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Drug Metabolism in Hemorrhagic Shock: Pharmacokinetics of Selective Markers of Cytochrome-P450 2C9, 2D6, and 3A4 Enzyme Activities in a Porcine Model[1]

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Background. **Cytochrome-P450 enzymes metabolize most administered drugs. A variety of clinical conditions affect the CYP system. However, the effect of hemorrhagic shock on CYP-mediated drug metabolism in clinical setting or in clinically applicable *in-vivo* models is largely unknown. Simultaneous administration of multiple CYP enzyme-selective drugs is a technique to ascertain a population's metabolic profile with a limited number of subjects.**

Materials and Methods. **Pigs were used as experimental animals as they possess CYP functionality similar to humans. Three probe drugs (dextromethorphan [CYP2D6], flurbiprofen [CYP2C9], and midazolam [CYP3A4]; doses: 0.5, 0.25, and 0.5 mg/kg, respectively) were administered intravenously to six Yorkshire-crossbred pigs in healthy state. Hemorrhagic shock was induced in six (four from healthy group after a 7-d washout period and two additional) pigs and the same doses of probe drugs were administered after a 14-h resuscitation phase. Blood samples were collected periodically in both phases and analyzed for parent drugs and metabolites (dextrorphan, 4'-hydroxy-flurbiprofen and 1'-hydroxy-midazolam) to calculate pharmacokinetic parameters. A comprehensive set of biochemical and physiologic markers of shock was also recorded.**

Results. **No changes in parent drug clearances were observed post-shock. Extensive metabolite formation with apparent higher exposure to total (conjugated and unconjugated) dextrorphan ($p = 0.08$), 4'-hydroxy-flurbiprofen ($p = 0.11$) and 1'-hydroxy-midazolam ($p = 0.09$) were observed post-shock.**

Conclusions. **The metabolic capacity of CYP enzymes did not appear to be severely hindered in resuscitative phase of hemorrhagic shock. Diminished renal secretory function caused by hemorrhagic shock may be the cause of metabolite accumulation in plasma.**

Key Words: **cytochrome-P450; dextromethorphan; flurbiprofen; glucuronide; midazolam; pig; pharmacokinetics; physiology; resuscitation; shock.**

INTRODUCTION

The first step in the metabolism of most drugs is an oxidative process catalyzed by the cytochrome-P450 (CYP) group of enzymes [1, 2]. The human CYP enzymes are classified in 18 families and 57 subfamilies [1]. The CYP1, 2 and 3 families are clinically most important as they are involved in the metabolism of a majority of pharmaceutical compounds in humans [1–3].

The expression levels and activities of different CYP enzymes (also called isoforms or isozymes) vary between species, between individuals of a species, and in some disease states. For example, about 5% to 10% of Caucasians lack functional CYP2D6 enzyme, making them poor metabolizers of the enzyme's substrates like dextromethorphan [4]. Another example is variability in CYP2C9 where the CYP2C9*2 and CYP2C9*3 allelic genotypes confer poor metabolizer trait for drugs such as warfarin [5]. Disease states seen in the critically ill patients, notably infection, have been shown to reduce the levels as well as the activity of the CYP enzymes. Incubation of human hepatocytes with inflammatory

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cytokines reduced CYP enzyme levels and activity [6, 7] as did the administration of lipopolysaccharide to animals [8, 9]. Administration of lipopolysaccharide to healthy humans has also been shown to reduce clearance of antipyrine, hexobarbital, and theophylline [10, 11].

Hemorrhagic shock leads to a complex syndrome of cardiovascular dysfunction, metabolic acidosis, and release of pro-inflammatory cytokines [12–14]. There is limited information regarding the effect of hemorrhagic shock on functioning of CYP enzymes. Various animal studies have demonstrated a decrease in total clearance of probe drugs (including fentanyl, midazolam, morphine, and propofol) in the hemorrhagic shock state [15–18]. These studies did not characterize the oxidative metabolism of the administered probe drugs and, therefore, do not allow interpretation of direct effect of hemorrhagic shock on CYP functioning.

The present study sought to examine the effect of hemorrhagic shock on CYP-mediated drug metabolism. Pigs were chosen as the large-animal model because there is evidence showing CYP function in pigs similar to humans [19–23]. Dextromethorphan (DEX), flurbiprofen (FLB), and midazolam (MDZ) were selected as probes of CYP activity. These compounds are metabolized to dextrorphan, 4'-hydroxy-flurbiprofen and 1'-hydroxy-midazolam by CYP2D6, CYP2C9, and CYP3A4, respectively, in humans and are well-established probe drugs to assess the activity of these CYP isoforms. Since about 80% of currently marketed drugs are metabolized by these three CYP isoforms [2], the administration of these three probe drugs can provide comprehensive information about CYP functionality in an individual. Simultaneous administration of multiple CYP probe drugs ("cocktail" strategy) has been used by various research groups [24, 25] and offers the advantage of elucidating comprehensive metabolic profile of a selected population.

The main objective of this study was to determine the effect of resuscitated hemorrhagic shock on the pharmacokinetics of three probe drugs that are metabolized primarily by the CYP enzyme system. This is the first study to investigate the pharmacokinetic disposition and inter-population variability therein of intravenously administered DEX, FLB, and MDZ in pigs. Experimental hemorrhagic shock was induced in pigs by use of a protocol developed by our group [26]. A comprehensive set of biochemical and physiologic markers of hemorrhagic shock were monitored and recorded in this study. The information about pharmacokinetics of these three probe drugs, along with biochemical and physiologic changes in pigs in hemorrhagic shock, are also likely to be useful in design of similar studies in future.

METHODS

We performed this study as a subset of comprehensive investigations of new treatment strategies for hemorrhagic shock. The animals described below came from the standard treatment arms of these studies. Results of these studies have previously been described [26–28]. The study protocol is shown in Fig. 1.

Materials and Chemicals

The sources of the chemicals and drugs have been described in detail in two previous publications [26, 29]. Injectable doses of DEX (hydrobromide salt) and FLB (sodium salt) were prepared immediately before each pharmacokinetic study by dissolving the powdered drugs in sterile 0.9% saline (Baxter Healthcare, Deerfield, IL) and filtering the resulting solution through a 0.45 μm Acrodisc syringe filter (Pall, East Hills, NY) under aseptic conditions. A commercially available generic 5 mg/mL injection (Baxter Healthcare) was used for dosing of midazolam. Blood samples were

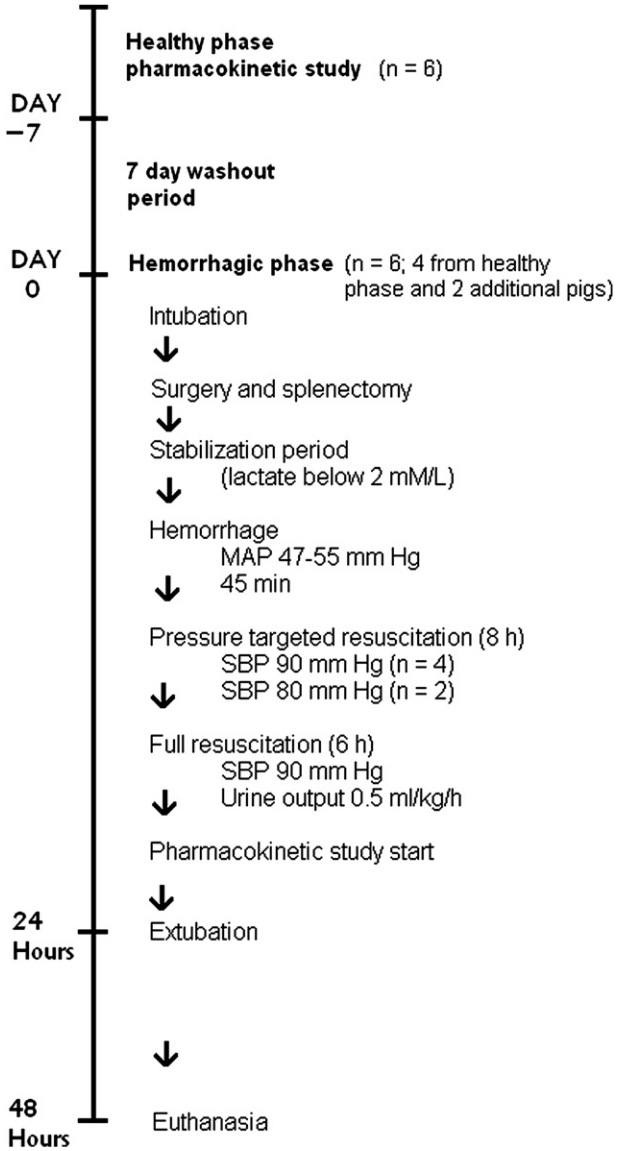

FIG. 1. Study protocol. (MAP and SBP refer to mean and systolic blood pressures, respectively).