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Pharmacokinetics of isoflavones from soy infant formula in neonatal and adult rhesus monkeys

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ABSTRACT

Consumption of soy infant formula represents a unique exposure scenario in which developing children ingest a mixture of endocrine-active isoflavones along with a substantial portion of daily nutrition. Genistein and daidzein were administered as glucoside conjugates to neonatal rhesus monkeys in a fortified commercial soy formula at 5, 35, and 70 days after birth. A single gavage dosing with 10 mg/kg bw genistein and 6 mg/kg bw daidzein was chosen to represent the upper range of typical daily consumption and to facilitate complete pharmacokinetic measurements for aglycone and total isoflavones and equol. Adult monkeys were also gavaged with the same formula solution at 2.8 and 1.6 mg/kg bw genistein and daidzein, respectively, and by IV injection with isoflavone aglycones (5.2 and 3.2 mg/kg bw, respectively) to determine absolute bioavailability. Significant differences in internal exposure were observed between neonatal and adult monkeys, with higher values for dose-adjusted AUC and C_{max} of the active aglycone isoflavones in neonates. The magnitude and frequency of equol production by the gut microbiome were also significantly greater in adults. These findings are consistent with immaturity of metabolic and/or physiological systems in developing non-human primates that reduces total clearance of soy isoflavones from the body.

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1. Introduction

In many ways, the major soy isoflavones, genistein and daidzein, are archetypical endocrine-active compounds that act through estrogen receptor binding (ER α and β). The role for such chemicals in the etiology of many human disease states has been discussed widely, if inconclusively (National Research Council, 1999; Nohynek et al., 2013; World Health Organization, 2013). Isoflavones, which occur in plants as their glucoside conjugates (Schemes 1 and 2), are extensively distributed in the diets of humans and livestock across cultures that embrace traditional and commercial foods. While estrogenic effects from consumption of isoflavone-rich foods are often associated with beneficial effects in adult humans (reviewed in Messina, 2014; Lagari and Levis, 2014), a body of evidence for both adverse and beneficial effects on breast cancer has been reported in adult animal models treated with isoflavones (reviewed in Helferich et al., 2008); however, other concern comes from exposures during the perinatal period when the potential for altered organizational programming may confer increased susceptibility for diseases later in life (reviewed in Chen and Rogan, 2004; NTP, 2010).

The fetal and neonatal windows of susceptibility to the estrogenic effects of isoflavones can also reflect maturation of processes that serve to detoxify genistein and daidzein through metabolism and excretion (Coughtrie et al., 1988; Falk, 1955; Ginsberg et al., 2002; Strassburg et al., 2002), so perinatal pharmacokinetics can be a critical element of risk assessment (Mattison et al., 2014). Consumption of soy infant formula has long been recognized as a unique human exposure setting given that: many organ systems in mammals continue to develop during the neonatal period (Strassburg et al., 2002; Clancy et al., 2007); a large percentage of total caloric intake is provided by a single source that is rich in isoflavones (20–50 mg total isoflavones per day); and an estimated 10–25% of all children in the U.S. are so fed (reviewed in Chen and Rogan, 2004; Cao et al., 2009; NTP, 2010; Vandenplas et al., 2014). The internal exposure measurements (mean spot plasma

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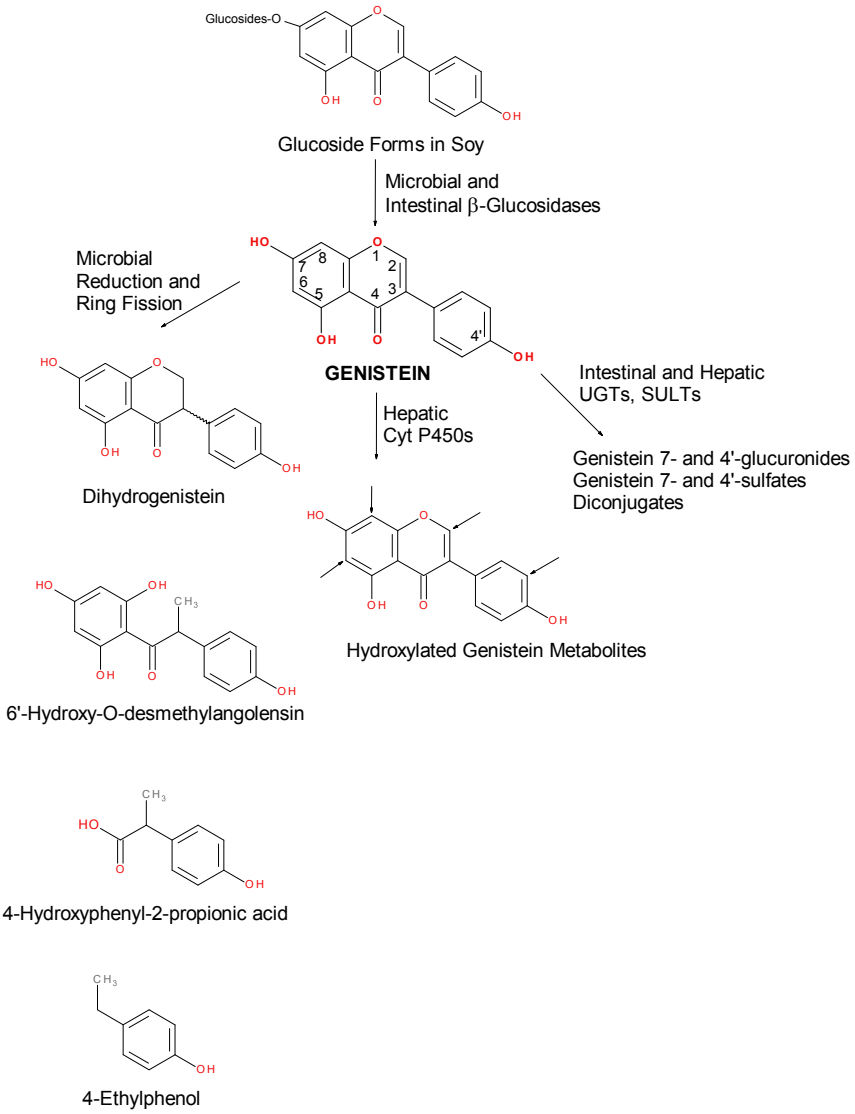

Scheme 1. Metabolism of genistein.

concentrations of total genistein and daidzein of 2.5 and 1.2 μM, respectively) reported by Setchell et al. (1997) in 4-month-old infants consuming soy formula (6–9 mg/kg bw total isoflavones per day) have provided a benchmark against which many animal studies have been designed and interpreted (e.g., NTP, 2008a; NTP, 2008b). These measurements in infants have been replicated and extended in a subsequent study by Cao et al. (2009). Nonetheless, in 2010, the National Toxicology Program and its expert panel concurred "that there is minimal concern for adverse effects on development in infants who consume soy infant formula" (https://www.niehs.nih.gov/health/topics/agents/sya-soy-formula/; NTP, 2010; McCarver et al., 2011), based in part on insufficient evidence for a conclusion from experimental animal studies of soy infant formula, soy diet, soy protein isolate, mixtures of isoflavones, daidzein, glycitein, or equol but also clear evidence of adverse developmental effects in experimental animal studies with genistein (e.g., NTP, 2008a; NTP, 2008b).

The serum pharmacokinetics of isoflavones and their glucoside conjugates have been studied in adult, pregnant, lactating, and neonatal rats, mice, and adult humans (Chang et al., 2000; Bloedon et al., 2002; Setchell et al., 2003; Doerge et al., 2006; Jefferson et al., 2009; Andrade et al., 2010). Unfortunately, limitations in study design often make many of these studies of limited value for risk assessment of human exposures through food, particularly soy formula (Doerge et al., 2002). The current study in rhesus monkeys (*Macaca mulatta*) uses an experimental design that includes: comparison of oral dosing with isoflavone glucosides in a soy infant formula between adults and several ages of neonates, starting shortly after birth; use of a dose within the range of proposed human exposure that is high enough to measure both aglycones (i.e., active) and conjugated (i.e., inactive) forms of isoflavones in serum; determinations using sensitive and specific LC/MS/MS methodology; and evaluation of oral and intravenous (IV) routes of administration in adults. The many known similarities to humans in physiology, metabolism, and pharmacology make the developing non-human primate model particularly appropriate to evaluate isoflavone serum pharmacokinetics and fill important data gaps for risk assessment of soy infant formula (Chellman et al., 2009), given the unlikely prospect of conducting such detailed studies in human infants (Setchell et al., 1997; Cao et al., 2009).