Computational Toxicology 3 (2017) 19–32

Contents lists available at ScienceDirect

Computational Toxicology

journal homepage: www.elsevier.com/locate/comtox

Physiologically-based pharmacokinetic analysis of benzoic acid in rats, guinea pigs and humans: Implications for dietary exposures and interspecies uncertainty

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ARTICLE INFO

Article history:
Received 27 February 2017
Received in revised form 1 June 2017
Accepted 13 June 2017
Available online 17 June 2017

Keywords:
Benzoic acid
Food additive
Metabolism
PBPK
Exposure assessment
Ingredient safety

ABSTRACT

Benzoic acid (BA) is a common preservative used in food and beverage products. In this study, we systematically reviewed the available pharmacokinetic data for BA within a variety of animal models to design multiple species-specific physiologically-based pharmacokinetic (PBPK) models. We focused specifically on rat, guinea pig, and human metabolic and dosimetric variations. Rate constants for the hepatic metabolism of BA to hippuric acid (HA) were predicted using elimination curves in conjunction with available liver perfusion data and appropriately optimized compartmental models. Following optimization simulations, the PBPK models were quantitatively validated by previously observed time-course datasets of BA and HA plasma concentrations after administration of different amounts of benzoate salts and/or BA, demonstrating the predictive strength and robustness of our computational approach. After validation of the computational models, we assessed resulting internal exposures that corresponded to repeated dosing schemes within each species (1, 5, 10, 50, 100 mg/kg(bw)/day). Simulated continuous daily exposure to BA at a dose of 5 mg/kg(bw)/day allowed for steady-state plasma concentrations of 0.1288 mg/L and 0.0426 mg/L in rats and humans, respectively. The individual steady-state values reached after different dosing schemes give rise to a human:rat steady-state-based margin of exposure (MOE) ratio range of 0.33–0.44 and a human:rat AUC-based MOE ratio range of 0.33–0.37 for pharmacokinetic extrapolation with respect to the dietary exposure schemes assessed. These data provide implications for reducing the pharmacokinetic component of the interspecies uncertainty factor associated with the current acceptable daily intake for assessing dietary exposures to benzoates.

1. Introduction

Benzoic acid (BA) and benzoate salts have been used as direct food and beverage additives for decades. These compounds effectively prevent microbial growth, as benzoates exert a high toxicological specificity toward bacteria and other food contaminants [1] while maintaining a remarkably high safety margin for humans and other mammalian species. Toxicological data for BA and related available experimental information date back as far as the 1940s, '50s and '60s [2]. In the majority of these existing studies, liberal dosing of BA or sodium benzoate elicited no toxicological responses or any stark physiological changes in rodent models. In a handful of chronic feeding studies from this era that have concrete dosing values, little to no toxicological responses were observed, where growth impairments had been assessed as a possible adverse outcome at exceptionally high doses [3–5]. In these developmental rodent models, the no observable adverse effect level (NOAEL) has been determined to be the highest doses used in these studies, which fall within the range of 550–2195 mg/kg (bw)/day. Regulatory agencies have also placed special emphasis on a multi-generational reproductive toxicity study that showed no offspring defects after up to ~500 mg/kg(bw)/day of oral BA administration [5]. This study has been regarded as having the pivotal datasets that govern the current worldwide regulations on benzoates as food additives, where the maximum acceptable daily intake (ADI) for humans has been conservatively set at 5 mg/kg (bw)/day [6] by extrapolating down from the highest tested dose in rodents (the NOAEL of 500 mg/kg(bw)/day) by the standard uncertainty factor of 100× (10× for intraspecies variations and 10× interspecies variations).

As these preservatives have been undergoing constant reevaluation, many intake assessments and estimates have been recently

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http://dx.doi.org/10.1016/j.comtox.2017.06.002
2468-1113/

produced [7–11]. A portion of these exposure studies, especially those in eastern Asia, have found that the largest probable estimations (i.e. those within the 95th percentile) were still substantially lower than the 5 mg/kg(bw)/day ADI. Among the most heavily benzoate-exposed populations in Taiwan, the highest daily dose was determined to be 3.1 mg/kg(bw)/day, or 62% of the ADI [7]. A similar study done in Hong Kong found that estimated benzoate exposure due to pre-packaged beverages was 0.97 mg/kg(bw)/day, or 19.4% of the ADI [8]. In contrast, there are a number of conservative assumptions made in the European Food Safety Authority (EFSA) 2016 opinion on benzoates that when compounded result in unrealistic estimated daily intake of benzoates [9]. Such compounded dietary estimates exceed the ADI, especially among European populations. In French populations, the highest average daily benzoate dose, based on consumption occurrence data, was calculated to be 0.79 mg/kg(bw)/day, with 2% of individuals estimated to have exceeded the ADI [10]. In a study of Flemish populations, the upper bound estimated intake was about 5.9 mg/kg(bw)day [11], however, as the authors point out, this is based upon the assumption that all the products contain the maximum allowable benzoate concentrations. Among a population in Denmark, the median values for average BA consumption all fell below the current ADI, yet the maximum high-end intake estimate did exceed the limit [12].

The Joint Expert Committee on Food Additives (JECFA) and the EFSA have both reported on these recent intake estimates [6,9], drawing special attention to the purported 95th percentile value of 7 mg/kg(bw)/day in adolescents and the purported 97.5th percentile value of 10.9 mg/kg(bw)/day in toddlers. The concerns associated with these high-end ADI-exceeding exposure estimates relate to the aforementioned developmental studies [3–5]; however, the current dietary levels still fall 100× below the doses required to elicit any significant response in rats. These estimations have led to calls to action for either (a) readjusting the maximum limit used in food and beverage products, or (b) reevaluating the safety database and thus the current ADI. With respect to the latter, one major area of uncertainty in defining and regulating intake limits is interspecies pharmacokinetic variability.

Following oral intake, BA is rapidly absorbed into the blood stream, metabolized into hippuric acid (HA), and readily excreted in many different mammalian species [13]. The distribution and elimination of BA in a physiological setting is primarily governed by metabolic processes, which are virtually all localized to the liver [14], and more specifically, to the mitochondrial matrix of hepatocytes [15]. The pharmacokinetic profile of BA, along with the primary metabolite HA, appears to be relatively conserved among mammalian species, as the same principle events have been observed in rats, mice, guinea pigs, pigs, monkeys, and humans. However, interspecies variations up until this point have only been assessed qualitatively, bearing no weight in the risk assessment process and causing regulatory agencies to rely on default uncertainty values.

We have thoroughly and quantitatively analyzed the pharmacokinetic database for benzoate preservatives to reach a more accurate measure of the interspecies internal exposure extrapolation. To do so, we have conducted a computational simulation study, employing physiologically-based pharmacokinetic (PBPK) models for BA, HA, and a variety of precursor compound exposures as they relate to estimated and assumed dietary exposures. It is important to note that the EFSA has determined that the ADME (i.e. absorption, distribution, metabolism, excretion) profile of BA is virtually the same as that of sodium or potassium benzoate [9], allowing full read-across for our models which are all based on biochemical characteristics of BA. These models and simulated dosing schemes have allowed us to reach a more accurate margin of exposure (MOE) ratio when considering pivotal toxicological rodent data for determining human safety.

2. Materials and methods

2.1. Study design and simulation approach

For this study, PBPK models were fully developed for three different mammalian species in order to assess pharmacokinetic differences from systemic exposures. To do so, similar modeling frameworks with established tissues that have been previously validated were implemented in Berkeley Madonna software (version 8.3.18; University of California, Berkeley, CA) and translated to a system dynamics format in AnyLogic multimethod simulation software (version 7.3.5; The AnyLogic Company, Chicago, IL). This adapted modeling interface provided (a) better visual development and formatting of the multi-species paradigm, (b) high-throughput parameter calibration tools, (c) useful graphing and data-capture tools for parameter variation experiments, and (d) computational discrete-event tools for complex dietary exposure scenarios, as discussed in subsequent sections of this paper. Simulation constructs are available upon request.

2.2. Multi-species PBPK development and parameterization

The models described herein were based upon important organs that have been previously compartmentalized (i.e. blood pool, liver, brain, adipose tissue, and gonads) with quantifiable properties, and remaining tissues have been categorized as either highly-perfused or poorly-perfused tissue conglomerates as described previously [16,17]. The models are also based upon the flow rates of blood to and from each of these compartments, the chemical-specific partitioning within each tissue, the oral absorption rate of the compounds, and chemical-specific metabolic and urinary elimination rates. These concepts are all (a) visualized in Fig. 1, taking into account the full model schematics specifically for BA and HA, and (b) mathematically constructed and elucidated by the differential equations listed in

Appendix A. Additional inputs for precursor compounds, benzyl acetate, benzyl alcohol and benzaldehyde, are schematically visualized in Fig. 1 as one aggregate input; however, their more complex bioavailable time-dependent inputs are described mathematically in Appendix B.

Model parameters have been categorically organized and tabulated for rats, guinea pigs, and humans (Table 1), where the only assumed and fixed parameter is body weight (*BW*). Rat weight in this model is assumed to be 0.25 kg, guinea pig weight is assumed to be 0.50 kg, and human weight is assumed to be 60 kg. Organization of parameters pertain to weight-dependent compartment volumes (*FXV*), chemical- and tissue-specific partition coefficients (*KX*), weight-dependent cardiac outputs (*Q*), tissue-specific blood flows as a fraction of total cardiac output (*QX*), first-order absorption and elimination constants (*KAb*, *KELB*, *KELH*), and Michaelian metabolic parameters for the critical enzymatic conjugation of BA to form HA (*VMAX*, *KM*). Generally, BA tissue concentration follows the most basic equation construct seen in the beginning of Appendix A (Eq. A.1) and displayed here:

$$\frac{d[BA]_X}{dt} = \left[\frac{\left(QX * Q * [BA]_{blood} - \frac{QX*Q*[BA]_X}{KX_{BA}}\right)}{BW * FXV}\right]$$

where *X* denotes the tissue or compartment of interest. In Table 1 and in Appendix A and Appendix A and Appendix B, liver parameters are denoted with the use of *L*, brain with *BR*, adipose tissue with *A*, highly-perfused with *HP*, poorly-perfused with *PP*, gonads with *G*,