between the two. In human patients with rheumatoid arthritis synovial PBZ concentrations were higher based on the greater inflammatory nature of the disease and a higher synovial fluid protein concentration (Farr *et al.*, 1982). In clinico-pharmacological study in humans, a relationship was present between dose, plasma concentration, and clinical effects of PBZ (Brooks *et al.*, 1975).

Many authors have suggested that the plasma concentrations of NSAIDs, do not correlate well with assessments of therapeutic response. This may reflect weaknesses in experimental design, capability of determining the changes in pain levels and inflammation, and in clinical studies the variability in the diseases being studied. It may be that concentrations in plasma bear only a distant relationship to those in the inflamed tissues where NSAIDs, presumably act (Famaey, 1985; Grennan *et al.*, 1985; Simkin, 1988). Compared with the CNS, NSAID readily penetrate into the joint and concentrations are not limited to the unbound fraction and will vary with the synovial environment. Studies in non-diseased joints are useful to describe the relative relationships and pharmacokinetics of the drug, but may have

little relationship in the diseased joint. Despite the many studies and years of its use in the horse, plasma synovial relationships in the non-diseased and naturally occurring diseased joint have not been reported.

NATURALLY OCCURRING OSTEOARTHRITIS

In a randomized controlled clinical trial, efficacy and safety of paste formulations of firocoxib (Equioxx®; Merial, Duluth, GA, USA) and PBZ in horses with naturally occurring osteoarthritis were compared. Horses were treated with firocoxib (0.1 mg/kg, orally every 24 h) or PBZ (4.4 mg/kg, orally every 24 h) for 14 days. Clinical improvement was defined as a reduction of at least 1 lameness score grade or a combined reduction of at least 3 points in scores for pain during manipulation or palpation, joint swelling, joint circumference, and range of motion. Results obtained suggested some greater improvement in some categories tested than others following firocoxib, but overall clinical efficacy of firocoxib and PBZ in horses were comparable (Doucet *et al.*, 2008).

Horses with naturally occurring forelimb and hind limb lameness were exercised on a treadmill and the degree of lameness evaluated by the use of kinematic analysis while trotting on the treadmill. Horses entered into the study were judged to have AAEP lameness scores of 1–3 based on a scale of upper severity score of 5 (Ross, 2003). In a cross-over study, PBZ paste was administered at 2.2 mg/kg (orally every 12 h for 5 days), alone or in combination with flunixin meglumine administered at 1.1 mg/kg, (i.v. every 12 h for 5 days). Lameness evaluations were performed before and 12 h after administration of two NSAID treatment regimens. Administration of a combination of the two NSAIDs alleviated lameness more effectively than did oral administration of PBZ alone. Based on the authors' conclusion, when evaluating all 28 horses, there was a significant clinical improvement after the administration of both drugs in all horses except five with forelimb lameness. PBZ alone did not result in significant clinical improvement in all horses. Results of this study suggested that the use of combinations of NSAID (stacking) did have a better effect at 12 h and would have a greater effect at 24 h. The authors suggested that 'stacking of drugs' should be a real concern (Keegan *et al.*, 2008).

The analgesic effects of PBZ in nine horses with chronic forelimb lameness were studied. The horses were administered saline for control or PBZ at 4.4 and 8.8 mg/kg i.v. daily for 4 d. Peak vertical force (force plate) was measured and AAEP clinical lameness scores were assigned before initiation of each treatment. All horses were evaluated 6, 12, and 24 h after the final dose. The vertical force was significantly increased at all post-treatment evaluation times after PBZ compared to control horses. Clinical lameness and vertical force scores were significantly decreased at 6 and 12 h at both doses and no differences were observed between the low or high dose. Scores were significantly decreased 24 hours after treatment only when PBZ was administered at the high dose (Hu *et al.*, 2005).

Force plate analysis and the AAEP lameness scoring system were used to evaluate the analgesic efficacies of flunixin (1.1 mg/kg), PBZ (4.4 mg/kg), or physiologic saline solution administered i.v. in 12 horses with navicular syndrome. Medications were administered once daily for 4 days with a 14-day washout period between treatments. At 6, 12, and 24 h after the fourth treatment, AAEP lameness evaluations and force plate data indicated significant improvement in lameness from baseline values in horses treated with flunixin or PBZ, compared with saline-treated control horses. The effect of flunixin or PBZ was maintained for at least 24 h but no differences from control were noted at 30 h. Flunixin meglumine and PBZ appear to have similar analgesic effects in horses with navicular syndrome (Erkert *et al.*, 2005).

The analgesic effects of the NSAID, ketoprofen at 2.2 and 3.63 mg/kg and PBZ at 4.4 mg/kg were compared in seven horses with bilateral forelimb chronic laminitis. Hoof pain was quantified objectively by means of an electronic hoof tester and lameness was subjectively graded on a modified Obel scale (Obel, 1948). Ketoprofen administered at 3.63 mg/kg (equimolar to 4.4 mg/kg of PBZ) reduced hoof pain and lameness score to a greater extent than the 2.2 mg/kg dose of ketoprofen or the 4.4 mg/kg of PBZ. These data suggest that ketoprofen at 1.65 times the recommended therapeutic dose was more potent than PBZ in alleviating chronic pain and lameness in horses. PBZ (4.4 mg/kg) and high dose of ketoprofen were still effective at 24 h (Owens *et al.*, 1995).

Horses (12) with navicular syndrome were fitted with 3° heel-elevation horseshoes and a force plate was used to measure baseline peak vertical ground reaction force of the forelimbs. Vertical force was measured 24 h and 14 days after shoeing and 24 h following the administration of PBZ (4.4 mg/kg, i.v. q 12 h) for 5 days. There was further significant improvement in vertical force measured 24 h following PBZ treatment. Heel-elevation shoeing alone and in combination with PBZ administration quantitatively decreased lameness in horses with navicular syndrome; injection of distal interphalangeal joint with triamcinolone acetonide did not significantly improve the vertical force measurements (Schoonover *et al.*, 2005).

INDUCED LAMENESS MODELS

The objective was to test the hypothesis that PBZ alleviates lameness in an adjustable heart bar-shoe model of equine foot pain following a single i.v. dose of 4.4 mg/kg. Heart rate and lameness score (1–5) were assessed every 20 min for 2 h and then hourly through 9 h. A lameness grade of four was produced for the study and no lameness was observed following the study when the setscrew was removed. In the PBZ-treated horses, the lameness score was lowest between 4 and 5 h post-treatment when the score was reduced from 4 to 1.5 compared with control horses. PBZ was efficacious in alleviating lameness in this model. The PBZ plasma concentrations were approximately 15 and 7 μg/mL at 4 and 8 h, respectively. The study period did not include observations beyond 9 h, but the lameness

score had not recovered to baseline values at that time (Foreman *et al.*, 2008).

Lipopolysaccharide-induced synovitis was produced in horses and treated with PBZ (4.4 mg/kg, i.v., q 12 h), or etodolac (23 mg/kg, i.v., q 12 h). Both reduced synovial fluid white blood cell counts at 6 and 24 h. In addition, both drugs significantly reduced PGE_2 levels at 6 h, but TXB_2 was only reduced by PBZ (Morton *et al.*, 2005). Using a standardized lameness model, flunixin was studied and PBZ was used in the same model as a positive control. At a dose of 4.4 mg/kg of PBZ and 1.1 mg/kg of flunixin peak effect occurred at 8–12 and 12 h, respectively. Flunixin analgesic activity persisted for 30 h and PBZ for 24 h (Houdeshell & Hennessey, 1977).

An induced arthritis model was developed to establish the relationship between the plasma concentration of PBZ and its pharmacological effects. A dose–effect relationship was shown for PBZ with an absence of effect for the 1 mg/kg dose and a maximum effect at about 2 mg/kg; at higher PBZ doses, the maximum effect was not modified, but its duration was increased from 8 h with a 2 mg/kg dose to about 24 h with an 8 mg/kg dose (Toutain *et al.*, 1994). This study and others cited in this review came to the same conclusion that the maximum dose was 2.2 mg/kg and higher doses did not increase the effect except the duration. Similar results were noted in humans, a dose-finding study determined that the most efficacious dose was 300 mg/day. Doses below this did not produce full benefit and no further improvement occurred with higher doses (Bird *et al.*, 1983).

The production of muscle inflammation by the injection of Freud's adjuvant did not affect the plasma kinetics and when administered 5 weeks apart there was no within horse variability indicating that the administration of PBZ did not affect the plasma kinetics of subsequent doses (Mills *et al.*, 1996). This was verified by clinical observations that the plasma concentrations in a large population of horses were consistent postrace when a routine administration schedule was established in a horse and previous administrations did not affect subsequent doses.

INDIRECT ASSESSMENT OF DURATION OF NSAID EFFECTS

It has been shown that the mechanism of the action of aspirin-like compounds was a direct inhibition of prostaglandin synthetase, thereby preventing prostaglandin biosynthesis (Vane, 1971; Moncada *et al.*, 1974; Vane & Botting, 1987). Products of prostaglandin biosynthesis such as prostaglandins and prostacyclin produce hyperalgesia associated with inflammation and may cause pain in some inflammatory conditions by sensitizing the chemical receptors of afferent pain endings to other inflammatory mediators such as bradykinin and histamine. NSAIDs are potent inhibitor of the conversion of arachidonic acid to arachidonic acid-derived mediators of inflammation. The site of action of NSAID is the cyclooxygenase pathway, therefore, blocking the synthesis and release of several chemical mediators of inflammation, collectively known as eicosanoids. NSAIDs in normal therapeutic doses do not block the lipoxygenase pathways which may be responsible for the reduction of leukocyte migration into the inflammatory site and the reduction of edema (Higgs, 1980).

Cyclooxygenase (COX-1) was the first enzyme recognized for catalyzing the synthesis of prostanoids from arachidonic acid, since this initial description a second isoform COX-2 has been described. PBZ is primarily a non-selective COX inhibitor; *in vitro* analysis in horse blood showed a greater COX-1 selectivity determined by the depression of TXB_2, compared to COX-2 selectivity determined by the depression of PGE_2 (Beretta *et al.*, 2005). This observation confirms that in the horse, PBZ is a more selective inhibitor of COX-1 than COX-2. This is relevant in that species difference have been noted in the concentrations of the stable metabolite TXB_2 released by COX-1 activation and the concentration of PGE_2 release by lipopolysaccharide activation of COX-2 and the selective inhibition by various NSAID (Brideau *et al.*, 2001).

There have been considerable advances in the development of pharmacokinetic/pharmacodynamic (PK/PD) models in veterinary and human medicine and investigators have studied the effects of the drug and concurrent changes in plasma or tissue concentrations of inflammatory mediators. Modern PK/PD studies link the effect(s) of the drug to its corresponding concentration in plasma (Lees *et al.*, 2004a,b,c; Toutain & Lees, 2004). General PD/PK models have been developed for describing drug actions on various active metabolites and hormones (Krzyzanski & Jusko, 2001; Puchalski *et al.*, 2001).

A number of studies have used the reduction in the metabolic products of inflammation as indirect models of the actions of PBZ and other NSAIDs at the molecular level to determine the degree and duration of action. Three types of models have been used:

1. Suppression of the release of inflammatory mediators in blood samples. A number of PK/PD models have been developed using this technique (Lees *et al.*, 1987a; Soma *et al.*, 1992).
2. Suppression of the release of inflammatory mediators in tissue cage and sponge models in which a sterile carrageenan solution was injected into the cage or sterile carrageenan-soaked polyester sponge strips were inserted subcutaneously. Both were based on the creation of a mild, reproducible and reversible inflammatory reaction that causes minimal distress to the experimental animals. The acute inflammatory exudates have been shown to contain proteins, white blood cells, and eicosanoids all because of the inflammatory reaction (Higgins & Lees, 1984; Lees & Higgins, 1984; Lees *et al.*, 1986; Higgins *et al.*, 1987a,b; Lees *et al.*, 1987a,b).
3. More recently, models in humans have used flow through methods to harvest inflammatory exudates. *In vivo* human bioassay can be used to study human volunteers and patients. Samples are collected from pertinent tissue sites such as the skin via aseptically inserted micro dialysis catheters. These experiments measured inflammatory substances in interstitial fluid collected from noninflamed and experimentally inflamed skin (Angst *et al.*, 2008a,b).

INDIRECT PLASMA MODELS

A study involving the inhibitory actions of NSAIDs on TXB_2 following a single dose of flunixin (1.1 mg/kg) or PBZ (4.4 mg/kg) was used to determine the duration of action of these drugs. Flunixin and PBZ produced similar degrees of reversible inhibition of TXB_2 at 4 (98% and 88%), 8 (77% and76%), and 24 (63% and 50%) h, respectively. At 48 h, inhibition of TXB_2 was no longer apparent (Lees *et al.*, 1987a,b).

In a similar study, the concurrent administration of flunixin meglumine (1.1 mg/kg, i.v.) and PBZ (2.2 mg/kg, i.v.) on the pharmacokinetics of each drug indicated that the pharmacokinetic variables calculated for each drug when administered alone and in combination were similar. Serum TXB_2 production was significantly suppressed for 8, 12, and 24 h after administration of flunixin and PBZ in combination. When these drugs were administered alone, the TXB_2 concentrations were not significantly different from control values at 24 h. Note in this study that the dose of PBZ was 2.2 mg/kg. (Semrad *et al.*, 1993).

INDIRECT TISSUE MODELS

Distribution of PBZ and its active metabolite, OPBZ, into tissue fluids was studied by measuring concentrations in plasma, tissue-cage fluid, peritoneal fluid and acute inflammatory exudates harvested from a polyester sponge model of inflammation in ponies. PBZ and OPBZ readily penetrated into inflammatory sites. After 6 h, the concentration of PBZ was higher in exudates than in plasma and remained so at 24 h. Mean concentrations of OPBZ in all fluids were lower than those of PBZ at all times, but OPBZ readily entered body fluids, especially into inflammatory exudates; suggesting that OPBZ may contribute to the anti-inflammatory effect. The estimated elimination half-life of PBZ from exudates was 24 h compared to 5 h from plasma. The authors suggested that the persistence of PBZ and OPBZ in tissues exudates extended the duration of PBZ effectiveness (Lees *et al.*, 1986). Other studies have shown that flunixin was also cleared more slowly from equine tissue inflammatory exudates than from plasma (Higgins *et al.*, 1987a,b).

Acute inflammation was induced in seven ponies by subcutaneous implantation of sterile carrageenan-soaked polyester sponge strips. Treatment comprised a single therapeutic dose of 4.4 mg/kg of PBZ administered intravenously at the time of sponge implantation. Exudates were harvested at 6, 12, and 24 h and examined for leukocyte and erythrocyte numbers. Leukocyte numbers were significantly increased from 6-h values at 12 and 24 h in both control and PBZ-treated animals but differences between control and treated ponies were not significant. The administration of PBZ produced significant reductions in exudate concentrations of PGE_2 and 6-keto-$PGF_1\alpha$, the stable products of prostacyclin at 6, 12, and 24 h. Concentrations of PBZ and OPBZ in exudates exceeded the plasma concentrations at 12 and 24 h. Concentrations of TXB_2, the stable products of TXB_2, were reduced in treated animals but these changes were not significant. Study results suggested an effect at 24 h based on the reduction of the two measured eicosanoids PGE_2 and 6-keto-$PGF_1\alpha$ (Higgins *et al.*, 1984).

In a 12-day treatment schedule, five ponies were administered an oral paste formulation of PBZ and five matched ponies were administered equivalent doses of a placebo paste. On day 12, a mild, nonimmune inflammatory reaction was induced subcutaneously. Exudates were collected at 4, 8, 12, and 24 h. There were no significant differences in exudate protein concentration and leukocyte numbers between the treatment groups, but exudate concentrations of 6-ketoF1α were reduced at 4, 8, and 12 h and those of TXB_2 at 8, 12, and 24 h in the PBZ treatment group. The increases in surface skin temperature were significantly less in PBZ-treated than in placebo-treated ponies between 4 and 24 h (Lees & Higgins, 1986).

The most widely accepted mode of action for NSAIDs is inhibition of prostaglandin synthetase. Leukocyte and erythrocyte accumulation in exudates is part of the inflammatory process. In the tissue cage and exudates studies, this was not significantly affected by the NSAID (Lees & Higgins, 1984, 1986). *In vitro* studies have shown that flunixin, PBZ, OPBZ, and indomethacin suppress leukocyte migration of which flunixin was the most potent of the drugs studied. The obvious difference between *in vivo* and *in vitro* studies is the more complex environment of the inflamed joint compared to the controlled environment of an *in vitro* study (Dawson *et al.*, 1987).

SUMMARY

This review presented a historical prospective and examined the information presented in four different models used to determine the pharmacological effects of NSAIDs, especially PBZ. They included naturally occurring lameness, reversible induced lameness, and indirect plasma and tissue models studying the suppression of the release of arachidonic-derived mediators of inflammation. The majority of studies suggest a persistent effect of PBZ at 24 h at 4.4 mg/kg. This reflects and substantiates the opinion of many clinical veterinarians, many of whom will not examine a horse for a prepurchase lameness examination unless the horse is shown to be free of NSAIDs and corticosteroids. Regulatory veterinarians responsible for prerace examinations of racehorses, wish to examine a horse prerace without the possibility of a NSAID or corticosteroid interfering with the examination and masking a possible musclo-skeletal condition. Based on scientific reports and the impression of clinical veterinarians, residual effects of PBZ remain at 24 h. The impact of this sustained effect on the health and welfare of the horse remains problematic.

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