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Influence of pharmaceutical quality on the bioavailability of active components from *Ginkgo biloba* preparations

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Abstract

To be effective, herbal medicinal products are expected to meet comparable standards concerning the assessment of efficacy, safety and biopharmaceutical quality as chemically defined synthetic drugs as food supplements. However, these requirements are often not fulfilled, particularly regarding the characterization of biopharmaceutical properties such as in-vitro dissolution and in-vivo bioavailability. With respect to the relevance of biopharmaceutical quality of herbal medicinal products, two different *Ginkgo biloba* brands (test product: Ginkgo biloba capsules; reference product: Ginkgold) were analysed for dissolution rates and bioavailability of the most relevant active ingredients. Dissolution rates at pH 1 and 4.5 were determined according to the USP 23. The relative bioavailability of ginkgolide A, ginkgolide B and bilobalide was investigated after single oral administration of 120 mg *Ginkgo biloba* extract as tablets or capsules. Bioavailability data (area under the curve and peak concentration in plasma) were clearly different and did not show bioequivalence of test and reference products. The slow in-vitro dissolution of the test product resulted in a large decrease in bioavailability. These results indicate for the first time that the pharmaceutical properties of a herbal medicinal product have a significant impact on the rate and extent of drug absorption, and very likely on efficacy in humans.

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Introduction

The use of plant extracts as dietary supplements or herbal drugs is gaining increasing popularity in the USA and Europe, leading to a substantial increase in the total sales of herbal medicinal products over the last 10 years. The actual size of the market for herbal medicinal products in the USA is difficult to assess, since these products are sold mostly in health food stores, by mail order, or by multi-level marketing organizations, for which accurate statistics are not available. Nevertheless, total sales were estimated at about $1.6 billion in 1994, with projections up to $3.24 billion in 1997 (Johnston 1997). Top sellers are Ginkgo, Ginseng, Garlic, Echinacea, St John's Wort and Saw Palmetto.

As part of this trend, antioxidants in the form of enriched foods or as dietary supplements (e.g. herbal extracts) came into the focus of interest. Oxidative stress increases with age and is a major causal factor of cellular damage and enhanced apoptosis in many aged tissues, including brain, which is particularly sensitive (Christen 2000; Sastre et al 2000; Leutner et al 2001). Because of the important role of oxidative stress as a major factor in brain aging and age-related neurodegenerative disease, it seems quite conceivable that the deleterious effects of oxidative stress can be reduced by increasing antioxidant levels, and that the putative additive effects of combinations of antioxidants might be particularly effective in this regard. This might be accomplished by increasing the dietary intake of fruits and vegetables (blueberry, spinach, strawberry, curcumin, vitamin E) (Cao et al 1998; Joseph et al 1998, 1999; Lim et al 2001) or other antioxidants (Ramassamy et al 1993) in the form of dietary supplements as herbal extract products. Although the rationale is clear, the usefulness of many of these products in humans has not yet been fully demonstrated. Their efficacy is also still a

matter of debate. It is quite clear that the basic condition for the use of herbal products is the sufficient presence of antioxidant molecules in the original extracts, their stability during preparation of tablets or capsules, and their sufficient bioavailability during use in humans.

One of the top sellers on the herbal market in the USA, Europe and other countries, *Ginkgo biloba* has become a widely used herbal remedy for increasing cognitive function in elderly people (Rai et al 1991; Kleijnen & Knipschild 1992), improving symptoms and the progression of vascular and neurodegenerative dementia, improving blood flow and treating tinnitus mainly of circulatory origin (Kanowski et al 1996; Le Bars et al 1997; Ernst & Stevinson 1999; Le Bars et al 2000). Moreover, patients suffering from Alzheimer's disease benefit from long-term therapy with *Ginkgo biloba* (Kanowski et al 1996; Le Bars et al 1997), with an efficacy considered to be clinically relevant (Oken et al 1998; Ernst 2002).

Ginkgo is considered to act at least partially via its free radical scavenging, antioxidant (Sastre et al 1998; Schindowski et al 2001) and anti-apoptotic properties (Ahlemeyer et al 1999; Bastianetto et al 2000), among other mechanisms such as platelet activating factor antagonism, anti-ischaemic and antihypoxic properties.

Flavonoids and terpenoids (ginkgolides and bilobalide) are considered to be the main active principles of the *Ginkgo biloba* extract. Flavonoids exhibit an extraordinary range of biochemical and pharmacological activities in mammalian cells, acting as antioxidants (Oyama et al 1994; Ishige et al 2001), free radical scavengers, enzyme inhibitors and cation chelators, and they possess anti-allergic, anti-inflammatory, antiproliferative and neuroprotective activity (Bastianetto et al 2000). Regarding the terpenoids, ginkgolide B has platelet activating factor antagonistic activity (Prehn & Krieglstein 1993), ginkgolide A and B have glucocorticoid synthesis regulating activity (Amri et al 1996), and ginkgolide B and bilobalide have anti-apoptotic activity (Ahlemeyer et al 1999; Zhou & Zhu 2000).

The in-vivo neuromodulatory effects of *Ginkgo biloba* have recently been supported by high-density oligonucleotide microarrays to define the transcriptional effects in the cortex and hippocampus of mice treated for 4 weeks with EGb 761 (Watanabe et al 2001). Thus, unlike many other herbal antioxidants, the pre-clinical activity of Ginkgo is supported by a large number of good studies. Moreover, most of its active ingredients have been identified, leading to the use of a standardized *Ginkgo biloba* extract (EGb 761).

EGb 761 is a standardized patented extract from *Ginkgo biloba* leaves (22–27% flavonol glycosides, 5–7% terpene lactones (2.8–3.4% ginkgolides A, B, C, and 2.6–3.2% bilobalide) and less than 5 p.p.m. ginkgolic acids because of their allergenic and neurotoxic properties; Ahlemeyer et al 2001). It is approved in the form of EGb 761 containing products as ethical medicine by German and French health authorities (Monographie: Trockenextrakt (35–67:1) aus Ginkgo-biloba-Blättern, extrahiert mit Aceton-Wasser. Fed. Register No.133, July 19 1994). In the USA, most herbal remedies are sold as dietary supplements under the Dietary Supplement Health and Education Act of 1994. As a consequence, there is no approval by the Food and Drug Administration, which means that for dietary supplements, activity and efficacy has not be documented by adequate experimental and clinical studies.

Clinical studies on the efficacy and safety of herbal drugs as well as investigations into the underlying pharmacological mechanism has also come into focus in recent years; the potential contribution of the biopharmaceutical properties of the dosage form on efficacy and safety has received much less attention (Blume & Schug 2000).

We have previously investigated the pharmaceutical quality of *Ginkgo biloba* extract containing products sold on the US market and found pronounced differences in biopharmaceutical quality (in-vitro dissolution rates of the terpene lactones) (Kressmann et al 2002). In the present study, we expanded our investigation to bioavailability studies in humans using two brands (used in the previous study) of different in-vitro pharmaceutical quality. We focused our investigations on the main active constituents, ginkgolide A, ginkgolide B and bilobalide, since specific analytical methods and pharmacokinetic data were available for these constituents but not for the individual flavonol glycosides (Biber & Koch 1999; Biber 2002). We clearly demonstrate the relevance and necessity of in-vitro and in-vivo biopharmaceutical studies of herbal antioxidants. Although the experiments were carried out using well-characterized Ginkgo extracts, they are relevant for all other extracts used as herbal antioxidants, where the customer can expect that at least some of the antioxidant ingredients reach the body.

Materials and Methods

In-vitro dissolution

Tests were performed using the paddle apparatus, which was checked for system suitability before the experiments as described in USP 23. Sinkers were attached to the capsules (test product), which would otherwise float. Investigations were conducted in 900 mL dissolution medium (0.1 M HCl for pH 1 and acetate buffer for pH 4.5) at a temperature of 37 ± 0.5°C and a rotation speed of 100 rev min^{-1}. For preparation of the acetate buffer, 2.99 g sodium acetate trihydrate and 1.66 g glacial acetic acid were dissolved in 1000 mL water. Terpene lactones (ginkgolide A, ginkgolide B and bilobalide) were quantified by high-performance liquid chromatography as described previously (Kressmann et al 2002).

In-vivo bioavailability study

Twelve healthy male volunteers were included taking the two different preparations in an open-label, randomized (order of treatments), single dose, crossover design (two periods with two treatments and two sequences). Volunteers were aged between 21 and 53 years (mean: 33.3 years), height 168–196 cm (mean: 183.1 cm), bodyweight

69–95 kg (mean: 78.6 kg) and body mass index 19–27 kg m^{-2} (mean: 23.5 kg m^{-2}). Smoking was not allowed during the hospitalization phase. No major physical activities were allowed during the entire study. The intake of alcoholic or caffeine-containing food or beverages was not permitted from 36 h before the first drug administration until the last blood sampling of each period. The intake of grapefruit-containing food or beverages was not allowed from 7 days before the first administration until the end of the last blood sampling of the last period. The study was planned with a descriptive evaluation. Thus, a biometrically based sample size estimation was not applicable. It was approved by an independent ethics committee (Board of Physicians, State of Thüringen) and all subjects gave their written informed consent. The study was performed in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki.

Treatment

All subjects received an oral dose of 120 mg extract under fasted conditions. There was a washout period of at least 1 week between periods 1 and 2. The test product was Ginkgo biloba capsules, two capsules (120 mg Ginkgo biloba extract), single dose (Batch no. 461-203; Centrum Herbals, distributed by Whitehall-Robins Healthcare, Madison, USA). The reference product was Ginkgold, two tablets (120 mg Ginkgo biloba extract EGb 761), single dose (Batch no. 0200102; manufactured by Dr W. Schwabe GmbH & Co., Karlsruhe, distributed by Nature's Way, Springfield, USA). The reference product contains the standardized *Ginkgo biloba* extract EGb 761, and the test product another standardized extract. As labelled, both are characterized by a content of 24% flavone glycosides and 6% terpene lactones. Both formulations exhibited minor differences in the content of ginkgolide A, ginkgolide B and bilobalide, which were characterized by a method described in an official pharmacopoeia, DAB 2000. The test vs reference product contained 1.07 ± 0.02 mg/capsule (1.78% of extract) vs 0.81 ± 0.03 mg/tablet (1.35% of extract) ginkgolide A, 0.50 ± 0.01 mg/capsule (0.83% of extract) vs 0.41 ± 0.08 mg/tablet (0.68% of extract) ginkgolide B, and 2.01 ± 0.02 mg/capsule (3.35% of extract) vs 1.89 ± 0.90 mg/tablet (3.15% of extract) bilobalide. Each capsule or tablet contained 60 mg extract. Data are mean values $\pm$ s.d. ($n = 3$).

Sample preparation

After each dosing, blood samples were collected for up to 15 h (at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12 and 15 h after administration) for measurement of ginkgolide A, ginkgolide B and bilobalide, using an established and validated analytical method. Each blood sample was centrifuged at least 20 min after collection for 10 min at 2700 *g* and 4°C. Then, 3.0 mL plasma was acidified with 300 μL 1 M HCl for stability purposes. The stability of the terpene lactones, in particular bilobalide, is dependent on pH: they are stable at very low pH values. All samples were stored at −20°C until analysis.

Drug analysis

Plasma concentrations of these compounds were quantitatively measured by gas chromatography/mass spectrometry using negative chemical ionization, by applying a very sensitive and validated method that allowed plasma concentrations as low as 1 ng mL^{-1} of each compound to be measured (Fourtillan et al 1995; Biber & Koch 1999).

In brief, internal standard (1-ethoxy-ginkgolide B; BN 50585 Wo 148, Schwabe, Karlsruhe, Germany) (final concentration 30 ng mL^{-1}) was added to 1.0 mL plasma and acidified with 20 μL 85% phosphoric acid. The samples were loaded on oasis cartridges (Oasis HLB sorbent, 30 mg; Waters, Eschborn, Germany), which were pre-conditioned with 1 mL each of methanol and water. After washing with 2×1 mL water and 2×1 mL 5% methanol, the substances were eluted with 1 mL ethyl acetate. The organic phase was evaporated and derivatization of the residue undertaken by adding 40 μL *N,O*-bis-(trimethylsilyl)trifluoracetamide (Pierce, Rockford, USA) for 1 h at 80°C. Then, 1 μL was injected for gas chromatography.

For analysis, a Trio-2000 quadrupole mass spectrometer from Fisons (VG Biotech, Manchester, UK) in combination with a 5890 gas chromatograph from Hewlett-Packard (Waldbronn, Germany) and a PC with the Mass-Lynx software version 2.1 was used. The separation was done on an HP1 column (dimethylpolysiloxan, 12 m × 0.2 mm × 0.33 μm). The injection temperature was 280°C, the initial column temperature was 150°C and was increased by the following gradient: 20°C min^{-1} up to 200°C, 10°C min^{-1} up to 280°C, which was maintained for 6 min. Helium was used as a carrier gas with a head pressure of 50 kPa. The transfer line to the mass spectrometer was heated up to 250°C. The ionization was done by negative chemical ionization using methane. The electron energy was set to 70 eV and source temperature was 180°C. The retention times of the trimethylsilyl derivatives of the terpene lactones were 11.8 min, 12.6 min and 6.7 min for ginkgolide A, ginkgolide B and bilobalide, respectively, and 12.0 min for methoxy-ginkgolide B, which was used as internal standard.

The study samples were measured in sequences comprising calibration and quality control samples. In each run, the plasma samples from a volunteer obtained after administration of test and reference medication (periods 1 and 2) were measured.

For calculation, single ion recording was done using the following masses (m/z): ginkgolide A 390.2; ginkgolide B 344.4; bilobalide 380.2; internal standard 416.2.

The Mass-Lynx software was used for integration and determination of the peak areas, calculation of the calibration curves and plasma concentrations. For the calibration curves, linear regression was used.

For validation of the method analysing the plasma samples specificity, accuracy and precision of calibration samples and of quality control samples, the lower limits of quantification, stability and recovery were investigated.

The method was specific as shown in chromatograms from six blank plasma samples. Calibration curves (six concentrations, n = 3 each) were linear over the range of 1–30 ng mL^{-1} for the ginkgolides and 1–60 ng mL^{-1} for bilobalide. The coefficient of determination was > 0.992 in all cases. Mean accuracy and precision (3-day validation) were $< 7\%$ and $< 11\%$, respectively. The limit of quantification (LLQ) was confirmed to be approximately 1 ng mL^{-1} for the ginkgolides and bilobalide. Accuracy and precision at LLQ were $< 5\%$ and $< 13\%$, respectively. Mean accuracy and precision for low, medium and high quality control samples were $< 11\%$ and $< 9\%$, respectively. No degradation occurred after storage at –18°C for 4 weeks and, after 3 freeze–thaw cycles (low and high quality control sample), no instability of the ginkgolides and bilobalide was found. Samples were stable after storage of about 24 h in the autosampler. A recovery of $> 90\%$ was calculated for the three substances.

Pharmacokinetics

The kinetic parameters were determined model-independently for each treatment phase using the Kinetica 2000 program (InnaPhase Corporation, Philadelphia, USA) except for λ_z and derived parameters. The parameters, area under the plasma concentration time curve ($AUC_{0-tlast}$), peak concentration in plasma (C_{max}), and the time to reach c_{max} (t_{max}), were determined directly from the measured concentrations. Terminal half-life of elimination ($t_{\frac{1}{2}}$) was obtained from $t_{\frac{1}{2}} = 0.693/\lambda_Z$. Terminal rate constant (λ_Z) was estimated by regression of the terminal log-linear phase concentration–time points. $AUC_{0-tlast}$ was calculated with the use of the linear trapezoidal rule. $AUC_{0-tlast}$ was extrapolated to infinity by adding C'_{last}/λ_z, which defines the extrapolated part of the AUC (AUC_{extra}). Therefore, $AUC_{0-\infty} = AUC_{0-tlast} + C'_{last}/\lambda_z$. C_{last} is the last quantifiable concentration. In addition, the extrapolated part of the AUC (AUC_{extra}) was calculated as follows: $AUC_{extra}\,(\%) = ((AUC_{0-\infty} - AUC_{0-tlast})/AUC_{0-\infty}) \times 100$.

Statistical analysis

Statistical analysis was performed after dose normalization as a valid case analysis including all twelve volunteers. No one had major protocol deviations, therefore nobody dropped out. It was carried out on the basis of a multiplicative model for all AUC and C_{max} values, while t_{max} and $t_{\frac{1}{2}}$ were evaluated on the basis of an additive model.

Analysis of variance was performed for AUC and C_{max} values. Statistical analyses of the primary characteristics were performed at a significance level of 0.05 (type 1 error probability).

Parametric and non-parametric point and interval estimates of the test/reference ratio were calculated for AUC and C_{max} values. Relative bioavailability of test vs reference was assessed by the ratios of geometric means (point estimates). The 90% confidence intervals (CI) served as interval estimates and were determined by parametric analysis (two one-sided *t*-tests) and non-parametric analysis (two one-sided Wilcoxon–Mann–Whitney tests). The non-parametric CI values were of descriptive type and were not considered in the decision.

Results

The differences found in the in-vitro dissolution performance at pH 1 of the reference product in comparison with the test product confirmed our previous findings for different batches of the products (Kressmann et al 2002). Moreover, similar results were seen at pH 4.5, with dissolution rates for the reference product after 15 min of $> 99\%$, but rates of $< 33\%$ after 60 min for the test product, with regard to ginkgolide A, ginkgolide B and bilobalide (data not shown).

Generally, pronounced differences could be detected between the test and reference formulations regarding the bioavailability of the investigated constituents, ginkgolide A, ginkgolide B and bilobalide. The mean plasma levels of ginkgolide A, ginkgolide B and bilobalide shown in Figure 1 were obtained after administration of two capsules each of the test and reference product, corresponding to 120 mg *Ginkgo biloba* extract, to twelve volunteers. There were clear differences in terms of extent of bioavailability (as AUC) for the test vs reference product.

The pharmacokinetic parameters for ginkgolide A, ginkgolide B and bilobalide are given in Table 1. For

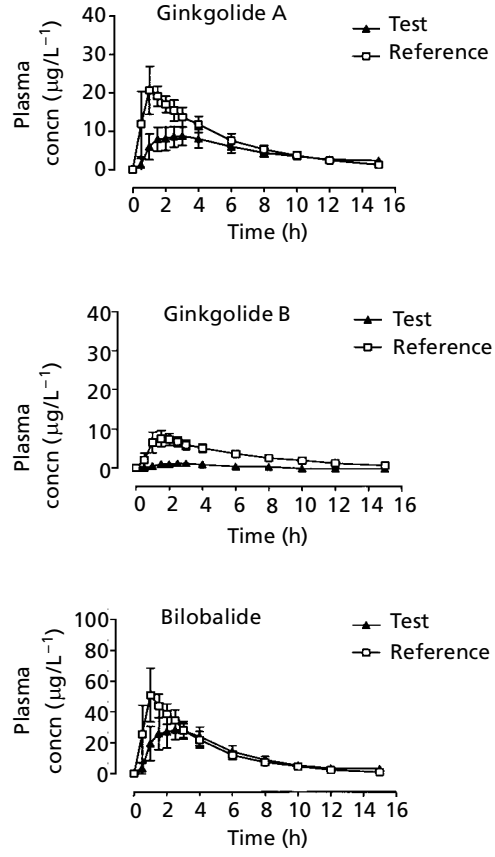

Figure 1 Plasma concentration–time profiles of test and reference products (mean ± s.d., n = 12).

Table 1 Pharmacokinetic parameters of ginkgolide A, ginkgolide B and bilobalide in 12 healthy male volunteers administered the test or reference product.

	Ginkgolide A		**Ginkgolide B**		**Bilobalide**	
	Test	Reference	Test	Reference	Test	Reference
C_{max} (ng mL^{-1})	9.43±2.80***	22.22±4.57	1.31±0.71***	8.27±1.82	33.11±10.11***	54.42±13.62
t_{max} (h)	2.21±0.66***	1.17±0.39	2.21±1.21	1.54±0.50	2.08±0.63***	1.21±0.45
$AUC_{0-\infty}$ (ng h mL^{-1})	83.92±19.44***	121.35±22.92	18.90±12.40***	59.88±11.39	191.56±43.59**	217.24±44.07
$t_{\frac{1}{2}}$ (h)	4.99±0.90**	3.93±0.40	9.91±6.02	6.04±1.48	3.52±0.73	3.19±0.40

Data are mean values±s.d. Statistical analyses of test vs reference values were performed by a paired two-tailed *t*-test (**$P< 0.01$, ***$P< 0.001$).

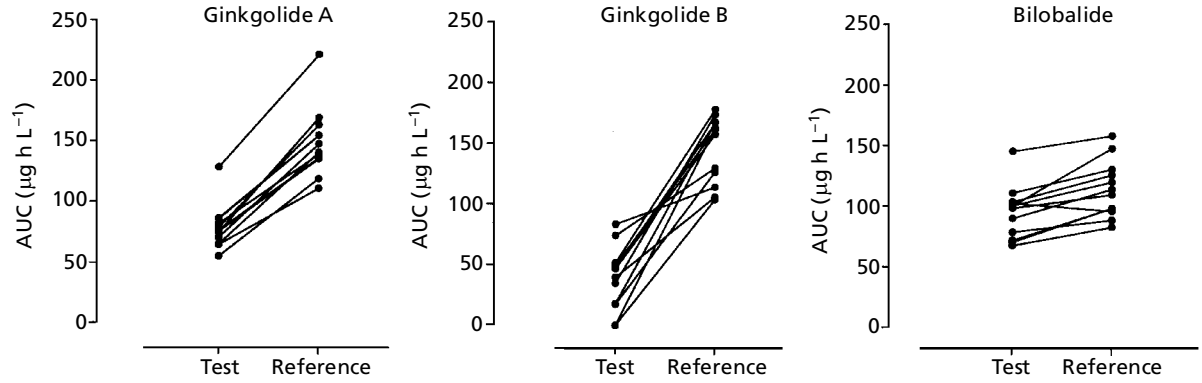

Figure 2 Comparison of individual $AUC_{0-\infty}$ data for ginkgolide A, ginkgolide B and bilobalide of test and reference products for 12 healthy male volunteers. After determination of pharmacokinetic parameters, a dose adjustment had to be performed before the statistical calculations for normalization of the content of ginkgolide A, ginkgolide B and bilobalide in the test and reference products. The normalization had been performed in such a way that the content of each constituent was used as factor for the adjustment as follows: $AUC_{0-\infty}$/content of formulation = $AUC_{0-\infty}$ adjusted or C_{max}/content of formulation = C_{max} adjusted. Therefore, the adjustment led to a normalization of the data to 1 mg for each constituent.

ginkgolide A, an arithmetically calculated $AUC_{0-\infty}$ value of 83.92 ng h mL^{-1} for the test preparation vs 121.35 ng h mL^{-1} for the reference product was found. For ginkgolide B, $AUC_{0-\infty}$ for the test product was 18.90 ng h mL^{-1}, and for the reference product was 59.88 ng h mL^{-1}. For bilobalide, $AUC_{0-\infty}$ values of 191.56 ng h mL^{-1} vs 217.24 ng h mL^{-1} for test and reference products, respectively, were found. Pronounced product-related differences were also observed for the C_{max} values. For ginkgolide A, calculation of the arithmetic C_{max} value resulted in 9.43 ng mL^{-1} for the test product and 22.22 ng mL^{-1} for the reference product, for ginkgolide B it was 1.31 ng mL^{-1} vs 8.27 ng mL^{-1}, and for bilobalide it was 33.11 ng mL^{-1} vs 54.42 ng mL^{-1} for test and reference products, respectively.

Moreover, pronounced differences could be demonstrated in terms of t_{max} values, as C_{max} occurred generally later for the test preparation compared with the reference for all three tested constituents. For ginkgolide A, this difference was more than 1 h (test: 2.21 h; reference: 1.17 h). For bilobalide, t_{max} values were 2.08 h for the test product and 1.21 h for the reference product, and for ginkgolide B, t_{max} values were 2.21 h for the test product and 1.54 h for the reference product. Differences in the calculated half-life time, $t_{\frac{1}{2}}$, regarding ginkgolide A can be explained by large standard deviations in the lower concentration range of the plasma levels (< 10 ng mL^{-1} with respect to the test product), close to the limit of detection. Small differences in the plasma levels result in relatively large differences in the slope of the terminal log-linear phase of elimination.

Pharmacokinetic parameters showed a rather low individual variability (% CV) of the parameters $AUC_{0-\infty}$ and C_{max} for the reference formulation over a range of about 20–25%. This variability was regularly higher in the case of the test product (about 20–30% for ginkgolide A, 50–60% for ginkgolide B and bilobalide).

Both formulations exhibited minor differences concerning the content of ginkgolide A, ginkgolide B and bilobalide, in agreement with our previous data using different batches (Kressmann et al 2002).

To ensure an appropriate comparison, the pharmacokinetic parameters had to be dose-adjusted to 1 mg before the statistical calculations. Therefore, the above-mentioned

Table 2 Statistical analysis of equivalence in terms of C_{max} und AUC_{tot}.

	C_{max}			AUC_{tot}		
	Ginkgolide A	Ginkgolide B[a]	Bilobalide	Ginkgolide A	Ginkgolide B[a]	Bilobalide
Recommend interval for bioequivalence		70–143%			80–125%	
Point estimate	32%	13%	57%	53%	26%	83%
90% Standard confidence interval (test/reference)	27.61–36.18%	12.68–17.87%	49.23–65.38%	49.46–55.25%	19.09–35.94%	77.59–88.08%

[a]For ginkgolide B, only 10 subjects were evaluated because of dropouts. The statistical method for testing relative bioavailability (e.g. bioequivalence) is based on the 90% confidence interval for the ratio of the population means (test/reference), for the parameters under consideration, as described in the note for guidance on investigation of bioavailability and bioequivalence CPMP/EWP/QWP/1401/98 (www.emea.eu.int/index/indexhl.htm).

differences became even more pronounced by dose normalization. All $AUC_{0-\infty}$ values for the test and reference products of each subject (crossover design) are given after dose normalization in Figure 2.

In the case of subjects 11 and 12, no relevant plasma concentrations of ginkgolide B could be observed, and calculation of pharmacokinetic parameters failed. Therefore, the statistical evaluation was performed only for subjects 1–10.

The statistical calculation (Table 2) showed tight ranges of CI values for $AUC_{0-\infty}$ and C_{max} for all three constituents. However, none of the CI values were observed to be inside the limits of 80–125% for $AUC_{0-\infty}$, or 70–143% for C_{max}. Ginkgolide A showed a point estimate and CI of 53% and 49.46–55.25% for $AUC_{0-\infty}$, and of 32% and 27.61–36.18% for C_{max}. The values were 26% and 19.09–35.94% for $AUC_{0-\infty}$ of ginkgolide B and 13% and 12.68–17.87% for C_{max}. The closest correspondence of test and reference formulations could be observed for bilobalide, with a point estimate of 83% and CI of 77.59–88.08% for $AUC_{0-\infty}$, and 57% and 49.23–65.38% for C_{max}.

Although the statistical analysis was of a descriptive type only, by calculation of CI values, the evaluation failed to prove bioequivalence, and even showed clear differences between the products (despite the relatively small sample size). The statistical analysis demonstrates that the 90% CI limits accepted for bioequivalence were outside for all substances measured (Table 2).

Comparing in-vitro and in-vivo study results, it was evident that the slow in-vitro dissolution of the test product ($< 30\%$ in 60 min) resulted in a decrease in bioavailability, especially for ginkgolide B.

Discussion

Although the market of herbal medicines in the USA is still increasing and about one-third of the adult American population are taking phytomedicines, the problem of pharmaceutical quality and bioavailability of herbal remedies sold as dietary supplements in the USA has been addressed in only a few publications. For several products on the dietary supplement market, substantial deviations from the label have been found, for example for Ginseng (Cui et al 1994), Feverfew (Heptinstall et al 1992), Ephedra (Gurley et al 2000), as well as in previous publications for *Ginkgo biloba* (ConsumerLab 2000; Kressmann et al 2002), indicating significant problems regarding pharmaceutical and/or biopharmaceutical quality.

We have previously demonstrated that many *Ginkgo biloba*-containing dietary supplements that claim a pharmaceutical quality according to the German Commission E monograph on *Ginkgo* (Fed. Register no. 133, 1994) actually fail to fulfil this specification (Kressmann et al 2002). Thus, they are not essentially similar to the innovator product (reference product in the in-vivo study) containing EGb761, which is in accordance with specifications of the German Commission E. Moreover, pronounced differences in dissolution profiles were found for some of the products. Since the relevance of different dissolution profiles found for some products in comparison with the innovator was not known, the present study comparing bioavailability in humans was initiated. The two products investigated were selected on the basis of their poor or good pharmaceutical quality (Kressmann et al 2002).

The comparison of the pharmacokinetic parameters after administration of the two products clearly demonstrated the relevance of biopharmaceutical in-vitro and in-vivo data. In comparison with the reference product, the test product showed a very poor dissolution profile at pH 1 and 4.5 in terms of ginkgolide A, ginkgolide B and bilobalide, which is in agreement with the in-vivo performance. The formulation seems to have a significant impact on the in-vivo bioavailability of ginkgolide A, ginkgolide B and bilobalide, although this effect is different for the individual compounds. Although the in-vitro/in-vivo relationship was excellent for ginkgolide B, about 75% reduction of the dissolution rate at 60 min (pH 4.5) for the test product vs

reference and about 70% reduction of $AUC_{0-\infty}$, it was not so good for ginkgolide A (about 75% and 30%), and much less for bilobalide (about 63% and 12%).

The pharmacokinetic parameters found for the reference product are in agreement with the results of Fourtillan et al (1995) with respect to ginkgolides A and B. For bilobalide, larger plasma concentrations were found, as in this study we preserved the plasma samples with hydrochloric acid to prevent the loss of bilobalide. Superiority of the reference could be demonstrated by the pharmacokinetic (Table 1) and statistical evaluation for all $AUC_{0-\infty}$ and C_{max} values (Table 2). In the case of ginkgolide B, bioavailability decreased nearly 90% after administration of the product with the low in-vitro performance.

It should be mentioned that very little is known about the metabolism and the possible presence of active metabolites of ginkgolide A, ginkgolide B and bilobalide, which may also contribute to the pharmacological activity of EGb and which were not measured in this study. Furthermore, for the neuroprotective effect of EGb, mainly the levels of the constituents in the extracellular fluid of the brain rather than the plasma levels are of relevance, although it is unlikely that plasma and brain availability of the constituents vary significantly between the two brands.

Regardless of their use as drugs or dietary supplements, the activity of herbal antioxidants depends on the presence of sufficient amounts of specific active ingredients and their bioavailability. If this is not the case, the claims made regarding human health can not be fulfilled, with the consequence that the consumer pays for a poor quality product.

References

Ahlemeyer, B., Möwes, A., Krieglstein, J. (1999) Inhibition of serum deprivation- and staurosporine-induced neuronal apoptosis by *Ginkgo biloba* extract and some of its constituents. *Eur. J. Pharmacol.* **367**: 423–430

Ahlemeyer, B., Selke, D., Schaper, C., Klumpp, S., Krieglstein, J. (2001) Ginkgolic acids induce neuronal death and activate protein phosphatase type-2C. *Eur. J. Pharmacol.* **430**: 1–7

Amri, H., Ogwuegbu, S. O., Boujrad, N., Drieu, K., Papadopoulos, V. (1996) In vivo regulation of peripheral-type benzodiazepine receptor and glucocorticoid synthesis by *Ginkgo biloba* extract EGb 761 and isolated ginkgolides. *Endocrinology* **137**: 5707–5718

Bastianetto, S., Ramassamy, C., Dore, S., Christen, Y., Poirier, J., Quirion, R. (2000) The *Ginkgo biloba* extract (EGb 761) protects hippocampal neurons against cell death induced by β-amyloid. *Eur. J. Neurosci.* **12**: 1882–1890

Biber, A. (2002) Pharmacokinetics of *Ginkgo biloba* extracts. *Pharmacopsychiatry*, In press

Biber, A., Koch, E. (1999) Bioavailability of ginkgolides and bilobalide from extracts of *Ginkgo biloba* using GC/MS. *Planta Med.* **65**: 192–193

Blume, H. H., Schug, B. S. (2000) Biopharmaceutical characterisation of herbal medicinal products: are in vivo studies necessary. *Eur. J. Drug Metab. Pharmacokinet.* **25**: 41–48

Cao, G., Russell, R. M., Lischner, N., Prior, R. L. (1998) Serum antioxidant capacity is increased by consumption of strawberries, spinach, red wine or vitamin C in elderly women. *J. Nutr.* **128**: 2383–2390

Christen, Y. (2000) Oxidative stress and Alzheimer disease. *Am. J. Clin. Nutr.* **71**: 621S–629S

ConsumerLab (2000) http://www.consumerlab.com/results/ginkgobiloba.asp (accessed 06 May 2002)

Cui, J., Garle, M., Eneroth, P., Björkhem, I. (1994) What do commercial ginseng preparations contain? *Lancet* **344**: 134

Ernst, E. (2002) The risk-benefit profile of commonly used herbal therapies: Ginkgo, St. John's Wort, Ginseng, Echinacea, Saw Palmetto, and Kava. *Ann. Intern. Med.* **136**: 42–53

Ernst, E., Stevinson, C. (1999) *Ginkgo biloba* for tinnitus: a review. *Clin. Otolaryngol.* **24**: 164–167

Fourtillan, J. B., Brisson, A. M., Girault, J., Ingrand, I., Decourt, J. P., Drieu, K., Jouenne, P., Biber, A. (1995) Proprietes pharmacocinetiques du bilobalide et des ginkgolides A et B chez le sujet sain apres administrations intraveneuses et orales d'extrait de *Ginkgo biloba* (EGb 761). *Therapie* **50**: 137–144

Gurley, B. J., Gardner, S. F., Hubbrand, M. (2000) Content versus label claims in ephedra-containing dietary supplements. *Am. J. Health Syst. Pharm.* **57**: 963–969

Heptinstall, S., Awang, D. V. C., Dawson, B. A., Kindack, D., Knight, D. W., May, J. (1992) Parthenolide content and bioactivity of feverfew (*Tanacetum parthenium* L.). Estimation of commercial and authenticated feverfew products. *J. Pharm. Pharmacol.* **44**: 391–395

Ishige, K., Schubert, D., Sagara, Y. (2001) Flavonoids protect neuronal cells from oxidative stress by three distinct mechanisms. *Free Radic. Biol. Med.* **30**: 433–446

Johnston, B. A. (1997) One-third of nation's adults use herbal remedies. *Herbalgram* **40**: 49

Joseph, J. A., Shukitt-Hale, B., Denisova, N. A., Prior, R. L., Cao, G., Martin, A., Taglialatela, G., Bickford, P. C. (1998) Long-term dietary strawberry, spinach, or vitamin E supplementation retards the onset of age-related neuronal signal-transduction and cognitive behavioral deficits. *J. Neurosci.* **18**: 8047–8055

Joseph, J. A., Shukitt-Hale, B., Denisova, N. A., Bielinski, D., Martin, A., McEwen, J. J., Bickford, P. C. (1999) Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. *J. Neurosci.* **19**: 8114–8121

Kanowski, S., Herrmann, W. M., Stephan, K., Wierich, W., Horr, R. (1996) Proof of efficacy of the *ginkgo biloba* special extract EGb 761 in outpatients suffering from mild to moderate primary degenerative dementia of the Alzheimer type or multi-infarct dementia. *Pharmacopsychiatry* **29**: 47–56

Kleijnen, J., Knipschild, P. (1992) *Ginkgo biloba. Lancet* **340**: 1136–1139

Kressmann, S., Müller, W. E., Blume, H. H. (2002) Pharmaceutical quality of different *Ginkgo biloba* brands. *J. Pharm. Pharmacol.* **54**: 661–669

Le Bars, P. L., Katz, M. M., Berman, N., Itil, T. M., Freedman, A. M., Schatzberg A. F. (1997) A placebo-controlled, double-blind, randomized trial of an extract of *Ginkgo biloba* for dementia. *JAMA* **278**: 1327–1332

Le Bars, P. L., Kieser, M., Itil, K. Z. (2000) A 26-week analysis of a double-blind, placebo-controlled trial of the *Ginkgo biloba* extract EGb 761 in dementia. *Dement. Geriatr. Cogn. Disord.* **11**: 230–237

Leutner, S., Eckert, A., Müller, W. E. (2001) ROS generation, lipid peroxidation and antioxidant enzyme activities in the aging brain. *J. Neural. Transm.* **108**: 955–967

Lim, G. P., Chu, T., Yang, F., Beech, W., Frautschy, S. A., Cole, G. M. (2001) The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. *J. Neurosci.* **21**: 8370–8377

Oken, B. S., Storzbach, D. M., Kaye, J. A. (1998) The efficacy of *Ginkgo biloba* on cognitive function in Alzheimer disease. *Arch. Neurol.* **55**: 1409–1415

Oyama, Y., Fuchs, P.A., Katayama, N., Noda, K. (1994) Myricetin and quercetin, the flavonoid constituents of *Ginkgo biloba* extract, greatly reduce oxidative metabolism in both resting and Ca^{2+}-loaded brain neurons. *Brain Res.* **635**: 125–129

Prehn, J. H. M., Krieglstein, J. (1993) Platelet-activating factor antagonists reduce excitotoxic damage in cultured neurons from embryonic chick telencephalon and protect the rat hippocampus and neocortex from ischemic injury in vivo. *J. Neurosci. Res.* **34**: 179–188

Rai, G. S., Shovlin, C., Wesnes, K. A. (1991) A double-blind placebo controlled study of *Ginkgo biloba* (''tanakan'') in elderly outpatients with mild to moderate memory impairment. *Curr. Med. Res. Opin.* **12**: 350–355

Ramassamy, C., Girbe, F., Christen, Y., Costentin, J. (1993) *Ginkgo biloba* extract EGb 761 or trolox C prevent the ascorbic acid/Fe^{2+} induced decrease in synaptosomal membrane fluidity. *Free Radic. Res. Commun.* **19**: 341–350

Sastre, J., Millan, A., Garcia, de la Asuncion, Pla, R., Juan, G., Pallardo, F., O'Connor, E., Martin, J. A., Droy-Lefaix, M. T., Vina, J. A. (1998) *Ginkgo biloba* extract (EGb 761) prevents mitochondrial aging by protecting against oxidative stress. *Free Radic. Biol. Med.* **24**: 298–304

Sastre, J., Pallardó, F. V., Vina, J. (2000) Mitochondrial oxidative stress plays a key role in aging and apoptosis. *IUBMB Life* **49**: 427–435

Schindowski, K., Leutner, S., Kressmann, S., Eckert, A., Müller, W. E. (2001) Age-related increase of oxidative stress-induced apoptosis in mice. Prevention by *Ginkgo biloba* extract (EGb 761). *J. Neural. Transm.* **108**: 969–978

Watanabe, C. M., Wolffram, S., Ader, P., Rimbach, G., Packer, L., Maguire, J. J., Schultz, P. G., Gohil, K. (2001) The in vivo neuromodulatory effects of the herbal medicine *Ginkgo biloba*. *Proc. Natl Acad. Sci. USA* **98**: 6577–6580

Zhou, L. J., Zhu, X. Z. (2000) Reactive oxygen species-induced apoptosis in PC12 cells and protective effect of bilobalide. *J. Pharmacol. Exp. Ther.* **293**: 982–988