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Interspecies difference of luteolin and apigenin after oral administration of *Chrysanthemum morifolium* extract and prediction of human pharmacokinetics

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The aims of the present study were to study the interspecies difference in the pharmacokinetics of luteolin and apigenin occurring in *Chrysanthemum morifolium* extract (CME) among rats, beagle dogs, mini-pigs, and humans, and compared the human pharmacokinetic parameters with the data predicted from the above three animals. The plasma concentrations of luteolin and apigenin were determined with a RP-HPLC method. An interspecies difference of pharmacokinetics was found, especially between rats and other species, the plasma concentration of luteolin was much lower than that of apigenin in rats, although the content of luteolin in CME was higher than that of apigenin, whereas the plasma concentration of luteolin was much higher than that of apigenin in dogs, mini-pigs and humans. Animal scale-up of some pharmacokinetic parameters of luteolin and apigenin were also performed after rats, beagle dogs, mini-pigs and humans were orally given CME at dosages of 400 mg/kg, 102 mg/kg, 90 mg/kg, and 20 mg/kg, respectively. Linear relationships were obtained between log mean retention time (MRT) and log species body weight (W) (kg), and log elimination half-life ($t_{1/2}$) (h) and logW. The corresponding allometric equations were $MRT = 9.382W^{0.1711}$ ($R^2 = 0.9999$) and $t_{1/2} = 4.811W^{0.1093}$ ($R^2 = 0.9013$) for luteolin, $MRT = 12.53W^{0.0356}$ ($R^2 = 0.9980$) and $t_{1/2} = 7.940W^{0.0294}$ ($R^2 = 0.9258$) for apigenin, respectively. The predicted human pharmacokinetic parameters (MRT and $t_{1/2}$) by an allometric approach were 18.6 h and 7.46 h for luteolin, 14.3 h and 8.95 h for apigenin, respectively, which were close to the values obtained from humans (20 mg CME/kg) in the present study. The study has demonstrated the possibility to extrapolate the pharmacokinetic behavior of flavonoids from animals to humans.

1. Introduction

Flavonoids are a group of natural polyphenols widely distributed in plants. About 6500 naturally occurring flavonoids have been identified up to date (Teillet et al. 2008). They are important constituents of the human diet, and the daily intake of flavonoids is approximately 200∼1000 mg (Kuhnau 1976). Major dietary sources of flavonoids include, for example, beverages (cola, red wine, coffee, tea, beer), fruits and fruit juices, vegetables, nuts, potatoes and corn (Manach et al. 2005; Spencer 2008). Many epidemiological studies showed that flavonoid intake lowered the occurrences of coronary heart disease and cancer possibly (Hertog et al. 1995). In addition, flavonoids exhibited various pharmacological effects, such as the antioxidant, anti-inflammatory, anti-mutagenic, and anti-allergic activities (Matsuda et al. 2002; Miyazawa and Hisama 2003; Ukiya et al. 2001). To better understand the *in vivo* effects of the flavonoids, pharmacokinetic information is essential, but many studies mainly focused on the pharmacokinetics of the flavonoids in animals (Gao et al. 2011; Shi et al. 2011; Singh et al. 2011). To our knowledge, only few reports on pharmacokinetic difference of flavonoids between animals and humans were published, and the pharmacokinetic data of flavonoids in human is scarce.

Since a human pharmacokinetic study cannot be performed without permission, researchers developed the interspecies scaling method to predict human pharmacokinetic information from animals. Interspecies pharmacokinetic scaling can be described as an operation where a pharmacokinetic parameter of interest can be scaled across species in an orderly manner (change in a parameter correlates with body weight) (Mahmood 2007). The concept of allometric scaling states that varied anatomical, physiological and biochemical parameters in mammals (such as tissue volumes, blood flow and process rates) can be scaled across species as a power function of the body weight (Poggesi 2004). The interspecies scaling method was applied to predict plasma concentration-time profiles (Dedrick 1973) and main PK parameters (distribution volumes, elimination half-life and clearances) (Boxenbaum 1982; Mordenti 1986). This approach implies the use of pharmacokinetics not only as a simple description of drug disposition but also as a tool for describing dynamic processes (Rowland 1977). Extrapolation from animal data to pharmacokinetic parameters in humans has emerged as an important tool applied in drug development. This extrapolation is helpful in facilitating the process of dosing transitions from animals to humans and accelerating the drug testing

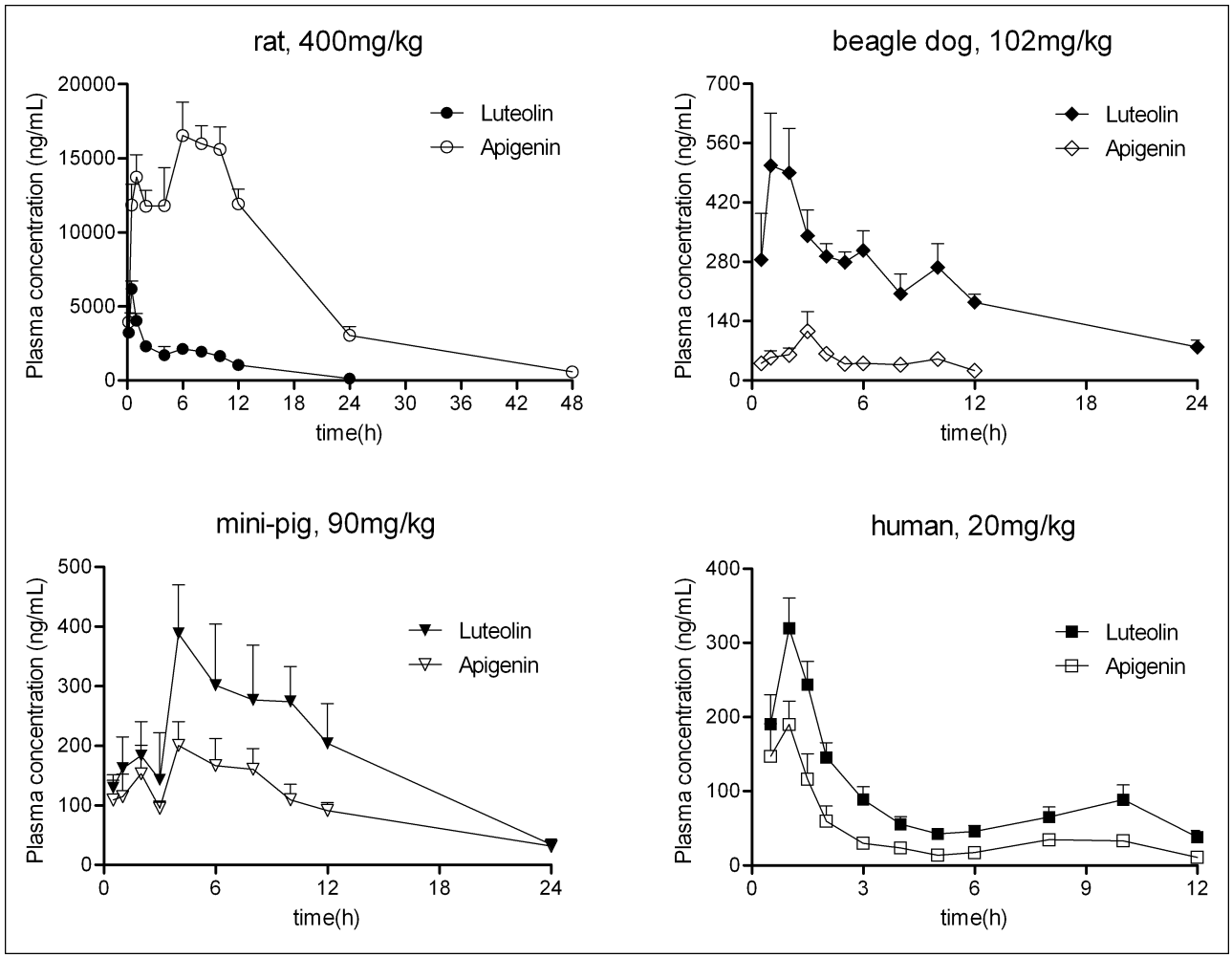

Fig. 1: Plasma concentration-time profiles of luteolin and apigenin following oral administration of CME. Data were expressed as mean ± SD, n = 3∼6 for animals, and 8 for healthy volunteers

and approval process. The ultimate goal of interspecies pharmacokinetic scaling is to determine the first-in-human dose based on predicted pharmacokinetic parameters in humans.

The flowers of *Chrysanthemum morifolium* Ramat. (CM) are widely used as a food supplement, or herb tea, and are considered as a healthy food by many consumers (Lai et al. 2007). Particularly, *Chrysanthemum* flower tea is a common beverage in China (Liu et al. 2012). In general, *Chrysanthemum morifolium* extract (CME) has been demonstrated to be safe as a traditional herbal medicine (Kim et al. 2009; Li et al. 2010) with effects on anti-myocardial ischemia and vasodilation (Jiang et al. 2004; Jiang et al. 2005). Previous studies showed that luteolin-7-O-β-D-glucoside and apigenin-7-O-β-D-glucoside were the main components contained in CM (Liu and Wu 2001) which could be hydrolyzed by the intestinal hydrolase into luteolin (3',4',5,7-tetrahydroxyflavone) and apigenin (4', 5, 7-trihydroxyflavone) when orally administered (Lu et al. 2010). Apigenin and luteolin belonging to a less-toxic and non-mutagenic flavone subclass of flavonoids, usually exist as glycosylated forms in celery, chamomile, and aromatic plants (Havsteen 2002). Their beneficial effects to human health have been extensively studied *in vitro* and *in vivo* (Hiremath et al. 2000; Middleton et al. 2000; Tormakangas et al. 2005).

Since CM is not only a herbal medicine but also a healthy food, tablets of CME have be permitted for clinical trials. Therefore, we studied the interspecies differences in the pharmacokinetics of luteolin and apigenin among rats, beagle dogs, mini-pigs, and humans after oral administration of CME, and applied the interspecies scaling method to predict the human pharmacokinetic information of luteolin and apigenin from the data of three animals, and compared the results with the pharmacokinetic parameters obtained from humans. The study will be potentially useful for providing a reference for further study of other flavonoids in humans.

2. Investigations and results

The plasma concentrations of luteolin and apigenin in the samples were analyzed by a modified HPLC method. The precision, accuracy, recoveries of assay and the stabilities of luteolin and apigenin in plasma were all fitted to the guidance for assay applied in preclinical pharmacokinetics study issued by State Food and Drug Administration of China. The linearity ranges of luteolin and apigenin were 0.0455 ∼ 8.09 μg/mL and 0.0259 ∼ 25.7 μg/mL, with the lowest limit of quantitation (LLOQ), 45.5 ng/mL and 25.9 ng/mL, respectively.

The plasma concentration-time profiles of luteolin and apigenin after oral administration of CME to rats (400 mg/kg), dogs (102 mg/kg), mini-pigs (90 mg/kg), and humans (20 mg/kg) are shown in Fig. 1. These three animals received orally CME at the equivalent dose, while humans got one third of the animal dose. Luteolin was still detectable after 48 h in rats, while it was lower than LLOQ after 24 h in mini-pigs, and 12 h in dogs and humans. Moreover, the plasma concentrations of apigenin were greater than that of luteolin in rats, although the content of luteolin in CME was higher than that of apigenin. However, the concentration of apigenin in dogs, mini-pigs and humans

Table 1: Pharmacokinetic parameters of luteolin after an oral administration of CME

Parameter	Rats (n = 5)	Dogs(n = 3)	Pigs(n = 6)	Humans(n = 8)
Dose/(mg/kg)	400	102	90	20
$AUC_{0-\infty}$/(mg/L/h)	33.4 ± 1.8	6.32 ± 1.2	4.91 ± 1.9	2.04 ± 0.47
V_z/F(L/kg)	5.13 ± 0.41	16.0 ± 4.5	17.5 ± 10	12.1 ± 6.5
Cl_Z/F(L/h/kg)	0.934 ± 0.048	1.26 ± 0.28	1.63 ± 0.73	0.79 ± 0.22
t_{max}/(h)	0.500 ± 0.00	1.33 ± 0.58	5.33 ± 1.6	1.06 ± 0.18
C_{max}/(mg/L)	6.18 ± 1.2	0.610 ± 0.20	0.522 ± 0.21	0.332 ± 0.12
MRT/(h)	7.15 ± 0.70	12.7 ± 4.4	16.3 ± 7.8	17.2 ± 8.0
$t_{1/2}$/(h)	3.80 ± 0.20	6.40 ± 3.2	6.51 ± 2.4	7.80 ± 4.1

Data were expressed as the mean ± SD (n = 3∼8).

were much higher than those of luteolin (Fig. 1). After oral dosing, absorption of luteolin in rats was the most rapid among the three animals, with t_{max} achieved 0.5 h post-dose, and the longest t_{max} value (8.0 h) of apigenin was observed in rats, meanwhile, the $t_{1/2}$ of elimination for luteolin in rats was relatively shorter (3.8 h), compared with those $t_{1/2}$ in dogs, mini-pigs and humans (Table 1). The lowest CLz/F and Vz/F values (0.934 L/h/kg and 5.13 L/kg) of luteolin in rats were observed, and the same went for apigenin (Table 2).

Figure 2 represents the application of the allometric method to luteolin and apigenin pharmacokinetic data and depicted our experimentally measured MRT and $t_{1/2}$ values in rat, beagle dog, mini-pig, and human plotted versus body weight (W) on log-log coordinates. Despite significant differences ($P < 0.01$) in the extent of the clearance of luteolin and apigenin in the three animal species examined, the data were used in the interspecies scale-up study and the prediction of humans pharmacokinetics with interspecies extrapolation. The direct regression of log MRT and log $t_{1/2}$ of luteolin and apigenin in animals versus log W (body weight) produced equations ($\log MRT = 0.9723 + 0.1711\ \log W$, $R^2 = 0.9999$ and $\log t_{1/2} = 0.6822 + 0.1093\ \log W$, $R^2 = 0.9013$ for luteolin, $\log MRT = 1.098 + 0.0356\ \log W$, $R^2 = 0.9980$ and $\log t_{1/2} = 0.8998 + 0.0294\ \log W$, $R^2 = 0.9258$ for apigenin). The predicted MRT value in humans (18.6 h for luteolin and 14.5 h for apigenin at a mean body weight of 55 kg) (Table 3) was close to the value obtained from our study following oral administration of CME at 20 mg/kg (17.2 h for luteolin and 13.8 h for apigenin). The predicted $t_{1/2}$ value in humans with interspecies extrapolation (7.46 h for luteolin and 8.93 h for apigenin) was also in close proximity to the value in the study (7.80 h for luteolin and 9.04 h for apigenin).

3. Discussion

The present study observed the interspecies differences of pharmacokinetics of luteolin and apigenin in CME. Figure 1 depicts the plasma concentration of luteolin in rats after oral administration of CME (400 mg/kg) which was much lower than that of apigenin, although the dosage of luteolin (equal to 30 mg/kg) was much higher than that of apigenin (equal to 21 mg/kg). In contrast, it was much higher than that of apigenin in dogs (102 mg/kg of CME), mini-pigs (90 mg/kg of CME), and humans (20 mg/kg of CME). Based on the linearity of PK for luteolin and apigenin at the dose of 100∼400 mg/kg of CME in rats (Ye et al. 2011) and considering the safety, we selected 20 mg/kg for humans (about one third of the animal equivalent dose). It has been elucidated in our previous study that luteolin glucoside and apigenin glucoside in CME would be hydrolyzed by hydrolase in the intestine of rats before being absorbed (Lu et al. 2010), and apigenin was absorbed more easily than luteolin in rat intestine (Chen et al. 2011), which could partly explain that the plasma concentration of luteolin was much lower than that of apigenin in rats. Additionally, a catechol ring of luteolin made it a preferred substrate of catechol-*O*-methyltransferase (COMT) which is widely distributed *in vivo* (Chen et al. 2011). Therefore, methylation of luteolin also caused its lower exposure than apigenin in rats. Though, luteolin would also be methylated by COMT in dogs, mini-pigs and humans, the activity of COMT varies from species to species, and it was higher in rats and quite lower in humans (Mannisto and Kaakkola 1999). Thus, the contribution of COMT on disposition of luteolin in humans might be much less than that in rats. Other factors induced interspecies differences of pharmacokinetics of luteolin and apigenin need to be studied further.

Allometric concepts can predict human pharmacokinetics using data from several animal species. For this purpose, these methods were applied to explore possible relationships between physiological variables such as body weight and pharmacokinetic parameters. After oral administration, the mean V_Z/F values of luteolin ranged from 5.13 L/kg in rats to 16.0 L/kg in dogs, and of apigenin ranged from 0.758 L/kg in rats to 56.5 L/kg in dogs, respectively, with the mini-pigs and humans exhibiting intermediate values (12.1–17.5 L/kg) (Table 1 and Table 2). The V_Z/F values were equal to or greater than that of body water (0.6 L/kg), suggesting that luteolin and apigenin might distribute widely into tissues (Lin et al. 1996). Particularly, V_Z/F value of apigenin in dogs were extremely large, 56.5 L/kg, which indi-

Table 2: Pharmacokinetic parameters of apigenin after an oral administration of CME

Parameter	Rats (n = 5)	Dogs(n = 3)	Pigs(n = 6)	Humans(n = 8)
Dose/(mg/kg)	400	102	90	20
$AUC_{0-\infty}$/(mg/L/h)	303.5 ± 46	0.837 ± 0.045	3.14 ± 0.61	0.859 ± 0.25
V_z/F(L/kg)	0.758 ± 0.18	56.5 ± 14	17.3 ± 5.7	15.0 ± 8.0
Cl_Z/F(q)	0.070 ± 0.011	5.68 ± 2.1	1.53 ± 0.26	1.30 ± 0.34
t_{max}/(h)	8.00 ± 2.0	3.00 ± 0.00	4.67 ± 2.0	1.06 ± 0.50
C_{max}/(mg/L)	19.1 ± 3.7	0.127 ± 0.072	0.306 ± 0.072	0.235 ± 0.11
MRT/(h)	11.8 ± 1.4	13.4 ± 6.7	14.0 ± 6.5	13.8 ± 3.1
$t_{1/2}$/(h)	7.51 ± 1.1	8.56 ± 1.6	8.60 ± 2.6	9.04 ± 3.6

Data were expressed as the mean ± SD (n = 3∼8).

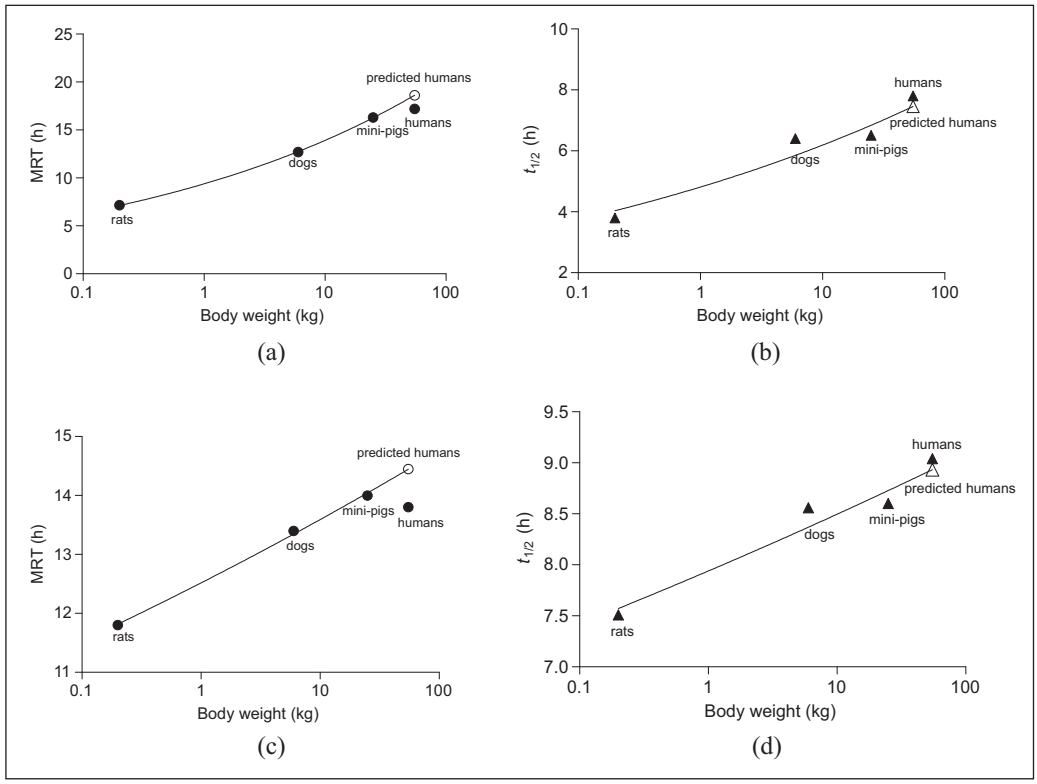

Fig. 2: Allometric relationship between (a) MRT, (b) $t_{1/2}$ of luteolin and (c) MRT, (d) $t_{1/2}$ of apigenin and species body weight (mean weight of rats, dogs, pigs, and humans studied). The regression line determined by the least-squares analysis of the three animal data were logMRT = 0.9723 + 0.1711 logW (R^2 = 0.9999) and $\log t_{1/2}$ = 0.6822 + 0.1093 logW (R^2 = 0.9013) for luteolin, logMRT = 1.098 + 0.0356 logW (R^2 = 0.9980) and $\log t_{1/2}$ = 0.8998 + 0.0294 logW (R^2 = 0.9258) for apigenin, respectively. The hollow symbols denote the predicted human values

Table 3: Predicted human pharmacokinetic parameters resulting from interspecies scaling of luteolin and apigenin

	Human value			
	Observed		Predicted	
Parameters	Luteolin	Apigenin	Luteolin	Apigenin
MRT/(h)	17.2	13.8	18.6	14.5
$t_{1/2}$/(h)	7.80	9.04	7.46	8.93

cated apigenin might be extensively bound to tissue protein in beagle dog (Tozer 1981). In addition, the Cl_z/F value of apigenin in rats were extremely small, only 0.07 L/h/kg. And the dog and mini-pig had the relatively higher clearance (Cl_z/F) of luteolin and apigenin, but relatively lower clearance in rat and human (Tables 1 and 2). Therefore, the pharmacokinetic studies revealed that significant interspecies differences exist in the rate of clearance of luteolin and apigenin among species.

Prediction of human pharmacokinetics has become an important part of the drug development process, to aid in estimating the potential therapeutic dose and safety margins before the first dose to humans. The pharmacokinetics of some drugs have been successfully extrapolated from animals to humans using allometric animal scale-up. Interspecies scaling of the Cl_z/F of drugs appeared to be relatively successful for drugs which are mainly excreted via the kidney (such as physical process) (Dedrick 1973; Paxton et al. 1990), especially for renal excretion small compounds (6–98 kDa) (Boxenbaum 1980). But, if the drug exhibits various mechanism of renal excretion among species, the scaling across species may not be simple and straightforward (Mahmood 2007). However, the scaling of the Cl_z/F among species was less successful if drug metabolism was the primary route for elimination (such as chemical process) (Dedrick 1973; Dedrick et al.1970), because species may differ in the nature of metabolite(s) and also in the rate and extent of metabolism. Among the various pharmacokinetic parameters studied in the literature, the apparent volume of distribution often yields the most statistically significant allometric relationship (Hayakawa et al. 2003; McGovren et al. 1988). In other words, V_z/F is often directly proportional to body weight (Shin et al. 2003). However, V_z/F of luteolin and apigenin in the present study did not play interspecies allometric scaling well. This might be in part due to the high serum protein binding of luteolin and apigenin in various species (Mordenti 1986). Cao et al. (2011) found that the binding constants (K_a) of luteolin and apigenin on human serum albumins (HSA) were $10^{8.6}$ and $10^{8.5}$, respectively, and the affinity of both for HSA were about 10∼10,000-times higher than that of common flavonoids. It has been reported that the predicted V_z/F is more successful in allometric scaling when the serum protein binding of a drug is low (Mordenti 1986). Moreover, the interspecies allometric scaling of CL_z/F of luteolin and apigenin did not work well. The reason might be that metabolism was the primary route of elimination in both cases (Dedrick 1973; Dedrick et al. 1970).

The data of the current study demonstrated that MRT and $t_{1/2}$ of luteolin and apigenin were well described by an allometric relationship. Allometric exponents were 0.1711 and 0.0356 for MRT of luteolin and apigenin, and 0.1093 and 0.0294 for both of $t_{1/2}$, respectively. Moreover, in comparison with observed data of human pharmacokinetics (MRT and $t_{1/2}$), the predicted data showed good uniformity. Extrapolation to human pharmacokinetics gave 18.6 h and 7.46 h for MRT and $t_{1/2}$ of luteolin, and 14.5 h and 8.93 h for apigenin, respectively, for a 55 kg body wt human. The predicted MRT and $t_{1/2}$ of luteolin approximated the mean human observed data (17.2 h for MRT and 7.80 h for $t_{1/2}$), and the same situation was observed in apigenin (13.8 h or MRT and 9.04 h for $t_{1/2}$). The validity of this scaling strategy was confirmed by the good accordance between the observed and

predicted value of luteolin and apigenin in humans (Table 3). Relatively high, persistent concentrations of luteolin and apigenin in plasma, followed by a relatively slow decrease, were observed in all species with MRT ranging from 11.8 h to 17.2 h, except for MRT (7.15 h) of luteolin in rats.

In summary, luteolin and apigenin exhibited marked interspecies differences in the pharmacokinetics of rats, beagle dogs, mini-pigs and humans after oral administration of CME. The mean MRT and $t_{1/2}$ showed general increase with species body weight and their significant interspecies correlations were obtained. Allometric interspecies scaling was applicable to predict MRT and $t_{1/2}$ of luteolin and apigenin in humans. Thus, the pharmacokinetic results obtained from animals may be used to predict the pharmacokinetics of CME in an untested species, especially humans. Moreover, the approach of allometric interspecies scaling may be employed to obtain preliminary estimates of the pharmacokinetic parameters of flavonoids in humans which can serve as a guideline for planning clinical studies.

4. Experimental

4.1. Chemicals and reagents

Luteolin and apigenin (purity > 99%) were obtained from J&K-ACIoS (Lot 62696) and Sigma-Aldrich Co. (Lot 111K1520), respectively. Methanol were of HPLC grade from Merck Co. Ltd. CME was provided by the Institute of Medicine, Zhejiang University, China, containing luteolin (7.60%, w/w) and apigenin (5.19%, w/w) by HPLC assay after hydrolysis treatment. CME tablets (100 mg CME/tablet) were provided by the Institute of Medicine, Zhejiang University, China. All other chemicals used were of the highest purity available.

4.2. Animals

All animal studies were performed according to an approved animal use protocol of Zhejiang University. Animals were maintained in the room with a controlled temperature (20 ± 1 °C), a relative humidity (50–60%), 12 h light-dark cycles, and ventilation. The filtered pathogen-free air, water and standard animal diet were provided during the pre-dosing period. All animals were fasted 12 h before CME administration, and water was provided *ad libitum*.

4.3. Oral administration of CME to rats, beagle dogs, mini-pigs and humans

Pharmacokinetic studies were conducted in male Sprague-Dawley rats, male beagle dogs, male mini-pigs, male and female humans. Blood samples were collected into heparinized tubes after CME administration, and centrifuged for 10 min at 4000 × g to separate the plasma. All plasma samples were stored at −20 °C until analysis.

Rats. Male Sprague-Dawley (SD) rats (n = 5, weighing 180∼220 g), 5 weeks old, were obtained from the Experimental Animal Center of Zhejiang Province, China. Rats were housed in the stainless steel wire-mesh cages. After fasting for 12 h, the rats were orally administered CME at a dose of 400 mg/kg suspended in 0.5% CMC-Na. A series of blood samples (0.2 mL/sample) were collected from the orbital venous sinus pre-dose and post-dose at 0.17, 0.5, 1, 2, 4, 5, 6, 8, 10, 12, 24, 48 h.

Beagle dogs. Beagle dogs (male, n = 3), weighing 5.0 ∼ 7.0 kg, were purchased from the Experimental Animal Center of Zhaoqing, Guangdong Province, China. The dogs were housed individually in stainless steel standard cages with free access to food and water. After fasting for 12 h, the dogs were orally administered CME tablets at a dose of 102 mg CME/kg, and blood samples (1.0 mL/sample) were collected from the leg vein of each animal pre-dose and post-dose at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24 h.

Mini-pigs. Bama mini-pigs (male, n = 6), weighing 24∼26 kg, obtained from the Experimental Animal Center of Guangxi University. Mini-pigs were kept individually in standard pig cages with free access to food and water. After fasting for 12 h, the mini-pigs received a single oral dose of CME tablets (90 mg CME/kg). A series of blood samples (1.0 mL/sample) were collected from the ear vein of each animal pre-dose and post-dose at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24 h.

Humans. Eight healthy humans (4 males and 4 females) were enrolled in the study. All subjects provided written informed consent prior to study participation. Subjects were aged 25 to 40 years old with 50 to 60 kg of body weight. All 8 subjects were deemed eligible by the principal investigator. The study was conducted in accordance with the ethical principle of the Helsinki Declaration and Good Clinical Practice. Subjects were prohibited from all medicines one week prior to study, also prohibited from drinking alcohol and smoking during study period. Subjects have only scheduled meals at appointed time (4 and 10 h after administration) while water was available *ad libitum*. Blood samples (0.5 mL/sample) were drawn from the vein in an arm at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12 h after oral administration of CME tablets (20 mg CME/kg) and blank samples were obtained prior to administration.

4.4. Analytical methods

The plasma concentrations of luteolin and apigenin in the samples were analyzed by a modified HPLC method developed in our previous study (Li et al. 2005). Briefly, frozen samples were allowed to thaw at room temperature, then 100 μL, 500 μL, 500 μL and 200 μL of rat, mini-pig, beagle dog and human plasma samples in 10 mL glass tubes were hydrolyzed with 2.0 mol/L HCl at 80 °C water bath for 1.5 h under continuous shaking. The hydrolyzed samples were cooled in an ice bath and then 3.0 mL of ethyl acetate was added for extraction of luteolin and apigenin. After centrifugation, 2.0 mL of supernatant was removed to another tube and evaporated to dryness under vacuum. The residue was dissolved with 250 μL mobile phase under vortexing. After centrifugation for 10 min at 15,000 × g, 50 μL of the supernatant was injected into the HPLC apparatus. All samples were analyzed by an Agilent 1100 HPLC system, with G1314A isocratic pump, a thermostatted column compartment, a variable-wavelength UV detector (VWD) and Agilent Chemstation software. Chromatographic separation was achieved on a Diamonsil ODS C_{18} column (250 mm × 4.6 mm, 5 μm) with a Security Guard C_{18} (10 mm × 4.6 mm, 5 μm) column. The optimum separation of HPLC was carried out with a mobile phase composed of methanol and 0.2% phosphoric acid aqueous solution (58:42, V/V) at a flow-rate of 1.0 mL/min. All analyses were carried out at 30 °C and the detective wavelength was 350 nm.

4.5. Pharmacokinetics analysis

t_{max} (time to reach the maximum concentration) and C_{max} (the maximum concentration) were determined directly from the plasma concentration-time curve of each animal and human. Pharmacokinetic parameters of luteolin and apigenin, such as elimination half-life ($t_{1/2}$), mean retention time (MRT), apparent volume of distribution (Vz/F) and clearance (Clz/F), were estimated via a non-compartmental approach using DAS package (version 2.0 pharmacokinetic software; Chinese Pharmacological Association, Beijing, China). The initial statistical analysis to evaluate differences in the mean data among the different species was conducted by a two-way ANOVA. $P < 0.05$ was considered to be statistically significant. All values were expressed as arithmetic mean values ± standard deviation (SD). For interspecies difference, the mean values of MRT, $t_{1/2}$ obtained from three animals and humans were plotted against body weight. Unweighted linear least-squares regression analysis was performed to fit the following relationship. The pharmacokinetic parameter was calculated using the formula

$$Y = aW^b,$$

where W is body weight, a is the allometric coefficient, and b is the allometric exponent. This equation may also be written in the following form, $\log Y = \log a + b \log W$, where $\log a$ is the y axis intercept and exponent b denotes proportionality between log Y and log W.

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