Equine hair analysis: current status and future prospects

The number of articles in the peer-reviewed literature on drug elimination in the mane and/or tail of horses is very few. Yet this issue of Equine Veterinary Journal adds 2 further articles (pp 113, 118) to the list. Perhaps something is afoot. An attraction of hair as a matrix for drug and other chemical analyses lies in the fact that it is readily collected, transported and stored. Certainly, the prospect of using sensitive methods of analytical chemistry, not merely to detect but to quantify drug (and indeed drug metabolite) concentrations in human hair has been with us for more than 20 years (Baumgartner et al. 1979). These authors detected opioid drugs in the hair of human addicts, using a radioimmunological method. The first report of the application of this technique in horses did not appear for a further 19 years (Beresford et al. 1998). Well before the 20th century, however, Casper (1857–58) detected arsenic in a suspected murder victim 11 years after death. Even more impressive is the detection of arsenic in the hair of the Emperor Napoleon more than 150 years after death (Forshufvud et al. 1961) and of cocaine in the hair of South American mummies more than 500 years old (Springfield et al. 1993). This retrospective aspect of hair analysis highlights unique features of hair in comparison with other matrices, such as blood, serum, urine, faeces or various organs and tissues.

First, hair is a living tissue but provides a much more permanent environment than other matrices. For the great majority of drugs, elimination from plasma, faeces, urine and tissues occurs much more rapidly, so that even the most sensitive techniques fail to detect (let alone quantify) most drugs and their metabolites after hours/days/occasionally weeks in body fluids and tissues. There are a few exceptions, involving strong (usually covalent) binding of drugs to plasma protein (e.g. the salicylanilide flukicide group of drugs) or tissues (e.g. the firm binding of polymyxins and aminoglycoside antibiotics to renal tissue).

Secondly, hair forms a very stable environment, enabling detection and quantitation months or years after drug administration. In this issue (p 113), Dunnett and Lees (2004) quantified, in horses with a known therapeutic history, the 2 constituents of a potentiated sulphonamide product (sulphadiazine and trimethoprim) 5 months after dosing. The same authors, in unpublished studies, have quantified the same drugs in equine hair 3 years after therapeutic dosing. The stability of entrapped drug in hair is not absolute, however. In a canine study, it was found that there was a slow decline in phenobarbitone concentration from follicle to tip in dogs with epilepsy receiving constant daily dosage over several months (Dunnett et al. 2002). This decline might be due to chemical breakdown at environmental temperatures, but is more likely to reflect repeated damage to individual hairs and consequent leakage of drug. Other potential factors leading to reduced drug concentration over time include decomposition caused by heat or UV light.

Thirdly, as revealed in the studies of Dunnett and Lees (2003, 2004), sectional hair analysis opens up the prospect of estimates of dose administered. Correlating administered dose to amount detected in hair will depend, in future studies, on relating plasma concentration as indicated by area under plasma concentration time curve (AUC) to concentrations in hair. This will vary markedly between drug groups and individual drugs within groups. This is well illustrated by potentiated sulphonamide products, for which sulphonamide:trimethoprim concentration ratios are of the order of 4:1 in formulated products and 20:1 in plasma but approximately 1:4 in hair. This marked difference is explained by the preferential binding of basic drugs such as trimethoprim to melanin in hair compared to weak acids like sulphonamides.

Fourthly, sectional hair analysis can also be used to give an approximate indication of date of drug dosing, the accuracy of the prediction decreasing with increasing time from days to weeks. One reason for this is that individual hairs grow at different rates, and a recent collaborative study of ours indicates that at any one time there is no growth (resting phase, telogen) in approximately 7% of equine hairs (M. Dunnett et al., unpublished data). In theory, prediction of period of dosing will also be adversely affected by seasonal variation in rate of growth of mane and tail hair. A preliminary investigation on hair growth rate by Popot et al. (2000) suggested a possible seasonal variation in mane growth. However, more detailed studies by Dunnett’s group indicate a uniform rate of mane and tail when measured over one calendar year (Tracy et al. 2002; Dunnett and Lees 2003).

Finally, determination of parent drug to metabolite ratios may be indicative of the route of drug administration. A recently completed study that investigated deposition of the fluoroquinolone antimicrobial enrofloxacin in mane and tail hair indicated enrofloxacin (parent drug):ciprofloxacin (metabolite) ratios of 21:1 after i.v. administration and 13:1 after dosing by the oral route (Dunnett et al. 2004).

The 2 articles on drug detection in mane and tail hair in this issue demonstrate both the feasibility and applicability of hair analysis to detect and quantify drugs and their metabolites in hair. A principal application is complementary analysis to urine testing for the detection of the illegal use of drugs in relation to competitive equine sports, including racing, eventing and showing, and also in relation to breeding...
programmes, where drugs may be used in attempts to improve conformation and enhance growth and development, for example in breeding stallion programmes. As Schlupp et al. (2004) point out in this issue (p 118), clenbuterol (a drug of the $\beta_2$-agonist class) is an anabolic agent, the use of which is banned in the horse but, because of high potency (and therefore use in small amounts) and relatively rapid clearance, detection in blood and urine is possible only for short periods; yet the consequences of its action of enhanced muscle growth are more persistent. Even with doses as low as 0.8 µg/kg bwt twice daily for 10 days, Schlupp et al. (2004) showed that the drug could be detected within 5 days in mane and tail hair, was maximal on Day 90 and still quantifiable at a concentration of 21 pg/mg on Day 360. On the other hand, the drug was not detectable in urine after Day 30.

For the future, there is much work to be done:

a) Investigate the potential of hair to provide a historical record of therapeutic or illegal drug exposure.

b) Develop and validate analytical methods for a wide range of therapeutic drugs and agents of abuse. Dunnett and Lees (2003) reported that methods were available for the detection and quantitation in equine hair of 20 drugs of several classes, including anti-inflammatory/analgesic, antimicrobial, sedative, vasodilator, anabolic and antifungal drugs.

c) Correlate hair concentrations with administered dose, plasma concentration and derived pharmacokinetic parameters, such as AUC.

d) Correlate hair drug concentration with factors such as lipid solubility, plasma protein binding, degree of ionisation (acid-base properties) and, most importantly, binding to melanin.

e) Investigate the pathways of incorporation; entry may be gained through capillary blood supply to follicles, or through sebum or sweat.

f) Apply under various circumstances the fruits of the new technology to such fields as prepurchase vetting, antidoping control, residue monitoring and compliance with therapeutic drug administration programmes.

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References


