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Original Research

Pharmacokinetic Assessment of the Marker Active Metabolites 4-Methyl-amino-antipyrine and 4-Acetyl-amino-antipyrine After Intravenous and Intramuscular Injection of Metamizole (Dipyrone) in Healthy Donkeys

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ABSTRACT

Metamizole (MT) is an analgesic and antipyretic drug labelled for use in humans, horses, cattle, swine, and dogs in some countries. Metamizole is rapidly hydrolyzed to the active primary metabolite 4-methyl-amino-antipyrine (MAA). MAA is formed in much larger amounts compared to other minor metabolites. Among the other secondary metabolites, 4-amino-antipyrine (AA) is also relatively active. The aim of this research was to evaluate the pharmacokinetic profiles of MAA and AA after administration of 25 mg/kg MT by intravenous (IV) and intramuscular (IM) routes in healthy donkeys. Six jennies were randomly allocated to two equally sized treatment groups according to a 2 × 2 crossover study. Blood was collected at predetermined times within 24 hours, and plasma was analyzed by a validated HPLC UV method. Plasma concentrations of MAA after IV and IM administrations of MT were detectable from 5 minutes to 10 hours in all the donkeys. Plasma concentrations of AA were detectable from 5 minutes to 8 hours, but in smaller amounts. C_{max} ($P < .01$), $AUC_{0\text{-last}}$, $AUC_{0\text{-}\infty}$, $AUMC_{0\text{-last}}$, and MRT ($P < .05$) were statistically different between the IV and IM groups. The AUC_{IM}/AUC_{IV} ratio of MAA was 1.37. The AA concentrations were lower than those found for MAA. The AA plasma versus time curves profiles after the two routes of administration of MT were variable (within the groups) and different (between the groups). T_{max}, λz, and $AUC_{0\text{-last}}$ were found to be statistically different between the groups ($P < .05$). The AUC_{IM} AA/AUC_{IV} AA ratio was 2.26.

1. Introduction

Metamizole (sodium N-[(2,3-dimethyl-5-oxo-1-phenyl-3-pyrazolin-4-yl)-N-methylamino] methanesulphonate) (MT), also known as dipyrone, is a pyrazolone derivative [1] introduced to pharmacotherapy in 1922 in Germany [2]. This is one of the nonopioid analgesic drugs possessing

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highest efficacy, used in both human and veterinary medicine for the treatment of pain and fever [3]. It is a weak COX-1 and COX-2 inhibitor [4] but a strong COX-3 inhibitor [5]. Recently, it has been reported that there is a potential for other likely mechanisms of action because the COX-3 inhibition may not truly explain the pharmacology of this drug [6]. Metamizole is on the human and veterinary market in several countries (European states, Asia, and South America) but has been withdrawn in others (Sweden, USA, Japan, UK, Australia, and Iran) because of safety concerns in humans. Although MT seems to be a relatively safe drug [7,8] compared to other nonopioid analgesics, there is some evidence, which is not unanimously accepted, suggesting that after prolonged administration MT might damage the hematopoietic system, triggering leukopenia, agranulocytosis, and even aplastic anemia in humans [9–11]. However, pharmacovigilance veterinary data have indicated that the incidence of adverse reactions in the target species is very low [12]. For veterinary use, MT is administered parentally in the dose range of 20 to 50 mg/kg body weight (package leaflet, Biovetalgin, BioWet, Drwalew, Poland).

There is a paucity of data on the pharmacokinetic properties of MT in animals, although the fate of MT administered to humans has already been described [13]. Metamizole is considered a prodrug which, in a hydrous environment, undergoes spontaneous breakdown to numerous metabolic products [13,14]. The parent drug is detectable in serum for just a few minutes after intravenous administration, but not after PO dosing. It is also not detectable in urine [14]. In humans, MT is rapidly hydrolyzed to the primary metabolite 4-methyl-amino-antipyrine (MAA). 4-Methyl-amino-antipyrine is further metabolized to 4-formyl-amino-antipyrine (FAA), which is an end-metabolite, and to 4-aminoantipyrine (AA) [13]. 4-Aminoantipyrine is acetylated to 4-acetyl-amino-antipyrine (AAA) [13–15] (Fig. 1). 4-Methyl-amino-antipyrine and AA are active metabolites [14,16]. The European Medicines Agency dossier reports that in bovine, porcine, and equid species, MAA has been selected as a marker residue for maximum residue limit calculation [12].

To the best of the authors' knowledge, only one report is present on the pharmacokinetics of MAA after MT intravenous administration in horses [17]. As the pharmacokinetics in horses can be different than in donkeys, the aim of the present study was to evaluate the pharmacokinetic profiles of MAA and AA after intravenous (IV) and intramuscular (IM) administrations of MT in healthy donkeys.

2. Material and Methods

2.1. Chemicals and Reagents

Pure MAA and AA analytical standard (>99.0% purity) were obtained from Toronto Research Chemicals (Toronto, Canada) and Sigma-Aldrich (St. Louis, MO, USA). The Internal Standard (IS) metoclopramide powder (>99.0% purity) was supplied by Sigma-Aldrich. Donkey control plasma samples were collected in untreated healthy donkeys belonging to the same herd as the six animals selected for the treatments.

2.2. Animal Treatment and Sampling

Six healthy adult Mammoth Jackstock jennies (*Equus asinus*), aged 7 to 11 years and weighing 210 to 290 kg, were enrolled in the study. The jennies were determined to be clinically healthy on physical examination, serum chemistry and hematological analyses. Animals were evaluated daily (for 1 week) for visible adverse effects by specialized personnel. Animal care and handling was performed according to the provision of the Directive 2010/63/UE (# 45/2014). Jennies were acclimatized to the stalls and handlers prior to commencing the study. Animals were deprived of food for 8 hours prior to the commencement of the experiment, while water was available ad libitum. Hay and water were available ad libitum from 2 hours after treatment administration.

Animals were randomly allocated to two treatment groups (A = 3 and B = 3) (six slips of paper marked with the numbers 1 to 6 in a box) according to an open, single-dose, two-treatment, and two-period crossover design experiment. Two jugular venous catheters, one in each side (for MT administration and for sample collection, respectively), were placed in each animal 1 day prior to commencement of the study. The group A animals received a single dose of MT (25 mg/kg) by intravenous injection (IV) (Biovetalgin, injectable solution 500 mg/mL, BioWet, Drwalew, Poland), whereas the group B animals received MT at the same dose by intramuscular injection (IM), injected in the middle quadrant of the neck muscle (Biovetalgin). The dose was selected based on package leaflet recommendations for equid species. An interval of 1 week (washout period) was observed to ensure complete metabolism and excretion of MAA and AA. After this period, the groups were rotated and the crossover study completed. By the end of the study, each donkey had received MT by both administration routes. The blood (3–5 mL) was collected via previously inserted catheters at assigned times (0, 15, 30, 45 minutes and 1, 1.5, 2, 4, 6, 8, 10, and 24 hours). The samples were centrifuged at 1,000*g* within 30 minutes of collection, and the harvested plasma was frozen immediately and stored at −20°C. Samples were analyzed within 1 week of collection.

2.3. HPLC-FL

The analytical method was based on a previously described method [18] with slight modifications [19]. The HPLC system was an LC Jasco (Como, Italy) that consisted of a quaternary gradient system (PU 2089 PLUS), in line with an ultraviolet detector (Jasco UV-975) set at 254 nm. The chromatographic separation assay was performed with a Luna C18(2) analytical column (250 mm × 4.6 mm inner diameter, 5 μ particle size [Phenomenex, Bologna, Italy]) preceded by a security guard column with the same stationary phase (C18(2) [Phenomenex]). The system was maintained at 25°C. The mobile phase consisted of acetonitrile:ammonium acetate (20 mM) solution, pH 5 (20:80, vol/vol) at a flow rate of 1 mL/min. The elution of the substances was carried out in isocratic mode.