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The Role of Alkamides as an Active Principle of *Echinacea*

Abstract

Alkamides are the major lipophilic constituents of *Echinacea* preparations, which are widely used in some European countries and in North America for common colds. In earlier investigations they have been shown to possess stimulatory effects on phagocytosis. Recent experiments have demonstrated that alkamides are detectable in human blood in relevant concentrations after oral administration of *Echinacea* preparations. Alkamides show structural similarity with anandamide, an endogenous ligand of cannabinoid receptors. Consequently, it was found that alkamides bind significantly to CB_2 receptors, which is now considered as a possible molecular mode of action of *Echinacea* alkamides as immunomodulatory agents. It was also demonstrated recently in several studies that alkamide-containing *Echinacea* preparations trigger effects on the pro-inflammatory cytokines. They were therefore suggested as a new class of cannabinomimetics. However, the therapeutic relevance of these findings is still not clear as clinical studies on the common cold show contradictory results. Among the many pharmacological properties reported, investigations concerning herb-drug interactions have been neglected for a long time. Latest research concludes that prolonged use of *Echinacea* poses a minimal risk for co-medications metabolized by the P450 enzymes.

Key words
Alkamides · *Echinacea purpurea* · *Echinacea angustifolia* · Asteraceae · immunomodulation · pharmacokinetics · cannabinoid receptor binding · herb-drug interactions

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Introduction

Echinacea preparations represent the most common herbal immunomodulators. They are marketed mainly for the treatment and prevention of the common cold and other upper respiratory tract infections (URTIs). An analysis reveals that completely different preparations are sold under the name *Echinacea* using different plant parts and different extraction solvents. The most abundantly used species are *Echinacea angustifolia*, *Echinacea purpurea* and *Echinacea pallida* [1]. Several reviews on the effectiveness of orally ingested *Echinacea* extracts in reducing the incidence, severity, or duration of acute URTIs have been published. The majority of trials investigated whether *Echinacea* preparations shorten the duration or decrease the severity of symptoms of the common cold (for reviews see [2], [3], [4]). A recent Cochrane review [3] concluded that especially preparations based on the aerial parts of *E. purpurea* might be effective for the early treatment of colds in adults but results are not fully consistent. Beneficial effects of other *Echinacea* preparations for preventative purposes might exist but have not been shown in independently replicated rigorously randomized trials.

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So far, polysaccharides, glycoproteins, caffeic acid derivatives and alkamides have been considered as the constituents most relevant for activity [5]. Alkamides produce a strong tingling effect in the mouth, which has been considered as a marker for high quality *Echinacea* by the American Indians [6], [7], [8], [9]. In recent years, research has focused on these lipophilic constituents of *Echinacea* because of their bioavailability. This review summarizes the evidence for the contribution of alkamides to the immunomodulatory effect of *Echinacea* and their possible role as active principles in the use of *Echinacea* for common cold.

Distribution of Alkamides in *Echinacea*

Alkamides are the major lipophilic constituents and can be found in high concentrations in the roots of *E. purpurea* and *E. angustifolia* and with decreasing concentrations in the aerial parts of *E. purpurea*, *E. pallida* and *E. angustifolia*. They vary between species and between different parts of the plant [10], [11]. A distribution study indicated that the root bark and secondary roots of *Echinacea angustifolia* contained the highest concentrations, whereas the stems and leaves of *E. angustifolia* were devoid of alkamides [12]. High quality *E. purpurea* root material contains up to 6 mg/g alkamides [13]. The main alkamides are the isomeric dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides (**8a**/**8b**) (Fig. **1**). Similar *N*-isobutylamides are also present in *Spilanthes* spp. and *Xanthoxylum* species [6], [7].

Pharmacology with Molecular Biological Aspects

A series of previous pharmacological experiments have shown that *Echinacea* extracts containing alkamides have significant anti-inflammatory and immunomodulatory properties. Among the many pharmacological effects reported, modulation of macrophages and PMN immune cells and effects on cytokine/chemokine expression in human cells have been demonstrated most convincingly [2], [4], [14], [15], [16], [17], [18], [19].

The lipophilic part of an ethanolic extract obtained from the roots of *E. purpurea* at a concentration of 10^{-3}% and from the roots of *E. angustifolia* (10^{-4}%), which contained the full spectrum of alkamides, stimulated phagocytosis of yeast particles by human polymorphonuclear neutrophils (PMN) *in vitro* by 37% and 34%, respectively. In mice, *per os* administration of lipophilic alkamide fractions from the roots of *E. angustifolia* and *E. purpurea* enhanced phagocytosis in the carbon-clearance assay by factors of 1.5 and 1.7, respectively [20], [21]. Other *in vivo* studies in rats have shown that administration of hydroalcoholic extracts (100 μL twice daily by oral gavage for 4 days) of *E. purpurea* roots and aerial parts containing defined concentrations of cichoric acid, polysaccharides and alkamides stimulated the phagocytic activity of macrophages dose-dependently [22]. An increase in lipopolysaccharide-stimulated nitric oxide release was observed in macrophages obtained from the spleens of rats previously treated with the standardized *Echinacea purpurea* extracts. A similar set of experiments demonstrated stimulation of alveolar macrophage function by alkamides administered to healthy rats [23]. In murine RAW264.7 macrophages, alkamides exerted an inhibitory effect on LPS-mediated activation, and a significant reduction in NO production was observed in comparison to cells treated with LPS alone. These data suggest that alkamides may have not only immunostimulatory but also anti-inflammatory activity [24]. An "Echinacea Premium Liquid alkamide fraction", which did not contain caffeic acid derivatives but only alkamides with undeca-2*E*-ene-8,10-diynoic acid isobutylamide as the major 2-ene alkamide and dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide as the major 2,4-diene alkamide, was found to significantly inhibit TNF-α production under LPS-stimulated conditions in the mouse macrophage cell line RAW 264. In these macrophages, only the alkamide mixture isolated from an ethanolic *Echinacea* extract ("Echinacea Premium Liquid") of *Echinacea purpurea* (300 mg/mL) and *Echinacea angustifolia* roots (200 mg/mL) significantly decreased LPS-stimulated NO production at a concentration of 2.0 μg/mL. However, the individual alkamides, undeca-2*E*-ene-8,10-diynoic acid isobutylamide and dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide as found in the isolated alkamide mixture did not reduce the LPS-induced NO production in the same assay [25].

Clifford has reported *in vitro* inhibition of cyclooxygenase-1 (COX-1) and, to a lesser extent, of cyclooxygenase-2 (COX-2) by alkamides isolated from *E. purpurea* roots [26]. *In vitro* inhibition of 5-lipoxygenase and cyclooxygenase (from sheep seminal microsomes) has also been reported for polyunsaturated alkamides from *E. angustifolia* roots and *Achillea* species [27]. COX-1 inhibitory activity was highest for undeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide (**2b**), dodeca-2*E*,4*Z*-diene-8,10-diynoic acid 2-methylbutylamide (**6**) and undeca-2*E*,4*Z*-diene-8,10-diynoic acid 2-methylbutylamide (**15**), with inhibitions of 60, 48 and 55%, respectively, at concentrations of 100 μg/mL. Undeca-2*Z*,4*E*-diene-8,10-diynoic acid isobutylamide (**2b**) possessed with 46% the strongest COX-2 inhibitory activity. Compounds **6** and **15** showed 31 and 39% inhibition of COX-2, respectively [26]. Inhibition of 5-lipoxygenase has also been described for extracts of roots of *E. purpurea*, *E. pallida* and *E. angustifolia* with IC_{50} values of 0.642, 1.08 and 0.444 μg root/mL, respectively. The alkamide concentrations in the root of each species were 0.05%, traces and 0.2%, respectively [28]. Inhibition of cyclooxygenase is known as an effective strategy to suppress pain and inflammation. Alkamides isolated from the roots of *E. angustifolia* inhibited COX-2-dependent prostaglandin E_2 formation, but did not inhibit COX-2 expression at the transcriptional or translational level. An analysis of 8 different alkamides revealed a contribution of undeca-2*Z*-ene-8,10-diynoic acid isobutylamide (**3b**), dodeca-2*E*-ene-8,10-diynoic acid isobutylamide (**5**) and dodeca-2*E*,4*Z*-diene-8,10-diynoic acid 2-methylbutylamide (**6**) to this response [29]. Recently, Gertsch et al. [14] demonstrated the modulation of TNF-α gene expression and multiple signal transduction pathways by *Echinacea* alkamides and postulated a mechanism related to cannabinoid receptors. Parallel *in vitro* kinetic experiments measuring both mRNA and protein levels over a timespan of 39 h after a co-incubation with LPS and *E. purpurea* tincture (Echinaforce™, endotoxin < 0.5 EU/mL) have been performed. LPS-stimulated TNF-α protein expression was potently modulated by the *Echinacea purpurea* preparation, resulting in a significant inhibition (40%) during the first 20 h, and subsequent stimulation of TNF-α protein expression (Fig. **2**).

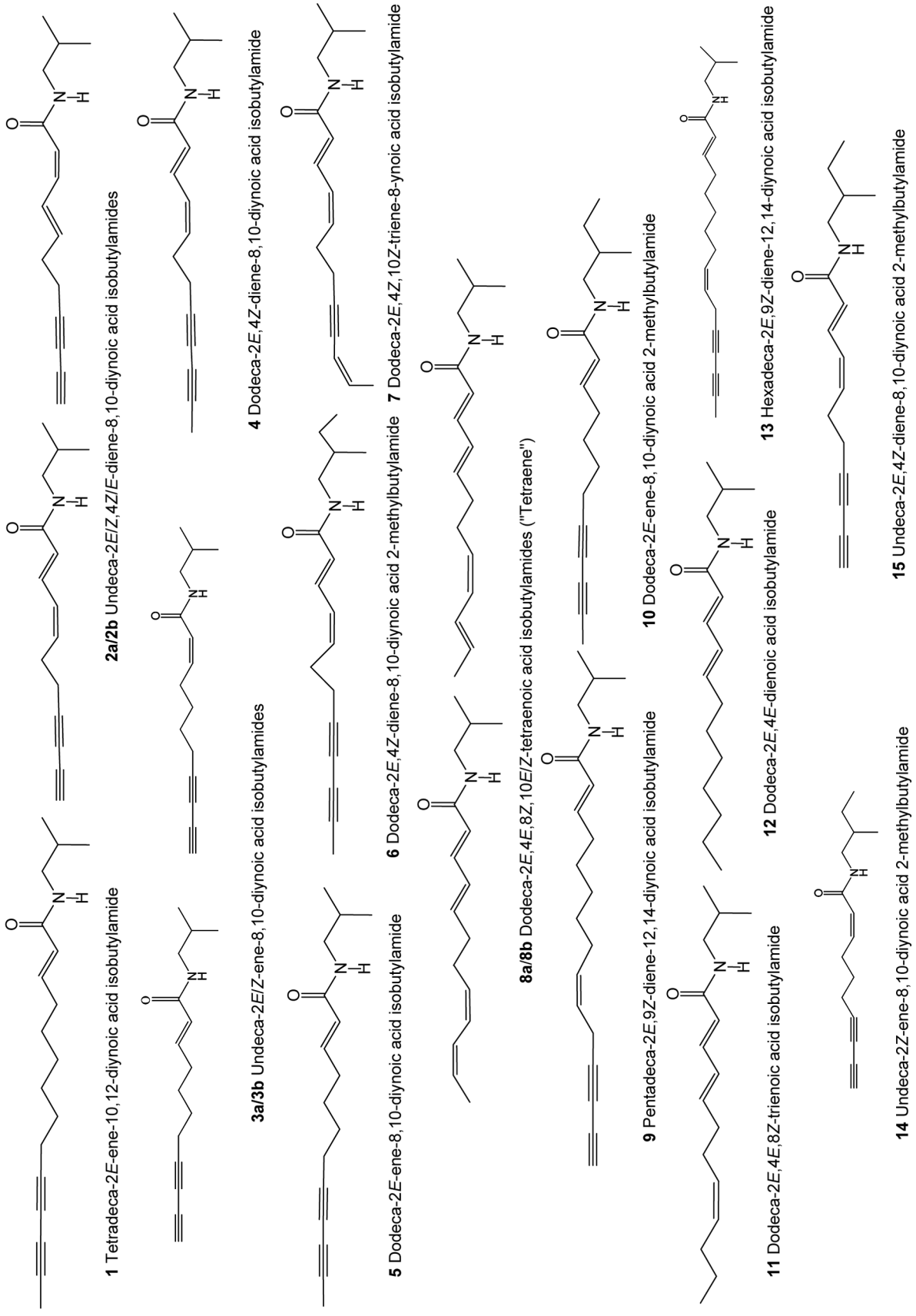

Fig. **1** Structures of the main *Echinacea* alkamides.

To ascertain whether CB_2 was the receptor subtype involved in the observed effects, a CB_2 antagonist was used in combination with the dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides. The specific antagonist strongly abolished TNF-α transcription and thus indicated a strict peripheral cannabinoid-mediated process. In parallel, receptor binding studies to rodent CB_1 and

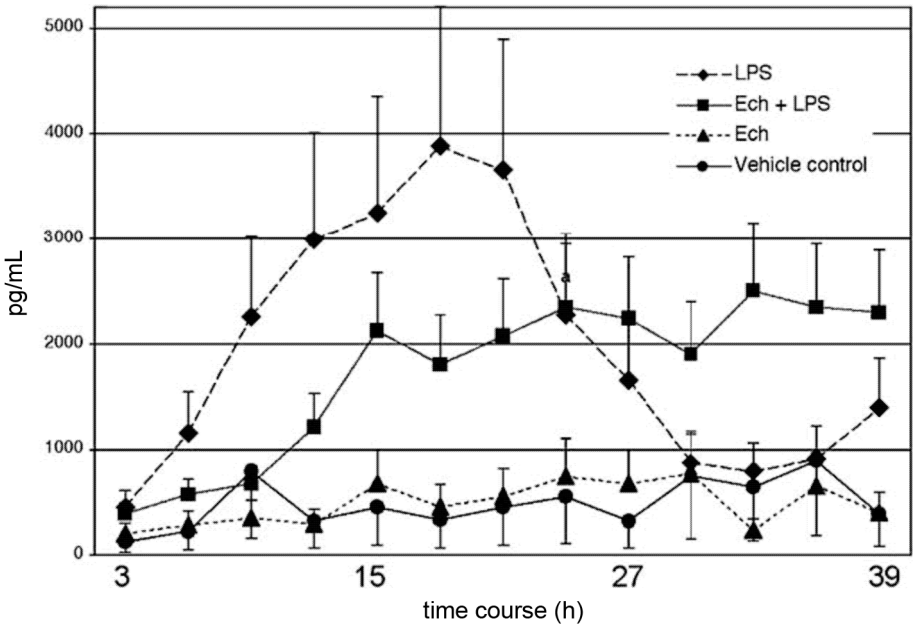

Fig. **2** Kinetic study showing TNF-expression in primary human monocytes/macrophages-enriched PBMCs (3×10^6 cells) from peripheral blood as protein levels, over a time course of 39 h. Echinaforce™ (25 μg/mL) and LPS (1 μg/mL) were both tested alone and in combination. Echinaforce™ was incubated for 1 h before addition of LPS. The protein concentrations were determined by ELISA. Data points were obtained every 3 h and are mean values ± S.E. from three independent experiments. Reprinted with permission by the publisher Elsevier, from reference Gertsch J, et al. FEBS Lett 2004; 577: 563 – 9 [14].

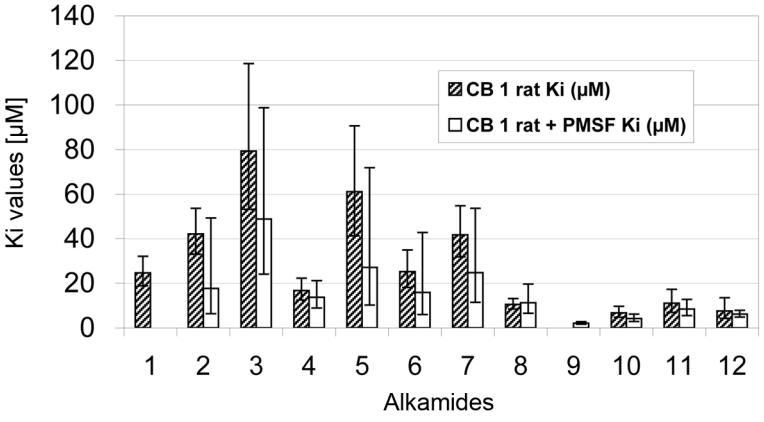

Fig. **3** Selectivity of alkamides from *E. angustifolia* for the CB_1 receptor from rat membranes with and without PMSF, obtained by a standard receptor binding assay using a [^{3}H]CP-55,940 as the radioligand and reported as mean Ki values [μM] with corresponding 95% confidence intervals determined from at least three independent experiments (from Woelkart K, et al. [30]).

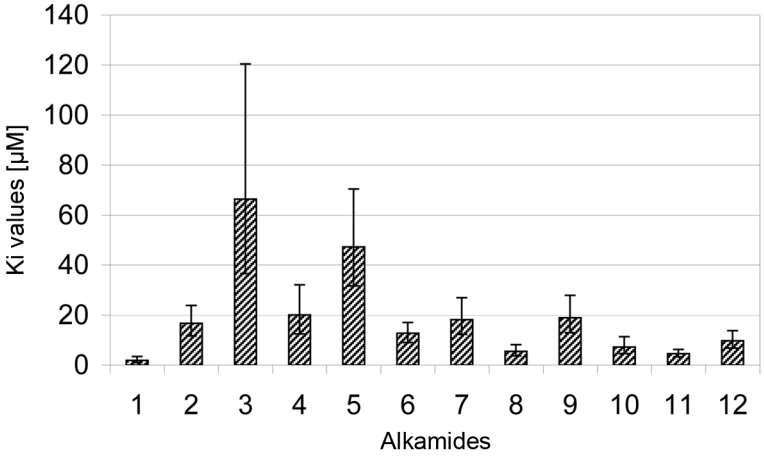

Fig. **4** Selectivity of alkamides from *E. angustifolia* for the CB_2 receptor from mouse membranes, obtained by a standard receptor binding assay using a [^{3}H]CP-55,940 as the radioligand and reported as mean Ki values [μM] with corresponding 95% confidence intervals determined from at least three independent experiments. (form Woelkart K, et al. [30]).

CB_2 receptors were conducted by Woelkart et al. [30]. Most of the *Echinacea* alkamides (**1**, **2a**, **2b**, **6**, **7**, **8a**, **8b**, **9**, **10**, **11**, **12**) showed affinities to CB_2 receptors with Ki values lower than 20 μM, some only five times less active than anandamide, the endogenous ligand [30] (Fig. **3** and **4**). The most recent evidence of CB-receptor-binding has been demonstrated by Raduner et al. [16]. At concentrations below 100 nM, dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide (**8a**) and dodeca-2*E*,4E-dienoic acid isobutylamide (**12**) potently displaced the radioligand from membrane recombinantly overexpressing CB_2 receptors with Ki values of 57 ± 14 nM and 60 ± 13 nM, respectively. In addition the interaction of alkamides with CB_2-receptors was explored in a homology model. In this binding arrangement, the importance of the aromatic ring of Tyr190 of the CB-receptor was highlighted [16]

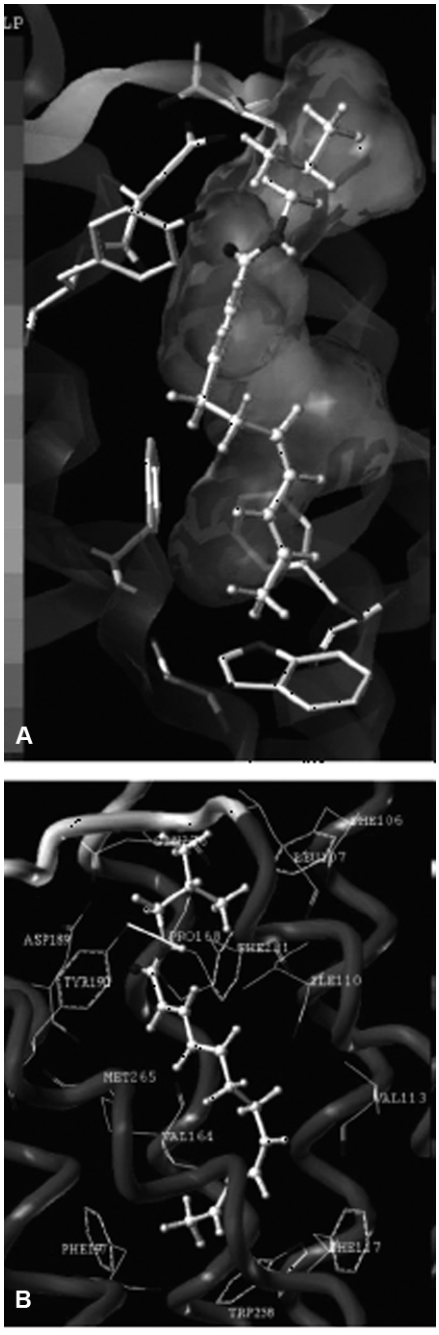

Fig. **5** Proposed binding conformation of dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide (**8a**) and dodeca-2*E*,4*E*-dienoic acid isobutylamide (**12**) in the CB_2-receptor. **A** The putative binding site for CB_2 ligands is located adjacent to helices III, V, VI and VII at the near extracellular side of the 7TM bundles. **B** The putative interaction of alkamides with the CB_2-receptor is shown. The oxygen atom in the hydroxy group of Tyr190 forms a hydrogen bond with the amide hydrogen of the alkamide, and the aromatic ring of Tyr190 exhibits *Π-Π* interactions with the C-2/C-3 double bond in the alkamide. Reprinted with permission by publisher ASBMB, J Biol Chem from reference Raduner S et al. [16].

(Fig. **5**, for a color version see Fig. **1S** in the Supporting Information). Effects on CB_2 receptor-containing immune cells in humans were evaluated in a randomized, single-dose, crossover *ex vivo* study with LPS-stimulated blood cells after *in vivo* administration of two *Echinacea purpurea* preparations standardized on 0.07 mg dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides (Echinaforce™ tincture and tablets). Both forms of medication led to a significant decrease in production of pro-inflammatory cytokines (IL-8 and TNF-α), while changes in IL-6 concentration were not statistically significant [15]. IL-8 and TNF-α are pro-inflammatory immunomodulators. Therefore the effect of the *Echinacea* preparations can be considered as an anti-inflammatory action and corresponds with the observed *in vitro* effects of alkamides. This can explain why the symptoms of a common cold, like sore throat can be reduced. Also the direct effects of alkamides on T-lymphocytes, which are key mediators of antiviral immunity, have been investigated by testing inhibition of IL-2 production. The *Echinacea purpurea* extract (95 : 5 ethanol : water) and two *Echinacea*-derived alkamides, dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide and dodeca-2*E*,4*E*-dienoic acid isobutylamide at concentrations of 0.6 to 25 μg/mL significantly suppressed the ability of activated Jurkat T cells to produce IL-2, an important factor involved in response to infection, which consequently leads to a faster resolution of cold symptoms [31].

An ethanolic and an ethyl acetate fraction of alkamides obtained from the roots of *Echinacea angustifolia* exhibited significant activity against three viruses often involved in colds and influenza (herpes simplex virus, influenza virus, and rhinovirus). This activity correlated with the presence of alkamides [32]. In a previous paper, it was reported that Echinilin™ (Factors R & D Technologies; Burnaby, Canada), a formulation prepared from freshly harvested *E. purpurea* plants and standardized on the basis of three known active components (alkamides, cichoric acid and polysaccharides) is effective for the treatment of a naturally acquired common cold [33]. However, the mechanism and the responsible constituents by which this effect was achieved remained unknown. In a recent clinical study, the effects of *Echinacea* were associated with a significant and sustained increase in the number of circulating total white blood cells, monocytes, neutrophils and NK cells. Furthermore, *Echinacea* treatment suppressed the cold-related increase in superoxide production by neutrophils [34], [35]. These data suggest that alkamides have the dual actions of anti-inflammatory and indirect antiviral effects, which together may influence the course of upper respiratory infection. In summary, a lot of recent pharmacological data suggest that *Echinacea* alkamides may not only have immunostimulatory but also anti-inflammatory and antiviral activity by a reduction in NO, TNF-α, IL-8, IL-2 and COX-dependent E_2 formation in different cell types and assays used.

Biopharmaceutics and Herb-Drug Interactions

In 2001, Dietz et al. demonstrated for the first time the bioavailability of dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides (**8a/8b**) in humans after oral administration of an ethanolic extract of *E. purpurea* [36]. Later Jager et al. could show the transport of dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides across Caco-2 monolayers, in an *in vitro* model for the intestinal epithelial barrier [37]. A similar study explored the transport of 12 alkamides from a proprietary preparation of *Echinacea*, which contained a 60% ethanol-water extract of *E. angustifolia* root (200 mg/mL) and *E. purpurea* root (300 mg/mL). Both the 2,4-diene and the 2-ene alkamides readily penetrated the monolay-

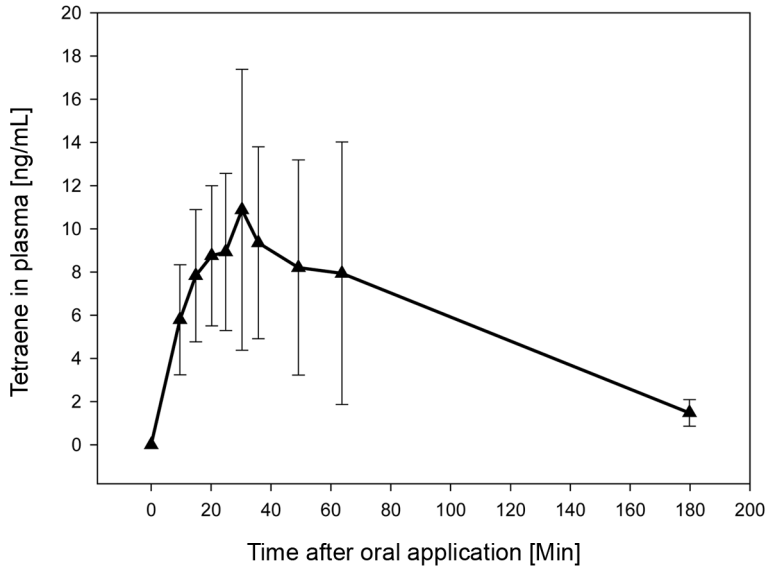

Fig. **6** Plasma concentration-time curve of tetraene **(8a/8b)** after a single oral dose of 2.5 mL 60% EtOH extract of *E. angustifolia* roots. Each point represents the mean ± standard deviation of eleven volunteers. Reprinted with permission by publisher Sage Publications, Inc., J Clin Pharmacol from reference Woelkart K et al. [39].

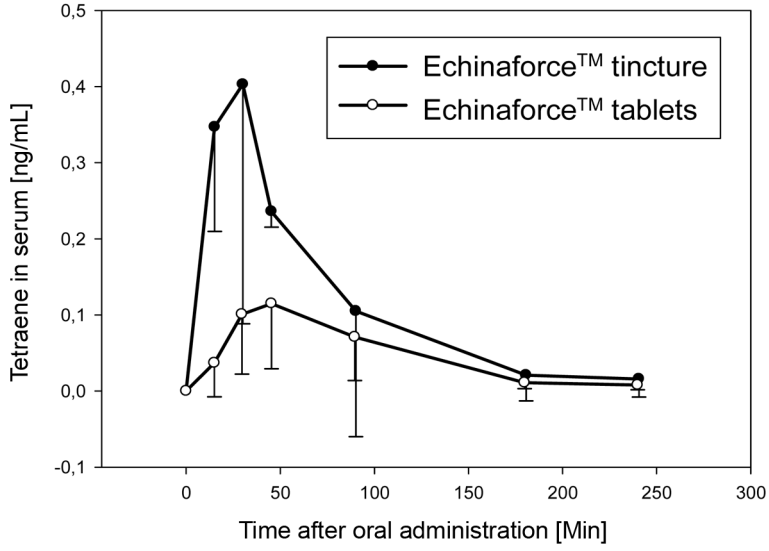

Fig. **7** Serum concentration-time curves of tetraene **(8a/8b)** after a single oral dose of 4 mL Echinaforce™ tincture and 12 Echinaforce™ tablets. Each point represents the mean ± standard deviation (SD) of the eight volunteers. Reprinted with permission by publisher Dustri-Verlag Dr. Karl Feistle GmbH & Co. KG, Int J Clin Pharm Th from reference Woelkart K et al. [15].

ers, although apparent permeability coefficient values varied (range 3×10^{-6} to 3×10^{-4} cm s^{-1}) depending on the structure. Saturated compounds and those with N-terminal methylation had lower permeability coefficients [38]. More recently, a pharmacokinetic study in humans showed that the absorption maximum (C_{max}) of dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides ("tetraenes") is reached already 30 min after oral administration of a 60% ethanolic extract from the roots of *E. angustifolia* (Fig. **6**). Initial concentrations of tetraenes could be detected in the blood as soon as 10 minutes after administration. Because of this fast absorption, the mucous membrane of the mouth is most likely already a major area of absorption [39]. In a subsequent study the absorption from tablets manufactured from ethanolic liquid extracts of *E. angustifolia* and *E. purpurea* which were taken immediately after a standard high fat breakfast was compared. Most alkamides were rapidly absorbed and were detected in plasma 20 min after tablet ingestion and remained detectable for up to 12 h. In contrast, caffeic acid derivatives could not be detected in any plasma sample at any time after tablet ingestion and therefore were reported not to be bioavailable. No obvious differences were observed in the pharmacokinetics of individual or total alkamides in two additional fasted subjects who took the same dose of the *Echinacea* preparation [40].

Based on the experience from these studies a cross-over study was performed to compare the pharmacokinetics and bioavailability of different formulations of an ethanolic extract from fresh *E. purpurea* prepared from 95% aerial parts and 5% roots (Echinaforce™ tincture and tablets). With this study design two pharmacokinetic curves for each galenic form in every subject have been obtained in order to exclude variability from the individual metabolic systems. The mean C_{max} of dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides (**8a**/**8b**) absorbed after oral administration of the tincture occurred at 30 min with a value of 0.40 ng/mL serum. In comparison the T_{max} of tablets was 45 min with a C_{max} of 0.12 ng/mL. (Fig. **7**) [15]. In all pharmacokinetic studies performed up-to-date, the time to reach the mean concentration maximum after administration of liquid *Echinacea* preparations (*E. angustifolia* or *E. purpurea*) was about 30 minutes (Table **1**). T_{max} after administration of *Echinacea* tablets, which has been investigated in two clinical studies, was varying within 45 minutes to 2.3 hours [40], [15]. As it has also been shown for

Table **1** Summary of all pharmacokinetic studies of *Echinacea* preparations performed up to now

Reference	*Plant species and preparation*	*Dose*	*Absorption*	*Detection*
[41]	*E. purpurea* mother tincture, Weber & Weber for the alkamides.	10, 20, 30, 40 and 50 mL mother tincture with 0.25, 0.49, 0.74, 0.99 and 1.24 mg dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides (tetraene) (**8a/8b**) (24,7 μg/mL)	Mean C_{max} of 14.0, 21.6, 32.2, 45.2 and 37.7 ng/mL, respectively with a T_{max} of 30, 30, 30, 27.5 and 50 minutes.	High performance liquid chromatography with PDA detection at 254 nm for the alkamides and 330 nm for cichoric acid.
	E. purpurea pressed juice, Schoenenberger for cichoric acid.	10, 30, 60, 90 and 120 mL of the pressed juice with 40, 120, 240, 360 and 480 mg cichoric acid (4 mg/mL)	Mean C_{max} of 0, 31.5, 43.3, 82.6, 129.4 ng/mL, respectively with a T_{max} of -, 2.0, 3.0, 3.6, 1.6 hours.	
[36]	*E. purpurea*; mother tincture	65 mL concentrated mother tincture with 4.3 mg dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides (tetraene) (**8a/8b**) (66.2 μg/mL)	44 ng/mL tetraene (**8a/8b**) one hour after application	High performance liquid chromatography with PDA detection (260 nm)
[39]	*E. angustifolia;* 60% ethanolic extract from the roots	2.5 mL of the ethanolic extract with 2.0 mg tetraene (**8a/8b**) (0.8 mg/mL)	The mean C_{max} reached after 30.3 (T_{max}) minutes was 10.9 ng/mL with an area under the curve of 1 029.1 ng equivalent min/mL.	Liquid chromatography, electrospray ionization ion-trap mass spectrometry with MS/MS detection in positive SRM (selected reaction monitoring) mode.
[40]	Echinacea Premium tablets, MediHerb, Australia, each containing extract equivalent to 675 mg of *E. purpurea* root plus 600 mg of *E. angustifolia* root prepared from the dried ethanolic extracts of the two *Echinacea* species.	Four Echinacea Premium tablets with a total of 43.68 mg alkamides, especially a total of 17.16 mg dodeca-2*E*,4*E*,8*Z*, 10*Z*-tetraenoic acid isobutylamide (**8a**)	C_{max} for total alkamides was 336 ng/mL, T_{max} was 2.3 hours and the AUC was 714 μg equivalent h/L. Especially for (**8a**) C_{max} was 221 ng/mL with a T_{max} of 1.9 h and a AUC of 476 μg eq h/L.	Liquid chromatography-mass spectrometry equipped with an APCI interface operating in positive ion SIM mode.
[15]	An extract of fresh organically grown *E. purpurea*, 95% herb and 5% roots with 65 Vol. % alcohol. (Echinaforce™, Bioforce AG, Switzerland)	4 mL Echinaforce™ tincture (0.018 mg/mL dodeca-2*E*,4*E*,8*Z*, 10*E*/*Z*-tetraenoic acid isobutylamides **8a/8b**) or 12 Echinaforce™ tablets (0.006 mg/tablet **8a/8b**), which both contained 0.07 mg of **8a/8b** (tetraene).	The mean maximum concentration (C_{max}) after administration of the Echinaforce™ tincture and Echinaforce™ tablets was reached after 30 minutes with 0.40 ng/mL and after 45 minutes with 0.12 ng/mL, respectively.	Liquid chromatography, electrospray ionization ion-trap mass spectrometry with MS/MS detection in positive SRM (selected reaction monitoring) mode.

Echinacea tinctures, the more concentrated preparations needed longer to attain T_{max}. T_{max} for the dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides (**8a/8b**) was obtained 2.3 hours after administration of 17.16 mg and 45 minutes after administration of 0.07 mg.

One reason could be the reported micelle formation of the alkamides [16]. The solubility of the lipophilic compounds is limited in aqueous solutions as detected by a Tyndall effect at concentrations above 10 μM and even low alkamide concentrations (~300 nM) resulted in the formation of detectable particles in buffer [16]. Although there are no investigations available concerning micellar structures of alkamides in blood, only crude estimates of (apparent) bioavailability can be calculated from the C_{max} for the dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides (**8a/8b**) from all performed pharmacokinetic studies. In addition, all performed pharmacokinetic studies [15], [39], [40], [41] have been single-dose studies and provide evidence that alkamides are bioavailable and that their pharmacokinetics are in agreement with the one dose three times daily regimen already recommended for *Echinacea*. To accurately determine the interdosing interval, a study with a multiple-dose protocol would be needed to determine the steady-state plasma concentration for each of the active compounds.

Besides bioavailability, information concerning the metabolism and plant-drug interactions is an important issue in the evaluation of *in vivo* activity of alkamides. An interaction can be particularly important when the total drug absorption is altered. Most drug interaction studies which investigated the metabolism by human liver microsomes *in vitro* have been carried out with pure alkamides so far [42]. Time- and NADPH-dependent degradation of alkamides was observed in microsomal fractions suggesting that they are metabolized by cytochrome P450 enzymes in human liver. There was a difference in the susceptibility of 2-ene and 2,4-diene pure synthetic alkamides to microsomal degradation. Obviously less degradation of the dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides was evident in the mixture of alkamides present in an ethanolic *Echinacea* extract, suggesting that metabolism by liver P450 s was dependent both on their chemistry and the combination present in the incubation mixture. Matthias et al. [43] suggested that *Echinacea* may affect the P450-mediated metabolism of other concurrently ingested pharmaceuticals. However, *in vivo* assessments of *Echinacea* preparations are essential to predict possible CYP-mediated interactions. Twelve young adults participated in an open-label, randomized study at the University of Arkansas. Each participant took 800 mg *E. purpurea* (plant part and type of extract not defined) twice daily for 28 days and was followed by

a 30-day washout period. Only cichoric acid was used as phytochemical marker compound. No significant effect on CYP activity in humans was observed [44]. Therefore, as in previous reports, *Echinacea purpurea* appeared to have a minor influence on CYP3A4 *in vivo*. On the other hand, Gorski et al. [45] demonstrated in an *in vivo* study with twelve healthy subjects the reduction of the oral clearance of substrates of CYP1A2 but not the oral clearance of substrates of CYP2C9 and CYP2D6 after taking 400 mg *E. purpurea* root extract four times a day for 8 days. *Echinacea* selectively modulates the catalytic activity of CYP3A at hepatic and intestinal sites. Therefore, care should be taken when *Echinacea* is coadministered with drugs dependent on CYP3A or CYP1A2 for their elimination [45]. More recently Yale et al. [46] investigated the inhibition of three of the most important drug metabolizing enzymes, cytochrome P450 3A4, 2D6, and 2C9 using high throughput CYP inhibition screening *in vitro* assays. Depending on the model substrate, a 70% methanol extract of *E. purpurea* aerial parts exhibited mild inhibition of CYP3A4 activity or even mild inducing effects. Little effect on CYP2D6 and moderate inhibition of CYP2C9 were also observed for the *E. purpurea* preparation. The phytochemical content of the herbal preparation was not reanalyzed before analyses [46]. The few *in vivo* studies performed up to now, used preparations which were not standardized or fully characterized. Therefore no relevant statements can be made whether critical inhibitory levels of alkamides can be reached in the liver under normal therapeutic use. In future the level of achievable serum concentrations of *Echinacea* constituents should be considered in *in vitro* studies.

Summary and Conclusion

It has been demonstrated in many animal, *in vitro* and *ex vivo* studies that an alkamide-enriched *Echinacea* fraction or isolated alkamides have significant anti-inflammatory and immunomodulatory properties. Modulation of macrophages, reduction of NO and TNF-α, and inhibition of the arachidonic acid metabolism could be directly related to alkamides. Dodeca-2*E*,4*Z*-diene-8,10-diynoic acid 2-methylbutylamide showed so far the best inhibition of COX-2-dependent prostaglandin E_2 formation. Although these animal and *in vitro* studies lend mechanistic credibility, the bioavailability and *ex vivo* studies give further information on therapeutic benefits in humans. Recent experiments have demonstrated that alkamides are detectable in human blood after oral ingestion of different *Echinacea* preparations. Effects on pro-inflammatory cytokines after LPS-stimulation could be shown in an e*x vivo* study. A single application of *Echinacea* led to a significant decrease in the production of IL-8 and TNF-α. There are also data showing that alkamides from *Echinacea* are a new class of CB_2-specific cannabinomimetics, which share the anti-inflammatory properties of anandamide and the cannabinoids from *Cannabis sativa*. However, as it has been shown in some studies that the anti-inflammatory effects exerted by cannabinomimetics are not strictly CB_2-dependent, this therefore raises the question about a possible common second target.

Furthermore, *Echinacea*-derived alkamides significantly suppressed T-lymphocytes, which are key mediators of antiviral immunity and exhibited activity against herpes simplex virus, influenza virus and rhinovirus, which are often implicated in colds and influenza. In a clinical study a standardized *E. purpurea* preparation was effective for the treatment of a naturally acquired common cold [33], while an alkamide-enriched *E. angustifolia* root extract failed in a recent experimental rhinovirus inoculation study [47]. It has be argued whether the dose of 300 mg of *Echinacea* root, given three times each day, containing 1.5 mg or less dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides was too low to obtain a significant effect on symptoms associated with rhinovirus infection [48]. Therefore, further clinical studies are needed to evaluate the therapeutic role of alkamides. Also the influence of alkamides on cytochrome P450 enzymes needs further *in vivo* evaluation. Most of the *in vivo* studies performed so far used phytochemically insufficiently characterized *Echinacea* preparations. Thus, with the present knowledge it is not possible to make a final statement on the therapeutic role of alkamides, but it is quite likely that they are relevant constituents of *Echinacea*.

References

[1] Brevoort T. The booming U.S. botanical market. Herbalgram 1998; 44: 33 – 46.
[2] Barrett B. Medicinal properties of *Echinacea*: a critical review. Phytomedicine 2003; 10: 66 – 86.
[3] Linde K, Barrett B, Woelkart K, Bauer R, Melchart D. Echinacea for preventing and treating the common cold. Cochrane Database Syst Rev 2006; Issue 1.
[4] Barnes J, Anderson LA, Gibbons S, Phillipson JD. *Echinacea* species (*Echinacea angustifolia* (DC.) Hell., *Echinacea pallida* (Nutt.) Nutt., *Echinacea purpurea* (L.) Moench): A review of their chemistry, pharmacology and clinical properties. J Pharm Pharmacol 2005; 57: 929 – 54.
[5] Bauer R. Chemistry, analysis and immunological investigations of *Echinacea* phytopharmaceuticals. In: Wagner H, editor. Immunomodulatory agents from plants. Basel, Boston, Berlin: Birkhäuser Verlag; 1999: 41 – 88.
[6] Greger H. Comparative phytochemistry of the alkamides. Amsterdam, Oxford, New York, Tokyo: Elsevier; 1988.
[7] Breyant BP, Mezine I. Alkylamides that produce tingling paresthesia activate tactile and thermal trigeminal neurons. Brain Res 1999; 842: 452 – 60.
[8] Ramsewak RS, Erickson AJ, Nair MG. Bioactive *N*-isobutylamides from the flower buds of *Spilanthes acmella*. Phytochemistry 1999; 51: 729 – 32.
[9] Bohlmann R, Ziesche J, Robinson H, King RM. Polyacetylene compounds – new amides from *Spilanthes alba*. Phytochemistry 1980; 19: 1535 – 7.
[10] Perry NB, Van Klink JW, Burgess EJ, Parmenter GA. Alkamide levels in *Echinacea purpurea*: a rapid analytical method revealing differences among roots, rhizomes, stems, leaves and flowers. Planta Med 1997; 63: 58 – 62.
[11] Bauer R, Remiger P, Wagner H. *Echinacea*. Vergleichende DC- und HPLC-Analyse der Herba-Drogen von *Echinacea purpurea*, *E. pallida* und *E. angustifolia*. Dtsch Apoth Ztg 1988; 128: 174 – 80.
[12] Kabganian R, Carrier DJ, Rose PA, Abrams SR, Sokhansanj S. Localization of alkamides, echinacoside and cynarin with *Echinacea angustifolia*. J Herbs Spices Med Plants 2002; 10: 73 – 81.
[13] Wills RBH, Stuart DL. Alkylamide and cichoric acid levels in *Echinacea purpurea* grown in Australia. Food Chem 1999; 67: 385 – 8.
[14] Gertsch J, Schoop R, Kuenzle U, Suter A. *Echinacea* alkylamides modulate TNF-α gene expression via cannabinoid receptor CB_2 and multiple signal transduction pathways. FEBS Lett 2004; 577: 563 – 9.
[15] Woelkart K, Marth E, Raggam R, Suter A, Schoop R, Koidl C et al. Bioavailability and pharmacokinetic studies on *Echinacea purpurea* preparations and their interaction with the immune system. Int J Clin Pharm Ther 2006; 44: 401 – 8.

[16] Raduner S, Majewska A, Chen J-Z, Xie X-Q, Hamon J, Faller B et al. Alkylamides from *Echinacea* are a new class of cannabinomimetics – CB_2-receptor dependent and independent immunomodulatory effects. J Biol Chem 2006; 281: 14192 – 206.
[17] Bauer R, Jurcic K, Puhlmann J, Wagner H. Immunologische *in vivo*- und *in vitro*-Untersuchungen mit *Echinacea* Extrakten [Immunological studies *in vivo* and *in vitro* of *Echinacea* extracts. Translated by Beate V Foit, Santa Cruz, CA, USA]. Arzneimittelforschung/Drug Res 1988; 38: 276 – 81.
[18] Bauer R, Remiger P, Jurcic K, Wagner H. Beeinflussung der Phagozytose-Aktivität durch Echiancea-Extrakte [Influence of *Echinacea* extract on phagocytotic activity. Reviewed in translation, translator unknown]. Z Phytother 1989; 10: 43 – 8.
[19] Rininger JA, Kickner S, Chigurupati P, McLean A, Franck Z. Immunopharmacological activity of *Echinacea* preparations following simulated digestion on murine macrophages and human peripheral blood mononuclear cells. J Leukocyte Biol 2000; 68: 503 – 10.
[20] Bauer R, Jurcic K, Puhlmann J, Wagner H. Immunologische *in vivo*- und *in vitro*-Untersuchungen mit *Echinacea*-Extrakten. Arzneimittelforschung 1988; 38: 276 – 81.
[21] Bauer R, Remiger P, Jurcic K, Wagner H. Beeinflussung der Phagozytose-Aktivität durch *Echinacea*-Extrakte. Z Phytother 1989; 10: 43 – 8.
[22] Goel V, Chang C, Slama JV, Barton R, Bauer R, Gahler R et al. *Echinacea* stimulates macrophage function in the lung and spleen of normal rats. J Nutr Biochem 2002; 13: 487 – 92.
[23] Goel V, Chang C, Slama JV, Barton R, Bauer R, Gahler R et al. Alkylamides of *Echinacea purpurea* stimulate alveolar macrophage function in normal rats. Int Immunopharmacol 2002; 2: 381 – 7.
[24] Chen Y, Fu T, Tao T, Yang J, Chang Y, Wang M et al. Macrophage activating effects of new alkamides from the roots of *Echinacea* Species. J Nat Prod 2005; 68: 773 – 6.
[25] Stevenson LM, Matthias A, Banbury L, Penman KG, Bone KM, Leach Det al. Modulation of macrophage immune responses by *Echinacea*. Molecules 2005; 10: 1279 – 85.
[26] Clifford LJ, Nair MG, Rana J, Dewitt DL. Bioactivity of alkamides isolated from *Echinacea purpurea* (L.) Moench. Phytomedicine 2002; 9: 249 – 53.
[27] Müller-Jakic B, Breu W, Pröbstle A, Redl K, Greger H, Bauer R. *In vitro* inhibition of cyclooxygenase and 5-lipoxygenase by alkamides from *Echinacea* and *Achillea* species. Planta Med 1994; 60: 37 – 40.
[28] Merali S, Binns S, Paulin-Levasseur M, Ficker C, Smith M, Baum B et al. Antifungal and anti-inflammatory activity of the genus *Echinacea*. Pharm Biol 2003; 41: 412 – 20.
[29] Hinz B, Woelkart K, Bauer R. Alkamides from *Echinacea* inhibit cyclooxygenase-2-dependent prostaglandin synthesis in human neuroglioma cells. Planta Med 2006; 72: 1062.
[30] Woelkart K, Xu W, Pei Y, Makriyannis A, Picone RP, Bauer R. The endocannabinoid system as a target for alkamides from *Echinacea angustifolia* roots. Planta Med 2005; 71: 701 – 5.
[31] Sasagawa M, Cech NB, Gray D, Elmer GW, Wenner CA. *Echinacea* alkylamides inhibit interleukin-2 production by Jurkat T cells. Int Immunopharmacol 2006; 6: 1214 – 21.
[32] Hudson J, Vimalanathan S, Kang L, Amiguet VT, Livesey J, Arnason JT. Characterization of antiviral activities in *Echinacea* root preparations. Pharm Biol 2005; 43: 790 – 6.
[33] Goel V, Lovlin R, Chang C, Slama JV, Barton R, Gahler R et al. A proprietary extract from the *Echinacea* plant (*Echinacea purpurea*) enhances systemic immune response during a common cold. Phytother Res 2005; 19: 689 – 94.
[34] Goel V, Lovlin R, Barton R, Lyon MR, Bauer R, Lee TDG et al. Efficacy of a standardized *Echinacea* preparation (Echinilin™) for the treatment of the common cold: a randomized, double-blind, placebo-controlled trial. J Clin Pharm Ther 2004; 29: 75 – 83.
[35] Barton R. Efficacy of echinilin for the common cold. Clin Infect Dis 2005; 41: 761 – 2.
[36] Dietz B, Heilmann J, Bauer R. Absorption of dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides after oral application of *Echinacea purpurea* tincture. Planta Med 2001; 67: 863 – 4.
[37] Jager H, Meinel L, Dietz B, Lapke C, Bauer R, Merkle HP et al. Transport of alkamides from *Echinacea* species through Caco-2 monolayers. Planta Med 2002; 68: 469 – 71.
[38] Matthias A, Blanchfield JT, Penman KG, Toth I, Lang CS, De Voss JJ et al. Permeability studies of alkylamides and caffeic acid conjugates from *Echinacea* using a Caco-2 cell monolayer model. J Clin Pharm Ther 2004; 29: 7 – 13.
[39] Woelkart K, Koidl C, Grisold A, Gangemi JD, Turner RB, Marth E et al. Bioavailability and pharmacokinetics of alkamides from the roots of *Echinacea angustifolia* in humans. J Clin Pharmacol 2005; 45: 683 – 9.
[40] Matthias A, Addison RS, Penman KG, Dickinson RG, Bone KM, Lehmann RP. *Echinacea* alkamide disposition and pharmacokinetics in humans after tablet ingestion. Life Sci 2005; 77: 2018 – 29.
[41] Osowski S. Echinacea – ein Immunstimulans? Zur Pharmakokinetik der *Echinacea purpurea*-Inhaltsstoffe Dodeca-2E,4E,8Z,10E/Z-tetraensäure-isobutylamid und Cichoriensäure sowie ihr immunmodulierender Einfluss. [dissertation]. Freiburg: Albert Ludwig University; 1998.
[42] Butterweck V, Derendorf H, Gaus W, Nahrstedt A, Schulz V, Unger M. Pharmacokinetic herb-drug interactions: are preventive screenings necessary and appropriate?. Planta Med 2004; 70: 784 – 91.
[43] Matthias A, Gillam EMJ, Penman KG, Matovis NJ, Bone KM, De Voss JJ et al. Cytochrome P450 enzyme-mediated degradation of *Echinacea* alkylamides in human liver microsomes. Chem Biol Interact 2005; 155: 62 – 70.
[44] Gurley BJ, Gardner SF, Hubbard MA, Williams DK, Gentry WB, Carrier J et al. *In vivo* assessment of botanical supplementation on human cytochrome P450 phenotypes: *Citrus aurantium*, *Echinacea purpurea*, milk thistle, and saw palmetto. Clin Pharmacol Ther 2004; 76: 428 – 40.
[45] Gorski JC, Huang S-M, Pinto A, Hamman MA, Hilligoss JK, Zaheer NA et al. The effect of *Echinacea* (*Echinacea purpurea* root) on cytochrome P450 activity *in vivo*. Clin Pharmacol Ther 2004; 75: 89 – 100.
[46] Yale SH, Glurich I. Analysis of the inhibitory potential of *Ginkgo biloba*, *Echinacea purpurea*, and *Serenoa repens* on the metabolic activity of cytochrome P450 3A4, 2D6, and 2 C9. J Altern Complement Med 2005; 11: 433 – 9.
[47] Turner RB, Bauer R, Woelkart K, Hulsey TC, Gangemi JD. An evaluation of *Echinacea angustifolia* in experimental rhinovirus infections. N Engl J Med 2005; 353: 341 – 8.
[48] Blumenthal M, Farnsworth NR, Leach M, Turner RB, Gangemi JD. *Echinacea angustifolia* in rhinovirus infections (multiple letters). N Engl J Med 2005; 353: 1971 – 2.