

THE EXCRETION OF IBUPROFEN BY THE HORSE – A PRELIMINARY REPORT

J. A. EVANS, M.A., M.V.B., Ph.D., M.R.C.V.S., M.I.C.I., M. B. T. LAMBERT, M.A., Ph.D., C.Chem., F.I.C.I
and J. MILLER, M.Sc.

Equine Forensic Unit, Department of Pharmacology, Trinity College, University of Dublin, Eire

ABSTRACT

The anti-inflammatory drug Ibuprofen [(±)-2-(p-isobutylphenyl) propionic acid] was estimated in the blood and urine of a horse using gas-liquid chromatography of the silylated derivative. Levels of the drug in the two body fluids were measured over a period of about 24 hours after administering a 12 gm dose of Ibuprofen. Plasma peak levels were observed within 30 to 60 min, and the drug was no longer detectable in the plasma by 8 hr. Urinary peak levels were observed 200 to 300 min after dosing, and the drug was no longer detectable in the urine by about 28 hr. It was observed that only 2% to 6% of the free unchanged drug was excreted in the urine.

Introduction

Ibuprofen, 2-(4-isobutylphenyl) propionic acid ("Brufen", The Boots Co. Ltd.) is a compound with analgesic, anti-inflammatory and anti-pyretic properties. The drug potency in a number of conventional animal tests is between 16 and 32 times that of acetylsalicylic acid ("aspirin") and it is effective in the treatment of arthritis (Adams, Cliffe et al, 1967, Adams, McCullough et al, 1969).

Ibuprofen levels in the plasma and urine of several species have been reported in the literature (Adams, Bough et al, 1969; Mills, Adams et al, 1973; Brooks, Schlagel et al, 1973). No values in the body fluids for similar experiments on the horse have been published.

The use of anti-inflammatory drugs in horses at the time of racing is forbidden by the Rules of Racing of Ireland, France, Britain and a number of other European Countries.

Materials and Methods

Estimation of Ibuprofen in Urine. The analytical method used here was first developed for the detection and estimation of Ibuprofen in urine and plasma of greyhounds. Mills, Adams et al, 1973, and Kaiser and Vangiesen in 1974 published a similar method using the methyl derivative.

Urine (25 ml) was adjusted to pH 2.0 and extracted with chloroform (40 ml) mixed, and the extracts pooled. The chloroform was dried with anhydrous sodium sulphate and evaporated to dryness. The residue dissolved in ethanol was transferred to a hypo vial (Phase Separation Ltd.), the alcohol evaporated and internal standard (n-tetradecane in tetrahydrofuran) added. The extracted Ibuprofen was silylated 2 hrs at room temperature with

N,O-bis (trimethylsilyl) acetamide and the resulting mixture injected in the column (3% SE30 on Chromosorb W(H.P.) 80-100 mesh at 165°C using nitrogen carrier gas and flame ionisation detector.

Plasma was diluted with an equal volume of water, adjusted to pH 2.0 and 8.0 ml. of the diluted plasma extracted with three lots of ether (10 ml). Thereafter the procedure was essentially as for urine.

Animal Experiments. A seven year old gelding of 390 kg weight was used for the experiment. The subject was given aqueous solutions of Ibuprofen orally. In the first experiment the animal was given a 4 gram dose of the pure drug at 11.00 hr each day for three days. The dose on the final and fourth day was increased to 12 grams. In the second experiment the animal was given only a single dose of 12 grams made up from 30 pulverised tablets. Samples were collected during the days of treatment and for up to thirty hours after the final dose.

The urine was collected in a harness (Weir & Gifford, 1971) and stored at -12°C until analysed.

Results

The results of the experiments are shown in Figs 1 to 5.

Discussion

Figure 1 shows that there were no materials present capable of interfering with either the silylated Ibuprofen or the internal standard. This was true both for the urine and plasma extracts. The third peak shown in the figure was an artefact produced by the tetrahydrofuran which however did not interfere with the method. It was important to use tetrahydrofuran of high purity and to

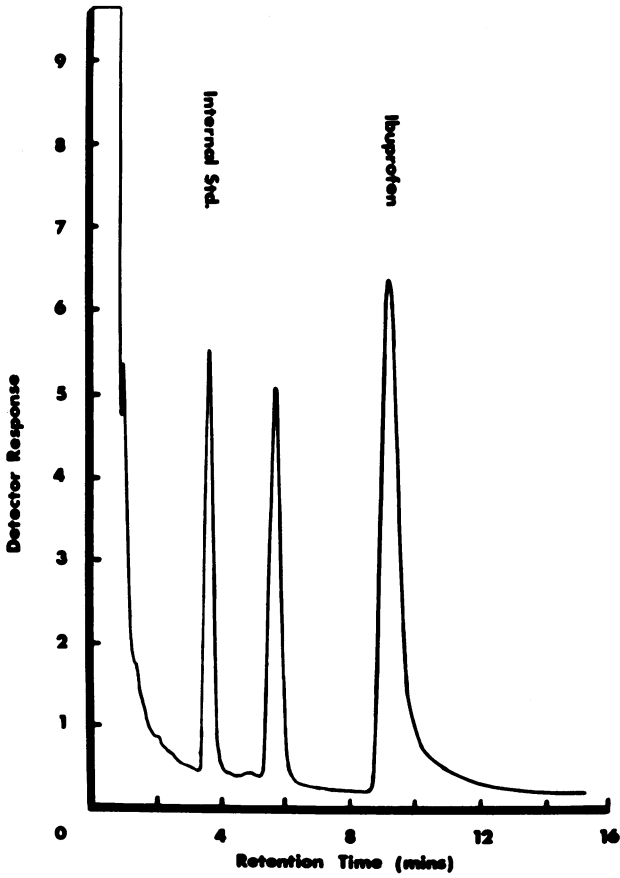


Figure 1: Gas-liquid chromatogram of horse urine extract.

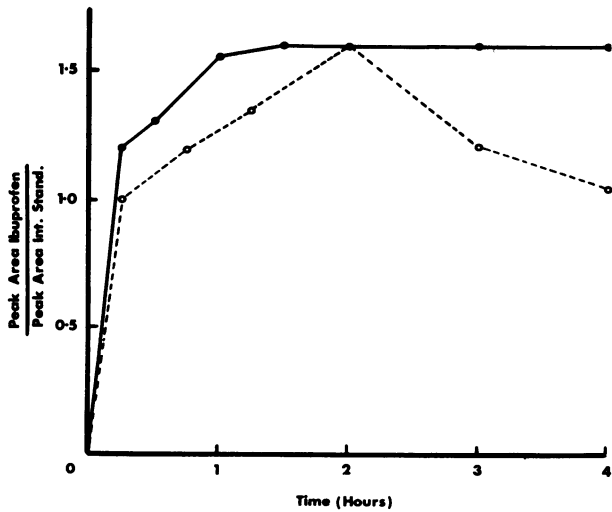


Figure 2: Effect of reaction time on the formation of the silylated Ibuprofen derivative.

● —●— moisture absent, ○ - - - - ○ moisture present.

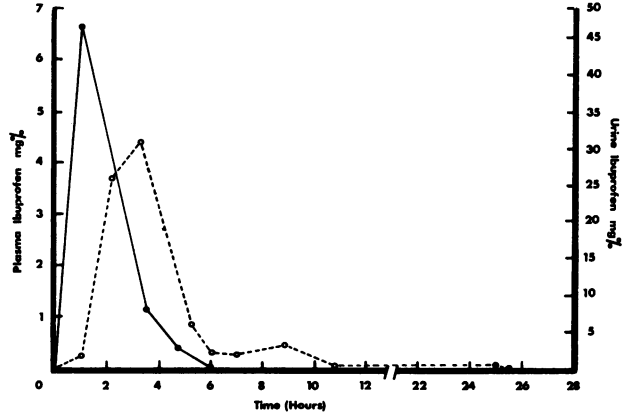


Figure 3: Plasma and urine concentrations of Ibuprofen versus time in the horse after a 12 gm. dose orally of pure Ibuprofen in water.

● —●— plasma, ○ - - - - ○ urine.

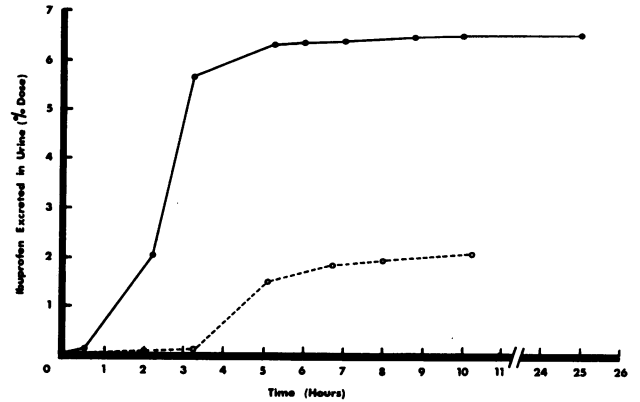


Figure 4: Ibuprofen excreted in horse urine as a percentage of dose versus time.

● —●— 12 gm. pure drug, ○ - - - - ○ 12 gm drug as pulverised tablets.

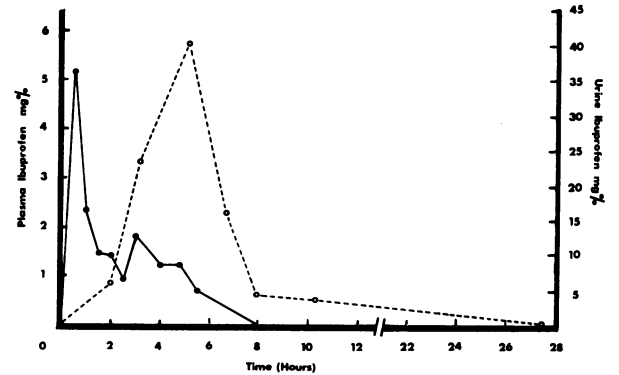


Figure 5: Plasma and urine concentrations of Ibuprofen versus time in the horse after a 12 gm. dose of Ibuprofen as pulverised tablets.

● —●— plasma, ○ - - - - ○ urine.

discard material which showed evidence of breakdown with increasing time. Figure 2 shows the effect of the (presumed) presence of moisture on the silylation reaction. Purging of the vial with nitrogen prior to silylation resulted in the reaction reaching completion by about 90 minutes and the derivative remaining stable for many hours. No deterioration was observed for those samples held for up to 24 hours. When moisture was present the same amount of product was formed, but at a slower rate, and breakdown of the derivative followed fairly rapidly.

The two main metabolites of Ibuprofen were not silylated under the conditions outlined. The derivatisation of these compounds required, in addition to BSA, the presence of the compound trimethylchlorosilane (Phase Separation Ltd.).

The drop in urinary drug values showed a biphasic pattern, as had been observed in the earlier experiment, with an 89% drop in values over the first 170 minutes, followed by a steady decrease over the next 20 hours. The drug had nearly disappeared by about 28 hours. The amount of unchanged drug excreted, as a percentage of

dose given, was very much smaller in the experiment using pulverised tablets, being only about 2% (Figure 4). The two figures given may not represent percentages of drug absorbed since there is no information concerning excretion by routes other than urine.

The rate of absorption is faster than that reported in the literature for man, rabbit and dog where figures of 90 minutes were reported (Adams, Bough et al, 1969; Mills, Adams et al, 1973) but slower than the 20 minutes reported for the rat (Adams, Bough et al, 1969).

The small percentage of unchanged drug appearing in the urine is in agreement with studies on other species (Mills, Adams et al, 1973).

Acknowledgements

The authors wish to thank The Boots Co. Ltd. for the gifts of Ibuprofen and two of its metabolites and to acknowledge the technical assistance of Messrs. J. Sullivan, J. Doherty and J. Cusack. The work was financed by the Racing Board, to which we are grateful.

REFERENCES

- Adams, S. S., Cliffe, E. E., Lessel, B and Nicholson, J. S. 1967. Some Biological Properties of 2-(4-Isobutylphenyl) propionic acid. *J.Pharm.Sci.* **56**, 1686.
- Adams, S. S., Bough, R. G., Cliffe, E. E., Lessel, B. and Mills, R. F. N. 1969. Absorption, Distribution and Toxicity of Ibuprofen. *Tox.Appl.Pharm.* **5**, 310-330.
- Adams, S. S., McCullough, K. F. and Nicholson, J. S. (1969). The Pharmacological Properties of Ibuprofen, an anti-inflammatory, analgesic and antipyretic agent. *Arch.int.Pharmacodyn.* **178**, 115-129.
- Brooks, C. D., Schlagel, C. A., Sekhar, N. C. and Sobota, J. T. 1973. Tolerance and Pharmacology of Ibuprofen. *Cur.Therap.Res.* **15**, 180-190.
- Kaiser, D. G. and Vangiessen, G. J. 1974. GLC Determination of Ibuprofen [(±)-2-(p-isobutylphenyl) propionic acid] in Plasma. *J.Pharm.Sci.* **63**, 219-221.
- Mills, R. F. N., Adams, S. S., Cliffe, E. E., Dickinson, W. and Nicholson, J. S. 1973. The Metabolism of Ibuprofen. *Xenob.* **3**, 589-598.
- Weir, J. J. R., and Gifford, F. 1971. Urine Collection Harness for Horses. *Vet.Rec.* **89**, 583-584.

DISCUSSION

TOBIN: I was very surprised at the difference between the urinary levels for the tablets and the pure drug. Have you any hypothesis or explanation?

LAMBERT: Not unless it is the fact that it was two completely different preparations, the bioavailability could be different. The drug was a crystalline powder supplied by the manufacturer. The tablets we pulverised because it is not easy to give 30 tablets and we thought it better to wash it down.