CELECOXIB IN THE HORSE: ITS RECOVERY, MASS SPECTROMETRIC IDENTIFICATION, DISPOSITION AND MAJOR URINARY METABOLITES

L. Dirikolu, A. F. Lehner, J. Jacobs, W. E. Woods, W. Karpiesiuk, J. D. Harkins, W. G. Carter, J. Boyles, C. G. Hughes, J. M. Bosken, C. Holtz, C. Natrass*, M. Fisher**, T. Tobin

*Maxwell H Gluck Equine Research Center and the Department of Veterinary Science, University of Kentucky, Lexington, Kentucky 40506; *Truesdail Laboratories, Tustin, California, USA; **Kentucky Racing Commission, Ironworks Pike, Lexington, Kentucky, USA*

Abstract

Celecoxib(4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1yl]benzene-sulphonamide) is a new non-steroidal anti-inflammatory drug (NSAID) that selectively inhibits cyclooxygenase-2 (COX-2) enzymes. COX-1, the major enzyme form found in healthy tissues, regulates homeostasis of the gastrointestinal tract and kidneys, while COX-2 is an inducible form involved in the pathogenesis of inflammation, pain and pyretic response. The majority of NSAIDs inhibit both COX-1 and COX-2 and their chronic usage can result in gastrointestinal ulceration and bleeding. Celecoxib offers therapeutic advantages and specifically inhibits COX-2 enzymes. Therefore, it is likely to be used in performance horses and suitable detection and confirmation methods for regulatory control are required.

Celecoxib (2.2 mg/kg, as Celebrex capsules) was administered orally to horses. Celecoxib was recovered by solid phase extraction from plasma and urine, derivatised with trimethylanilinium hydroxide (TMAH) and detected by GC/MS. Parent celecoxib was identified readily in post administration plasma samples, peaking at about 400 ng/ml 4 h after dosing and declining rapidly thereafter. Concentrations of parent celecoxib in post administration urines were low (<10 ng/ml). The major urinary metabolite detected was 4-carboxycelecoxib, with smaller amounts of a material tentatively identified as 4-hydroxy-methylcelecoxib. There was little evidence for the presence of glucuronidated metabolites of celecoxib. These data show that celecoxib is absorbed after oral administration in horses and provides a basis for its analytical detection and regulatory control in performance horses.

Introduction

Celecoxib is a newly developed non-steroidal anti-inflammatory drug (NSAID) recently released for use in human medicine. Celecoxib is a 1,5-diaryl pyrazole compound, 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulphonamide that specifically inhibits cyclooxygenase-2 (COX-2) enzyme (Hawkey 1999). This COX-2 enzyme is selectively induced by cytokines, endotoxin and mitogens during inflammation, giving rise to pain, swelling and stiffness (Penning *et al.* 1997). On the other hand COX-1, the major enzyme form found in healthy tissues, regulates homeostasis of the gastrointestinal tract and kidneys (Laneuville *et al.* 1994).

The majority of currently marketed NSAIDs inhibit COX-1 and COX-2 enzymes with little or no selectivity for any particular cyclooxygenase. Because of this, the chronic use of NSAIDs in animals results in gastrointestinal lesions with the development of ulcers and bleeding. These effects are associated primarily with the inhibition of the prostaglandin synthesis by COX-1 resulting in regulatory changes in blood flow at gastric mucosa, bicarbonate secretion and tumour necrosis factor alpha (TNF-α) production. More recently, work by Riendeau *et al.* (1997) supported the concept that selective COX-2 inhibitors are effective anti-inflammatory agents with fewer gastrointestinal side effects than other NSAIDs.

Based on our knowledge of the pharmacology of celecoxib in animals and man, celecoxib has the potential to be a useful therapeutic agent in the horse. The goal of this study was to develop detection methods for celecoxib and its major metabolites in equine biological samples to allow regulatory control of the use of this agent.

Materials and methods

Horses

Mature Thoroughbred mares weighing 450–500 kg were maintained on grass hay and feed (12% protein), which was a 50:50 mixture of oats and an alfalfa-based protein pellet. The animals were fed twice daily, vaccinated annually for tetanus and were de-wormed quarterly with ivermectin. A routine clinical examination was performed before the experiment to ensure that the animals were healthy and sound. During experimentation, horses were provided water and hay *ad libitum* and managed according to the rules and regulations of the University of Kentucky Institutional Animal Care Use Committee, which also approved the experimental protocol. Celecoxib capsules (Searle, Illinois, USA) were administered orally (1.1 grams; 500 kg x 2.2 mg/kg).

Blood samples were collected into heparinised tubes that were centrifuged at 913 *g* (4°C) for 15 min, and the plasma stored at -20°C until assayed. Urine collection was accomplished with a Foley catheter (24 Fr, Rusch, Inc., Georgia, USA) and attached plastic bag. Blank samples were collected during the 30 min prior to drug administration. Samples were collected at 0.5-1, 1-2, 2-4, 4-6 and 6–8 h after administration. At 24 and 72 h after administration, a Harris flush tube (24 Fr x 60 in; Seamless, Florida, USA) was used to collect a maximum of 300 ml urine. Urine was placed in aliquots and stored at -20°C until assayed.

Recovery of celecoxib from Celebrex capsules

Celecoxib was purified from Celebrex oral capsules for analytical standard as follows: 70 g capsule content was stirred in 800 ml dichloromethane for 2 h at room temperature. The suspension was filtered through celite under reduced pressure, washed with water (3 x 100 ml) and dried over magnesium sulphate. Dichloromethane was evaporated under reduced pressure and the resulting white precipitate was dried under vacuum for 5 h at room temperature (24°C). The celecoxib was pulverised by grinding with mortar and pestle, yielding 53 g of white powder. The resulting melting point (mp) was 158–159°C. Purity was assessed by GC/MS of TMS-derivatised aliquots of purified celecoxib as described below; this method eliminated contaminating excipients present in the Celebrex capsule.

Sample preparation

Aliquots (5 ml) of thawed urine were sonicated on ice for 90 s and warmed to 24°C. When urine was enzymatically treated with ß-glucuronidase, 1 ml of Sigma Type L-II Limpets, 5,000 units/ml, was added to 5 ml urine samples following sonication, along with 2 ml of 1M sodium acetate buffer, pH 5.0. The samples were mixed by vortex and incubated at 65°C for 3 h. After cooling overnight at 4°C, the samples were subjected to solid phase extraction (SPE) as described below.

Drug recovery

SPE was performed on biological samples by means of Bond Elut Certify columns (Varian, California, USA). Comparison of the Varian cocaine (basic drug) and barbiturate (acidic and neutral drug) procedures for extractions of 20 µg/ml celecoxib from 5 ml spiked blank urine demonstrated the exclusive utility of the barbiturate-type assay. Comparison of 4 column elution solvents (dichloromethane, methanol, pet ether and hexane:ethyl acetate, 1:1) demonstrated the utility of these solvents in the order dichloromethane > hexane-ethyl acetate > methanol >> pet ether, a pattern that was very similar to that for aprobarbital as comparison barbiturate. The final recommended SPE method with Certify columns is 1) combination of 5 ml urine sample with 2 ml 0.1 M KH_2PO_4, pH 6.0; 2) activation of column on a VacElut SPS-24 (Varian) vacuum manifold with 2 ml methanol, followed by 2 ml 0.1 M KH_2PO_4, pH 6.0; 3) application of specimen with slow passage under reduced vacuum; 4) column rinse with sequential 1 ml 0.1 M KH_2PO_4, pH 6.0:methanol, 80:20, 1 ml 1.0 M acetic acid, and 1 ml hexane; 5) column elution with 4 ml dichloromethane. Following elution, solvent containing the celecoxib-related materials was dried under a gentle flow of nitrogen gas in an Organomation Multivap (South Berlin, MA) with the water bath set at 40°C. Eluents were then subjected to derivatisation.

Derivatisation of celecoxib and related materials for GC/MS

Sample eluents extracted and dried as described above were derivatised with trimethylanilinium hydroxide (TMAH) (commercial preparation, Barb-Prep, Alltech, Illinois, USA) in the chromatograph injection port. Barb-Prep (50 µl) was added to tubes containing dried eluents, vortexed and transferred to auto-liquid sampler (ALS) vials for GC/MS analysis.

Derivatisation was also performed on celecoxib samples by reaction with N,O-bis (trimethylsilyl) trifluoroacetamide with catalysis with 1% (v/v) trimethylchlorosilane (BSTFA + 1% TMCS, Pierce,

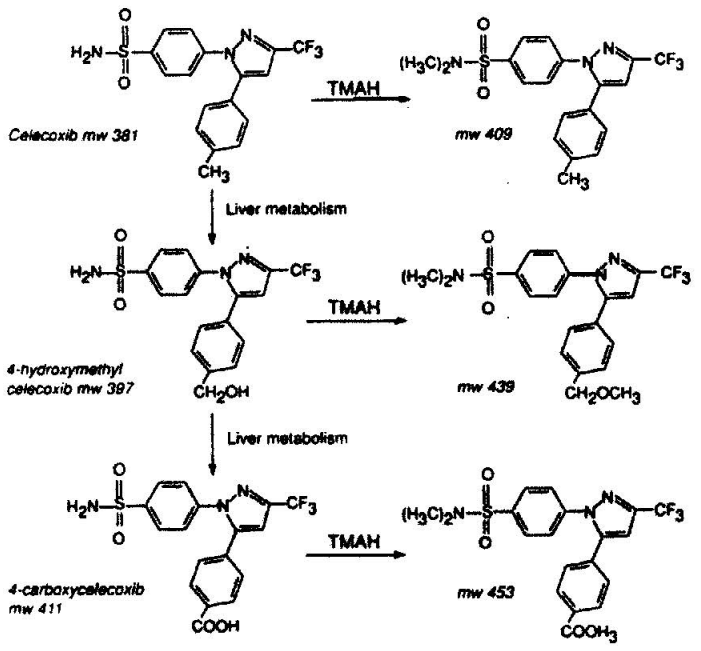

Fig 1: Equine metabolism of celecoxib and derivatisation chemistry for GC/MS. By analogy to known human metabolism, the proposed equine metabolism is presumably mediated by hepatic cytochrome P450 2C9 and is illustrated on the left side of the figure. Derivatisation to di- and trimethyl derivatives by trimethylanilinium hydroxide (TMAH) is illustrated on the right side of the figure.

Illinois, USA) by adding <0.1 mg samples to 50 µl reagent, vortexing and incubating in a sealed vial 30 min at 75°C.

Gas chromatography/mass spectrometery

Chromatography on sample extracts was initiated by injection of 1 µl aliquots into the 280°C injector port of a Hewlett-Packard (Georgia, USA) 6890/5972 GC/MSD equipped with autoliquid sampler. Barb-Prep-treated extracts were injected splitless with an initial oven temperature of 120°C (held 1 min), followed by oven ramping at 40°/min to 280° (held 13 min), with MS scanning from 50–550 m/z at 1.53 scans/s. The GC was equipped with a J&W (California, USA) 30 m DB-5MS 5% phenyl-95% methylpolysiloxane capillary column with 0.25 mm id and 250 µ film thickness. The carrier gas was helium set at a 1 ml/min flow rate.

Results and conclusions

Equine metabolism of celecoxib and derivatisation chemistry for GC/MS are shown in Figure 1. Celecoxib was also analysed by derivatisation with BSTFA/1%TMCS as described above to produce a mono-TMS derivative of mw 453. Figure 2 shows the GC trace and mass spectrum corresponding to this derivative. This method of derivatisation was useful in initial experiments in that it established that SPE of acid-neutrals (such as for barbiturates) was the preferable means of celecoxib extraction from biological fluids, but chromatography of TMS derivatives of celecoxib proved troublesome, including severe GC tailing and a tendency to form bis- and mono-TMS mixtures. For example, the spectrum in Figure 2 included a trace of a m/z 510 peak (bis-TMS minus CH_3) (data not shown), suggesting either possible overlapping bis-derivative or that the spectrum is in fact that of a bis derivative. Gross sample overload appeared to encourage the presence of a bis-TMS derivative with a major m/z 510 peak, suggesting that more than a single bis-TMS derivative may have been formed.

To avoid these problems, we utilised TMAH (Alltech 'Barb-Prep') injector port derivatisation. This circumvented the problems with BSTFA/1%TMCS, yielding a single clean dimethyl derivative of celecoxib of 409 mw. Figure 3 illustrates the mass spectra of dimethyl-celecoxib as prepared from standard (Fig 3b) and as extracted from equine urine (Fig 3a) 6 h post administration by SPE adapted from barbiturate acid-neutral methods. These data demonstrate that celecoxib recovered from equine urine matched our celecoxib standard both in EI-mass spectrum and in retention time.

Using this approach, SPE extraction of 4 ml unhydrolysed plasma samples post celecoxib administration was performed with external quantitation, ie dependence on ALS injector precision, by comparison to a series of blank plasma samples spiked with celecoxib (data not

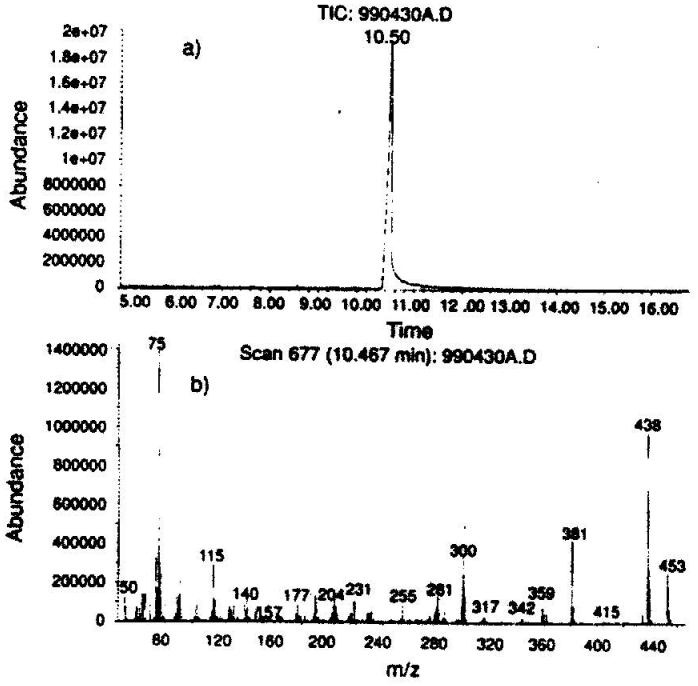

Fig 2: Celecoxib derivatised with BSTFA/1%TMCS to produce a mono-TMS derivative. Top (a) shows total ion chromatogram of purified celecoxib-TMS derivative. 500 ng, run splitless with starting oven temperature of 150°C (held 2 min), ramping at 30°/min to 280° (held 12 min). Bottom (b) shows mass spectrum of 10.5 min retention time (RT) peak, scanned from 50–550 m/z at 1.53 scans s.

shown). These data suggest peak concentrations of celecoxib of up to 400 ng/ml in equine plasma at 4 h post administration. Analysis of urine samples indicates that urine celecoxib concentrations are quite low, with peak concentrations of 8.5 ng/ml occurring at roughly 8 h post administration (data not shown). Most of these samples show little difference after glucuronidase treatment, suggesting that little glucuronidated celecoxib is excreted.

These very low concentrations of celecoxib in equine urine suggest extensive metabolism and therefore a high probability of urinary metabolites. Ion chromatography of full scan (m/z 50-550) acquisitions of SPE extracted urine showed 2 metabolites: 1) a major metabolite that has been assigned the structure 4-carboxycelecoxib based primarily on the presence of a m/z 453 molecular ion and revealed by ion chromatography for 453, the predicted molecular mass of a trimethylated 4-carboxy metabolite; and 2) a minor metabolite which has been tentatively assigned the structure 4-hydroxymethylcelecoxib revealed by chromatography for m/z 424, the predicted molecular mass of a trimethylated hydroxymethyl molecular ion minus a methyl group.

Figure 4a shows ion chromatography for the m/z 346 base peak of the 453 mw 4-carboxy metabolite and reveals that it occurs in a single isomeric species; in addition, the m/z 346 peak overlaps symmetrically with other principal components of the mass spectrum, particularly m/z 409 and 453 (overlap not shown), supporting the contention that these ions derive from a single compound. Figure 4b shows the mass spectrum corresponding to this metabolite. The assignment of this compound to the trimethylated 4-carboxycelecoxib structure derives support from analogy of fragmentation reactions to ones evident in dimethylated parent celecoxib, whose mass spectrum is shown in Figure 3b. Table 1 illustrates major ions with direct correspondence to celecoxib parent, such as m/z 390 for celecoxib and 434 for the 4-carboxy analogue involving simple loss of a fluorine atom. Others ions are supportive in that they involve aspects of the altered benzyl group which should not necessarily be in common. The only major exception is the pyrazole ring cleavage product which comprises the derivatised 4-carboxycelecoxib base peak and for which no corresponding peak is found in the dimethylcelecoxib spectrum. If the assignment is correct, then one might suspect long-range facilitation of this fragmentation by the methyl benzoate group. Alternatively, the assignment could be incorrect and necessitate different patterns of proton rearrangement on loss of the dimethylsulphonamide group $(CH_3)_2NSO_2$.

The structure assigned as 4-hydroxymethylcelecoxib is admittedly less straightforward in interpretation, particularly since we are not afforded the correspondences evidenced by the 4-carboxy metabolite, but

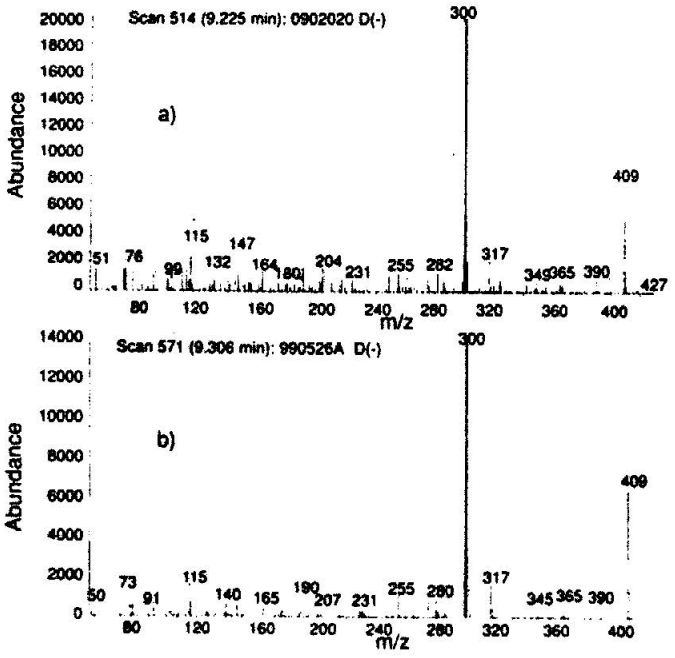

Fig 3: Mass spectra of celecoxib identified in equine urine 6 h post administration without glucuronidase (a) in comparison to celecoxib standard (b), both of which have been derivatised to dimethyl analogues with TMAH. The extracted equine compound had a 96% match to the standard by the H-P spectral matching algorithm.

examination of its chemistry is nonetheless of interest. Figure 5a shows by ion chromatography that, similar to the 4-carboxy metabolite, the m/z 424 ion of this compound occurs as a single peak. Although present in much lower yield than either unmetabolised celecoxib or the 4-carboxy metabolite of celecoxib, ion chromatography demonstrated overlap of the m/z 424 ion with other ions, including m/z 409, 393, 344, 330/331, 302, 286, 273/274, 215 and 188. The mass spectrum of the proposed metabolite is shown in Figure 5b.

The authors believe the spectrum shown in Figure 5b represents a celecoxib metabolite because of its appearance solely following celecoxib administration and its absence from blank urine, as further discussed below. In this case, the m/z 424 ion would be problematic if interpreted as the compound's molecular ion, in that it requires loss or gain of a nitrogen relative to celecoxib to satisfy the nitrogen rule for organic compound odd vs. even molecular weight determination. Owing to the difficulties of perceiving loss or gain of nitrogen, the simplest explanation of m/z 424 would involve metabolism followed by loss or gain of an odd mass group during mass spectral fragmentation. Assignment to a candidate N-acetyl metabolite of the sulphonamide group (Mandel 1979) is dissatisfying in that its mw would be 423, requiring intermolecular abstraction of a proton to achieve the m/z 424 M^+ molecular ion. In addition, Mass Spec Calculator Pro software (Quadtech Associates 1998) reveals no simple path from such a structure to the m/z 393 ion (presumed loss of CH_3O, ie 31 amu) among others.

TABLE 1: Comparison of mass spectral fragments from derivatised celecoxib and its principal derivatised metabolite, 4-carboxycelecoxib

Interpretation	Dimethylcelecoxib, ion (m/z)	Trimethyl 4-carboxy-celecoxib, ion (m/z)
Molecular ion, [M]	409	453
M - F (19)	390	434
M - N $(CH_3)_2$	365	409
Pyrazole ring cleavage with loss of N=C=C-CF_3	not found	346 (base peak)
M - [benzyl group $(C_6H_4CH_3)$+H]	317	not found
M - [$(CH_3)_2NSO$+H]	300 (base peak)	344
M - F (19) - [$(CH_3)_2NSO_2$+H]	281	325
M - [$(CH_3)_2NSO_2$+H] - OCH_2	not found	315
M - [$(CH_3)_2NSO_2$+H] -CH_3CO_2	not found	286
M - F (19) - [$(CH_3)_2NSO_2$+H]-$C_6H_4CH_3$	190	not found
M - F (19) - [$(CH_3)_2NSO_2$+H]-$C_6H_4CO_2CH_3$	not found	190

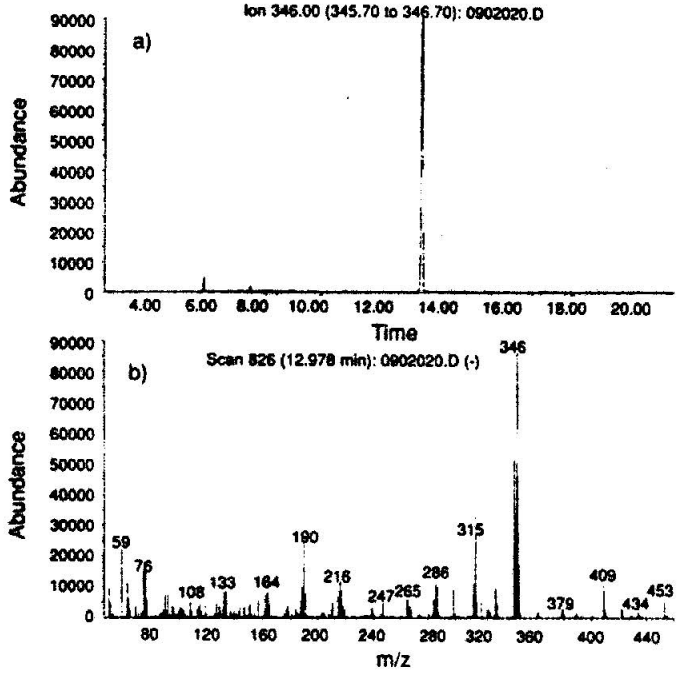

Fig 4: Ion chromatogram (a) of ion 346 m/z and mass spectrum (b) corresponding to the 12.976 min RT peak of proposed TMAH-derivatised trimethylated 4-carboxycelecoxib.

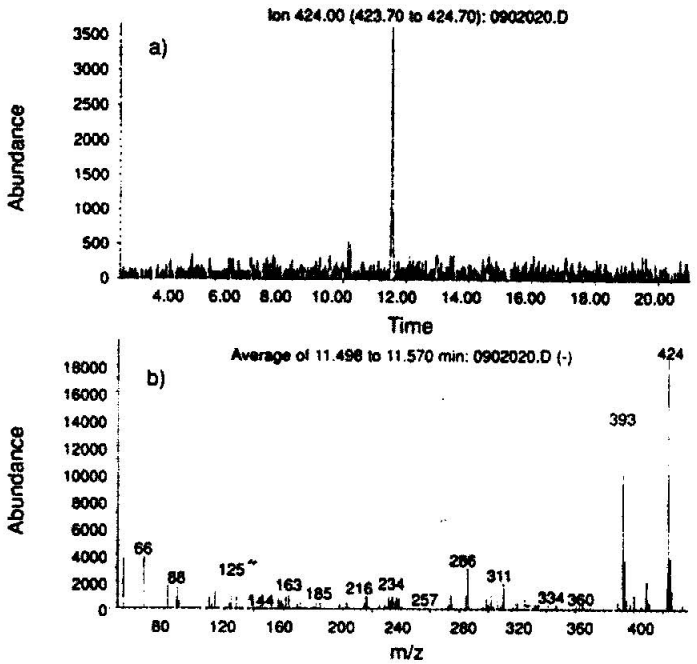

Fig 5: Ion chromatogram (a) of ion 424 m/z and mass spectrum (b) corresponding to the 11.536 min RT peak of proposed TMAH-derivatised trimethylated 4 hydroxymethylcelecoxib.

Oxidation of the exocyclic methyl group to an aldehyde has the disadvantages listed for N-acetylation above, particularly in that loss of a proton by alpha-cleavage would predominate to yield a m/z 422 RCO^+ ion highly stabilised by conjugation with the adjacent aromatic ring (McLafferty 1973); this leaves only hydroxylation of the methyl group to a 397 mw 4-hydroxymethylcelecoxib without derivatisation. Such hydroxylation has precedents in the drug metabolites hydroxymethyltolbutamide (Parkinson 1996) and 11-hydroxy-delta-9-tetrahydrocannibinol (Foltz *et al.* 1980) among others. Methylation with TMAH would then yield m/z 411, 425 or 439 molecular ions, with one, 2 or 3 methyl groups, respectively. The dimethyl would require loss of a

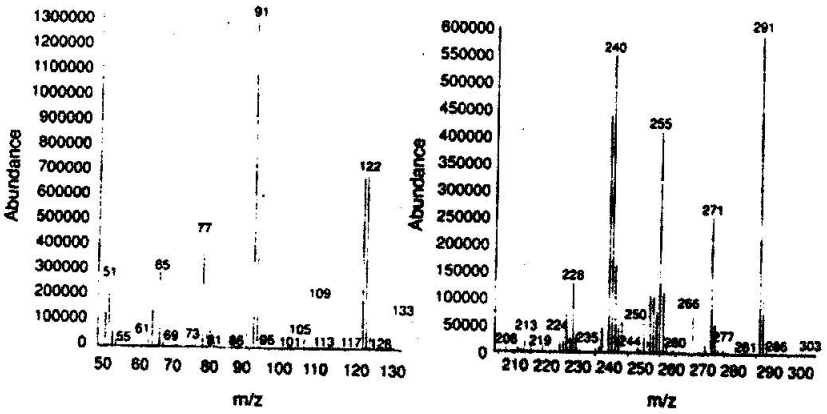

Fig 6: Methylation of model aromatic hydroxymethyl functionalities with TMAH: benzylic alcohol (initial mw 108, left), and 7-hydroxymethyl-12-methylbenz[a]anthracene (initial mw 272, right).

hydrogen radical to provide a positively charged m/z 424 species, whereas the trimethyl would require loss of a methyl radical, again to provide a positively charged m/z 424 intermediate. Precedents exist for both possibilities: parametric retrieval of NIST98 mass spectra reveals prominent loss of 1 amu from 1-propyn-3-ol and loss of methyl (15 amu) from 2-butyn-diol dimethyl ether, although in our case the methyl could come from one of 3 locations. Although the TMAH reagent is principally effective with amine and carboxylic acid compounds (Kovac 1993), we have succeeded in methylating benzylic alcohols with TMAH under our derivatising conditions (Fig 6), suggesting that methylation to a m/z 439 trimethylated 4-hydroxymethylcelecoxib is possible. Peak assignment with the aid of MS Calculator Pro software enabled reasonable interpretation of the Figure 5b mass spectrum (Table 2); the assignments specifically posit the existence of a m/z 439 molecular ion, although we were unable to visualise it. The assignment of this metabolite is satisfying in that it supplies an oxidative intermediate between celecoxib and its major 4-carboxycelecoxib metabolite. McLafferty (1973) supports the assignment of m/z 393 in that benzyl ethers typically give benzylic carbonium ions with loss of an OR radical, where OR would be OCH3 in our case. On the other hand, lack of correspondence of fragmentation schemes for the hydroxylated compound to the other 2 (Table 1) must probably be based on unusual instability of one of the derivative's methyl groups by long range effects of the benzyl ether, in lieu of a better argument.

Another possible explanation of the m/z 424 metabolite would involve ring hydroxylation of the celecoxib benzyl group ortho to the methyl group. We were able to methylate the phenolic group of the model compound o-cresol with TMAH under the conditions of our derivatisation (Fig 8). Thus, the proposed ring hydroxylated metabolite would be expected to yield a trimethyl derivative isobaric with the trimethyl derivative of the proposed 4-hydroxymethyl celecoxib, mw 439. Loss of methyl and methoxy radicals would then account for the fragment ions in Figure 5 (m/z 424 and 393); however, sequential loss of radicals is not a common mass spectrometric fragmentation process. Loss of 31 mass (o-me) unit is evident in the spectrum of methylated benzyl alcohol (Fig 6). We have nonetheless opted for the somewhat less

TABLE 2: Proposed assignment of peaks identified in the mass spectrum of the celecoxib matabolite tentatively designated as 4-hydroxymethylcelecoxib

Interpretation	Ion, m/z
439 (n.o.)	Molecular ion [M]
424	M - CH_3
409	m/z 424 - CH_3
393	m/z 424 - OCH_3
344	M - NC - CF_3
330	M - [$(CH_3)_2NSO_2$+H]
311	m/z 330 - F (19)
302	m/z 424 - [$C_6H_4CH_2OCH_3$+H]
286	M - [$(CH_3)_2NSO_2$+H] and - CH_3OCH_2
273	M - $N(CH_3)_2$ and [$C_6H_4CH_2OCH_3$+H]

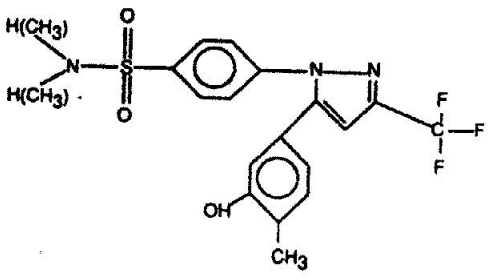

Fig 7: Structure of the proposed ring hydroxylated metabolite of celecoxib.

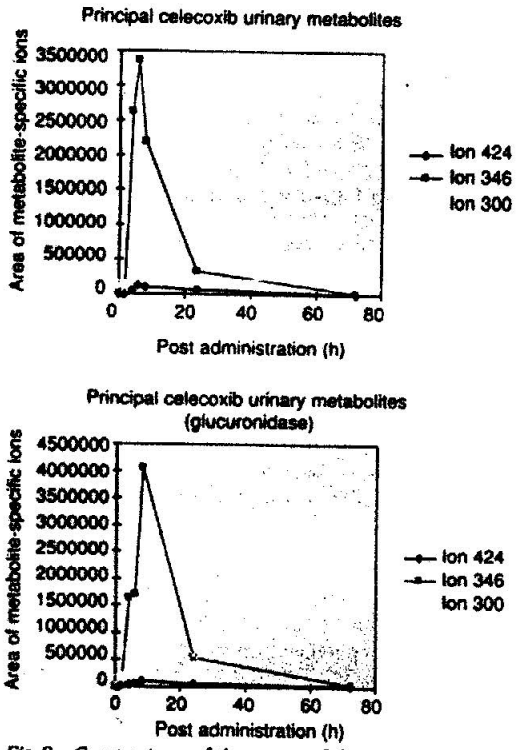

Fig 8: Comparison of the areas of the mass spectral base peaks of urinary celecoxib 4-carboxy metabolite (346 m/z), metabolised, unmetabolised celecoxib (300 m/z), and 4-hydroxymethyl metabolite (424 m/z) both without (top) and with (bottom) glucuronidase pre-treatment.

problematic 4-hydroxymethylcelecoxib structure as an explanation for the Figure 5 metabolite, in particular because it offers an oxidative intermediate to the 4-carboxy metabolite of Figure 4.

Figure 8 illustrates the relative significance of the 3 primary equine celecoxib metabolites by plotting the areas derived from ion chromatograms for each of their mass spectral base peaks. The carboxy and hydroxymethyl metabolites follow temporal patterns roughly similar to that of unmetabolised celecoxib (compare ions m/z 346 and 424 with 300), with peaks at roughly 6-8 h post administration. Although preliminary, these data suggest that the carboxy metabolite may be present in concentrations as much as 8-fold greater than those of celecoxib, whereas the hydroxymethyl metabolite appears to be present at only one quarter of the concentration of celecoxib, more or less.

Inspection and comparison of the data presented in the top and bottom section of Figure 8, with beta-glucuronidase pre-treatment of urine, suggests little support for the presence of these metabolites as glucuronides. In support of this interpretation, preliminary examination of urine

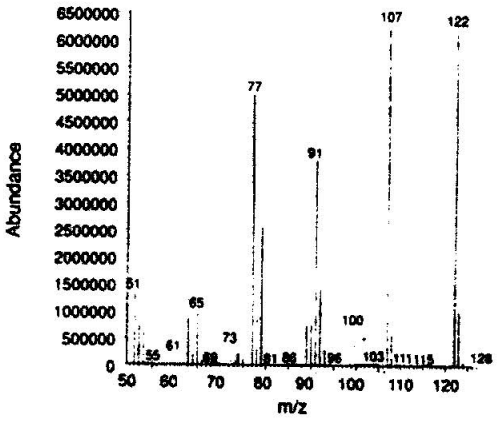

Fig 9: Methylation of a model phenolic functionality with TMAH: o-cresol (initial mw 108).

samples by electrospray ionisation tandem mass spectrometry failed to identify mass spectral peaks corresponding to the predicted molecular masses of celecoxib-glucuronides in either positive or negative mode electrospray.

In conclusion, these data suggest that celecoxib is well absorbed from the gastrointestinal tract in equines and provide a basis for analytical detection and regulatory control of this agent in performance horses. Parent celecoxib was identified readily in post administration plasma samples, peaking at about 400 ng/ml 4 h after dosing and declining rapidly thereafter. Concentrations of parent celecoxib in post administration urine were low (<10 ng/ml) suggesting extensive metabolism characteristic of this agent in horses. The major urinary metabolites detected were 4-carboxycelecoxib, along with a smaller amount of what we have tentatively assigned 4-hydroxy-methylcelecoxib, with little evidence for the presence of glucuronidated metabolites of celecoxib.

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References

Foltz, R.L., Fentiman, Jr., A.F. and Foltz, R.B. (1980) Delta-9-tetrahydrocannibinol (THC) and two of its metabolites, 11-hydroxy-delta-9-THC and 11-nor-9-carboxy-delta-9-THC. In: *GC/MS Assays for Abused Drugs in Body Fluids. Rockville, MD: NIDA Research Monograph* **32**, 62-89.

Hawkey, C.J (1999) COX-2 inhibitors. *The Lancet* **353**, 307-314.

Kovac, P. (1993) Alkylation. In: *Handbook of Derivatives for Chromatography*. Eds: K. Blau and J.M. Halket, 2nd edn. John Wiley and Sons, New York. pp 109-129.

Laneuville, O., Breuer, D.K., Dewitt, D.L., HLA, T., Funk, C.D. and Smith, W.L. (1994) Differential inhibition of human prostaglandin endoperoxide H synthase-1 and -2 by nonsteroidal anti-inflamatory drugs. *J. Pharmacol. exp. Ther.* **271**, 927-934.

Leach, H. and Ramsey, J.D. (1986) Gas Chromotography. In: *Clarke's Isolation and Identification of Drugs*. 2nd edn. Ed: A.C. Moffat. The pharmaceutical Press, London. p 189.

Mandel, H.G. (1979) Pathways of drug biotransformation: biochemical conjugation. In: *Fundamentals of Drug Metabolism and Drug Disposition*. Eds: B.N. La Du, H.G. Mandel, E.L.Way and N.Y. Huntington. R.E. Krieger Publishing, pp 149-186.

McLafferty, F.W. (1973) Mass spectra of common compound classes. In: *Interpretation of Mass Spectra*. 2nd edn. Ed: M.A. Reading. W.A. Benjamin, Inc. pp 97-188.

Parkinson, A. (1996) Biotransformation of xenobiotics. In: *Casarett and Doull's Toxicology*. Ed: C.D. Klaasen, McGraw-Hill, New York. pp 113-186.

Penning, T.D., Talley, J.J., Bertenshaw, S.R., Carter, J.S., Collins, P.W., Docter, S., Granetto, M.J., Lee, L.F., Malecha, J.W., Miyashiro, J.M., Rogers, R.S., Rogier, D.J., Yu, S.S., Anderson, G.D., Burton, E.G., Cogburn, J.N., Gregory, S.A., Koboldt C.M., Perkins, W.E., Seibert, K., Veenhuizen, A.W., Zhang, Y.Y. and Isakson, P.C. (1997) Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: identification of 4-[5-(methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide (SC-58635, celecoxib). *J. med. Chem.* **40**, 1347-1365.

Riendeau, D., Charleson, S., Cromlish, W., Mancini, J.A., Wong, E. and Guay, J (1997) Comparison of the cyclooxygenase-1 inhibitory properties of nonsteroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors, using sensitive microsomal and platelet assays. *Can J Pharmacol* **75**, 1088-1095.