PHARMACOGENOMICS: IMPLICATIONS FOR DRUG TESTING

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ABSTRACT

Studies in the field of pharmacogenomics have shown that there is large inter-subject variability in the response to drugs as a result of genetic differences between subjects which can contribute to variability in drug-metabolising enzymes and drug targets. Genetic differences between individual animals complicate the determination of what constitutes a pharmacologically insignificant trace of a substance because, in addition to the treatment effect size, the number of genetic groups, the size of each genetic group and the genetic effect of the substance must all be tested for. The provision of information regarding excretion/detection times for drugs is also complicated by genetic differences in rates of metabolism. In this paper, the implications of pharmacogenomics in the formulation of drug rules and provision of information regarding drug excretion times are discussed. It is concluded that policy development in relation to drugs in racing must include consideration of inherent genetic individuality.

INTRODUCTION

Prohibited substances are described in different ways in various racing jurisdictions. For example, in Australia and the UK, they are defined as substances capable of acting on one or more mammalian body systems, based on the definition of a prohibited substance in Article 6 of the International Agreement on Breeding, Racing and Wagering produced by the International Federation of Horseracing Authorities (International Federation of Horseracing Authorities 2006). Under the articles of the Japan Racing Association (JRA), prohibited drugs are those that can 'temporarily stimulate, or depress, race performance' (Japan Racing Association 2006). Under the model rules of the Association of Racing Commissioners International (ARCI) drugs are classified according to their likely impact on the outcome of a race, and their accepted use in horses (ARCI 2006).

Each of the definitions relies on an assessment of the potential of the drug in question to influence the physiological function of horses (in the case of the JRA and ARCI interpretations), or mammals more generally, as in the Article 6, UK and Australian definitions.

Advances in analytical methods have increased the likelihood that pharmacologically insignificant levels of drugs or their metabolites may be detected in samples taken from racing animals. The ARCI has specifically stated that members should 'address trace level detection so as not to lead to disciplinary action based on pharmacologically insignificant traces of these substances' (Tobin *et al.* 1999). Regulatory authorities have responded to the developments in analytical capacity in 2 ways. The first is the implementation of thresholds that are either explicitly stated, or implicit in the sensitivity of the analytical approach used, and below which disciplinary action is not pursued. The second is the publication of detection or withholding times that can provide a source of guidance in the 'safe' use of medications.

The appropriate implementation of either approach requires that consideration be given to developments in pharmacogenetics and pharmacogenomics. These rapidly developing areas of study focus on the contribution of individual genes (in the case of pharmacogenetics), or groups of genes (pharmacogenomics) in shaping an individual's response to drugs.

PHARMACOGENETICS AND PHARMACOGENOMICS

In people, many drug responses appear to be genetically determined. These genetic differences appear to be due to single nucleotide polymorphisms (SNPs) in genes encoding drug

transporters, drug-metabolising enzymes, and enzymes involved in DNA biosynthesis and repair (Shastry 2006). The considerable inter-individual variation in drug response between human patients is well known, and in some cases poses a serious threat to patient safety. Genetic differences may create variability in drug-target responses, and affect pharmacokinetics (Shastry 2006). Drug concentrations in plasma can vary more than 600-fold between 2 people of the same weight on the same drug dosage (Eichelbaum *et al.* 2006). Population variability in the plasma concentrations of phenylbutazone and oxyphenbutazone achieved in response to a standarised dose has been reported in horses (Chay *et al.* 1984). Eichelbaum *et al.* (2006) estimated that, in general, genetic factors account for 15–30% of inter-individual differences in drug metabolism and response, although for some drugs this may be as high as 95%.

The effect of genetic differences on drug metabolism is well recognised. In people, between 7% and 20% of randomly selected drugs are metabolised by enzymes known to exhibit functional genetic polymorphisms, and polymorphisms of drug-metabolising enzymes affect about 30% of all drugs used (Eichelbaum *et al.* 2006). The genes encoding for the cytochrome P450 group of enzymes have a significant number of alleles in people. Allelic differences in cytochrome P450 enzymes have been shown to affect the metabolism and excretion of drugs including warfarin in man (Voora *et al.* 2005). Siest *et al.* (2005) stated that genetically determined differences in the activity of one member of the cytochrome P450 group, CYP2D6, allow people to be categorised as poor metabolisers, or extensive metabolisers and that 'poor metabolisers are at increased risk for excessive or prolonged therapeutic effect or toxicity, while ultra-rapid metabolisers may not achieve sufficient therapeutic levels of the drug to be efficient'.

THRESHOLDS, HIGHEST NO EFFECT DOSES AND WITHHOLDING TIMES

Quierioz-Neto *et al.* (2002) stated that the urine concentrations of a drug in horse urine 'may bear little relationship to clinical effects', and that this observation is of 'significance when interpreting analytical results in racehorse drug testing'. They believed that 'the absence of established acceptable concentration thresholds does not allow a distinction to be made between an accidental positive result and a positive result stemming from the illegal administration of controlled substances', and proposed that 'the solution to this problem depends on the determination of the plasma and/or urinary concentrations of the active substance or its metabolites', at which there would be no pharmacological effect on performance.

Assessment of physical parameters such as heart rate, locomotor activity and heat lamp/hoof withdrawal, together with the abaxial sesamoid block test have been used to qualitatively assess whether drugs administered at specific dosages have a potentially performance enhancing effect (Kamerling *et al.* 1985; Harkins *et al.* 1999; Quierioz-Neto *et al.* 2001, 2002; Dirikolu *et al.* 2003). The maximum doses at which no physiological response can be identified have been described as the highest no-effect dose (HNED). By measuring urine concentrations of a drug and/or its metabolites following administration of the HNED, no effect urine thresholds for various drugs have been determined (Harkins *et al.* 1998, 1999; Quierioz-Neto *et al.* 2001).

However, genetic differences in the horse population may make the HNED approach inappropriate. Because genetic variation can affect the response to a drug, the HNED may be genotype-specific. Unless HNED analyses are performed across the entire range of genotypes in a species, it is possible that a drug present at the concentration calculated as the HNED may have a pharmacological effect on some horses. Population-wide analyses are clearly impractical, at least in the short term, due to the costs associated with large scale testing, and the fact that the contributions of various alleles and gene combinations to differences in drug responses are at present unknown. Although Thoroughbreds and Standardbreds can be conceptualised as relatively homogeneous ethnic group, this does not preclude the importance of genetic variation within the breeds. In people, genetic differences are greater within socially defined racial groups than between groups (Kaplan and Bennett 2003).

Calculations of withholding or detection times may be similarly flawed. Given the considerable evidence from human studies that genotypic variation leads to phenotypic differences in the clearance times of drugs, it is reasonable to assume that detection periods and withholding times are also genotype-specific.

The issue of applicability of HNED and withholding time calculations across species must also be considered. In people, there are differences between ethnic groups in polymorphisms of genes encoding for drug-metabolising enzymes and transporters. The potential exists for between species variations – HNED and withholding period determinations performed on Thoroughbreds may not be applicable to Standardbreds.

Regulatory response

The developments in the field of pharmacogenetics and pharmacogenomics raise a number of issues that need to be addressed by administrators in the framing of rules. The first is the fact that, where the rules define drugs in terms of their potential to alter performance, genotype may at some time be raised as a defence against prosecutions for breaches of the drug rules. If, as a consequence of its genotype, a horse has a less than normal response to a drug, should the presence of that drug at the proscribed levels in that particular horse constitute an offence against the rules? The more general approach taken by the UK and Australian authorities in defining prohibited substances avoids this problem, and may be the most suitable approach, if only for ease of administration. Under the ARCI model rules, the potential of a drug to influence a horse's racing performance is one extenuating factor to be considered during investigations. The question arises as to whether this requires assessment of the horse's genotype-specific response to a drug, and whether such evidence should be considered a mitigating factor.

The absolute nature of the drug rules in many jurisdictions precludes the fact that a horse may be a genetically slow metaboliser of a drug being used as a defence, even if it can be shown that the drug was administered according to the general withholding period/detection period guidelines. However, the authorities producing such guidelines should be aware of the potential impact of genetically-based differences in drug metabolism on the determination of generalised detection periods. The challenge will be in incorporating such variation in the published data.

The purpose of this paper is not to suggest solutions, but merely to highlight issues that result from scientific advances. At present, it appears that little emphasis is placed on the study of pharmacogenetics and pharmacogenomics in horses. Searches of the CAB, Medline and International Pharmaceutical Abstracts databases using combinations of the keywords, pharmacogenetic, pharmacogenomic, horse and equine returned no results. However, it is likely that with the passage of time, these topics will be of increasing importance to regulatory authorities, and that their potential implications should be considered in the management of regulatory matters.

Authorities going down the threshold path need to be aware that the calculated thresholds may not represent a true HNED. However, the use of thresholds has merit, particularly in issues of quality control. By defining the required sensitivity of analysis, procedures can be standardised between laboratories. However, it should be understood that reporting thresholds do not represent thresholds at which an absence of effects on performance can be guaranteed.

In conclusion, developments in our understanding of factors affecting drug responses and pharmacokinetics may diminish the relevance of the conceptual bases on which approaches to drug control have been based to date. The use of reporting thresholds as an administrative tool, rather than the HNED approach, reflects more accurately the current state of scientific knowledge.

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