FIGURA 1. *Echinacea purpurea*.
Foto: Bernat Vanaclocha.

PL03 Recent progress in the research on traditional herbal medicinal products

Rudolf Bauer
Institute of Pharmaceutical Sciences, Karl-Franzens-Universität Graz (Austria)

Abstract

In the European Union, the use of traditional herbal medicinal products has recently been regulated in Directive 2004/24/EC. According to this regulation, clinical studies and pre-clinical tests are not obligatory, but quality needs to be demonstrated in any individual case.

Echinacea and butterbur (Petasites) will be used as examples for demonstrating the progress in medicinal plant research. Alkamides, the major lipophilic constituents of Echinacea, have recently been found to be rapidly absorbed after oral application. Using LC-MS their pharmacokinetics have been studied and *ex-vivo* effects have been measured. Alkamides have also been shown to bind to cannabinoid receptors (CB2) which may represent a molecular mechanism of action of Echinacea. Extracts of the rhizomes of *Petasites hybridus* have been shown to inhibit 5-lipoxygenase and cyclooxygenase-2 and COX-2 expression. They are useful for the prevention of migraine and for the treatment of asthma and seasonal allergic rhinitis.

Key words: Traditional herbal medicinal products, *Echinacea angustifolia*, *Echinacea pallida, Echinacea purpurea, Petasites hybridus*.

Resumen

En la Unión Europea, el uso de medicamentos tradicionales a base de plantas medicinales ha sido regulado recientemente en la Directiva 2004/24/EC. De acuerdo con esta directiva, los estudios clínicos y los ensayos preclínicos no son obligatorios, pero la calidad requiere ser demostrada de forma individual en cada caso. La equinácea y el petasites pueden tomarse como ejemplos para demostrar el avance en la investigación sobre plantas medicinales.

Se ha demostrado recientemente que las alquilamidas, principales constituyentes lipofílicos de la equinácea, se absorben rápidamente tras su administración oral. Utilizando LC-MS, se ha estudiado su farmacocinética y se han medido sus efectos *ex vivo*. También se ha demostrado que las alquilamidas tienen la capacidad de unirse a los receptores canabinoides (CB2), lo cual puede constituir un mecanismo de acción molecular de la equinácea. Los extractos de rizoma de *Petasites hybridus* inhiben la 5-lipoxigenasa y la cicloxigenasa-2, así como la expresión de esta última. Son útiles en la prevención de la migraña y en el tratamiento del asma y de la rinitis alérgica estacional.

Key words: Medicamentos tradicionales a base de plantas, *Echinacea angustifolia, Echinacea pallida, Echinacea purpurea, Petasites hybridus*.

In the European Union, the use of traditional herbal medicinal products has recently been regulated in Directive 2004/24/EC [1]. According to this regulation, clinical studies and pre-clinical tests are not obligatory for the registration of herbal products, as long as the efficacy of them is plausible on the basis of long-standing use and experience. However, quality needs to be demonstrated in any case. Nevertheless, clinical and pharmacological studies are necessary for a rational use and to convince the medical profession that these products have a reasonable activity.

Echinacea species (Asteraceae) have a long history of medicinal use. *Echinacea angustifolia* DC., *Echinacea pallida* (Nutt.) Nutt. and *Echinacea purpurea* (L.) Moench are frequently used as herbal immunomodulators. Traditionally, roots and aerial parts of these plants have been used to treat wounds as well as insect and snake bites. Today echinacea is mainly used for infections of the upper respiratory tract. In order to obtain consistent quality of batches, HPLC analysis of the major constituents, alkamides and caffeic acid derivatives is necessary [2]. Determination of the active polysaccharides/glycoproteins would be useful as well. Specific ELISA methods have become recently available for that purpose [3].

Echinacoside and cynarine are the major polar constituents in the roots of *E. angustifolia* and frequently used for the standardization of corresponding *echinacea* preparations. Also in the current draft of the USP/NF monograph of *E. angustifolia* roots, echinacoside and cynarine are the compounds which have to be determined in an assay for total phenolics. The concentration of these compounds may change in certain preparations during storage (FIGURE 2). Cichoric acid is the major polar caffeic acid derivative in the roots of *Echinacea purpurea* and in the aerial parts of echinacea roots. Recent investigations have shown that these caffeic acid derivatives

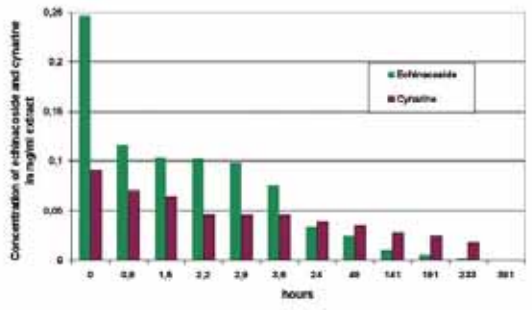

FIGURE. 2. Content of echinacoside and cynarine in 60 % EtOH *Echinacea angustifolia* root extract, during storage at 4°C over 16 days [5].

are rapidly oxidised by polyphenol oxidase present in the plant material [4, 5]. Therefore, it is necessary to control this enzymatic activity in order to obtain products with consistent content of caffeic acid derivatives.

Alkamides, the major lipophilic constituents, can be found in high concentrations in the aerial parts of *E. purpurea*, *E. pallida* and *E. angustifolia*, and in the roots of *E. purpurea* and *E. angustifolia*. They can be analysed by DAD-HPLC and, more sensitive and more specifically by LC-MS.

The pharmacokinetic properties of echinacea alkamides have recently been studied using ion trap SEI MS/MS [6,7]. The studies have demonstrated that echinacea alkamides are detectable in human blood already 10 minutes after oral application (FIGURE 3).

There is also evidence that alkamide containing echinacea preparations trigger effects on the pro-inflammatory cytokine TNF-α and chemokine IL-8 from a recent *ex vivo* study. [7] Gertsch *et al.* [8] could demonstrate modulation of TNF-α gene expression and multiple signal transduction pathways for echinacea alkamides and postulated a mechanism related to cannabinoid receptors. *In vitro* kinetic experiments measuring both TNF-α mRNA and protein levels over a time-span of 39 h after a co-incubation with LPS and *Echinacea purpurea* tincture (Echinaforce™) have also been performed. LPS-stimulated TNF-α protein expression was modulated by the *Echinacea purpurea* tincture, resulting in a significant inhibition (~ 40 %) during the first 20 h, and subsequent stimulation of TNF-α protein expression. Because of the structural similarity of echinacea alkamides and anandamide (AEA), the endogenous ligand of CB receptors, receptor binding studies with alkamides to rodent CB1 and CB2 receptors were conducted in parallel. It could be demonstrated that echinacea alkamides have in fact high affinity to CB receptors (FIGURE 4) [9]. Pentadeca-2*E*,9*Z*-diene-12,14-diynoic acid isobutylamide showed the highest affinity for CB1 with a Ki value of 2.0 µM, while Tetradeca-2*E*-ene-10,12-diynoic acid isobutylamide with a Ki of 1.9 µM was the most selective and most affine ligand for CB2. Most of the echinacea

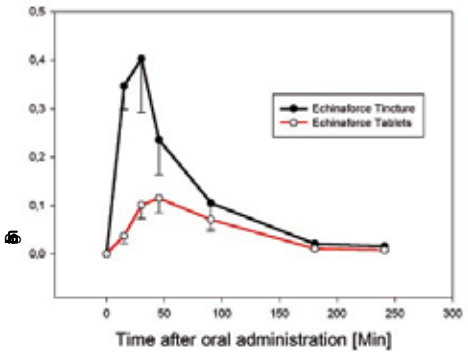

FIGURE 3. Comparison of AUC from Dodeca 2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides in serum after oral administration of 4 ml Echinaforce™ tincture and 12 Echinaforce™ tablets [7].

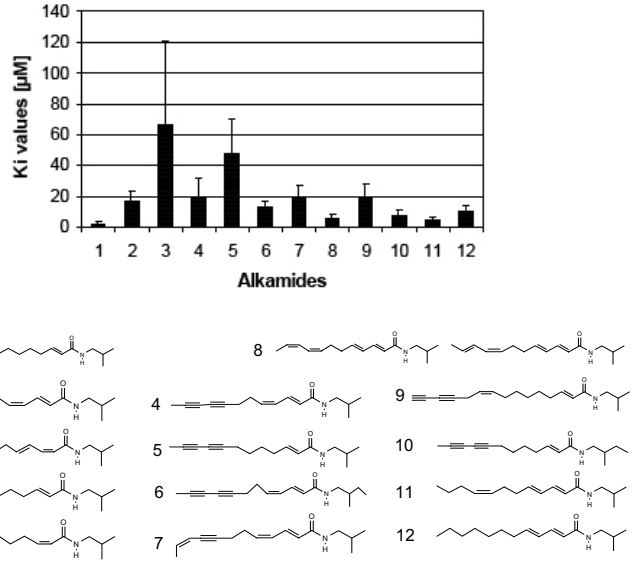

FIGURE 4. Selectivity of alkamides **(1-12)** from *Echinacea angustifolia* for CB2 receptor from mouse membranes, obtained by a standard receptor binding assay using a H.-CP-55,940 as the radioligand and reported as mean Ki values µM with corresponding 95 % confidence intervals determined from at least three independent experiments [9].

alkamides showed affinities to CB2 receptors with Ki values lower than 20 µM, some only five times less active than anandamide [9]. The most recent evidence of CB2-receptor-binding has recently been demonstrated by Raduner et al. [10]. At concentrations below 100 nM, dodeca-2*E*,4*E*,8*Z*,10*Z*- tetraenoic acid isobutylamide and dodeca-2*E*,4*E*-dienoic acid isobutylamide potently displaced the radioligand with Ki values of 57 ± 14 nM and 60 ± 13 nM, respectively.

Obviously alkamides from echinacea, as well as anandamide influence the cytokine milieu in human whole blood at low nM concentrations. Moreover, echinacea alkamides can exert both anti- but also pro-inflammatory effects in human blood, depending on the stimulus applied, drug concentration used, and degree of unsaturation of the lipophilic tail of the specific alkamide and can be considered as a new class of cannabinomimetics.

Although the outcome clinical studies with echinacea preparations is not consistent [11, 12], and the evidence may differ from product to product, a recent Cochrane review [13] came to the conclusion, that especially preparations based on the aerial parts of *Echinacea purpurea* have some evidence be effective for this purpose. According to a meta-analysis with three selected experimental infection studies, the likelihood of experiencing a clinical cold was 55 % higher with placebo than with echinacea [14].

Butterbur (*Petasites hybridus*) products enjoy increased interest due to a number of recently published double-blind and placebo-controlled clinical trials in migraine prevention and treatment of seasonal rhinitis. due to the toxicity of pyrrolizidine alkaloids, only lipophilic supercritical carbon dioxide extracts of the rhizomes and the leaves are used as medicinal products today.

In vitro experiments have identified a group of sesquiterpenes, mainly petasin and iso-petasin as the pharmacologically active components from the *Petasities hybridus* plant. Those compounds have spasmolytic as well as anti-inflammatory activities. Petasin and isopetasin relax smooth muscle and tracheal rings *in vitro* through effects involving calcium channels and calcium mobilization [15, 16]. Both compounds have also been demonstrated to possess anti-inflammatory effects by inhibiting leukotriene synthesis and the cyclooxygenase-2 enzyme [17, 18]. Recently, several clinical trials have been published with a special CO_2 extract made from the rhizomes of *Petasities hybridus* for migraine prevention [19-21]. An open trial in asthma patients suggests that this extract might also be effective in improving lung function, reducing corticoid use and asthma symptoms [22]. These trials were conducted with an extract containing a minimum of 15% petasin/isopetasin (Petadolex®, Weber & Weber, Germany). In addition, a different special CO_2 extract made from butterbur leaves has been shown to be effective in randomized controlled trials for the treatment of seasonal allergic rhinitis [23, 24]. That extract is standardized similarly to 8 mg of total petasines per tablet (20-40 mg Ze339-extract Tesalin®, Zeller AG, Switzerland).

Due to the new popularity of butterbur, many different butterbur products have appeared on the market, especially in the USA. We have analysed the most common products in terms of petasin/isopetasin content by HPLC-DAD/MS. TABLE 1 summarizes the products analysed and the determined petasin/isopetasin content.

From 6 tested products only 2 products contain what is specified on the label. Using underdosed products may lead consumers to the erroneous conclusion, that herbal drugs are ineffective. Therefore, efficient quality control and GMP is needed also for consistent quality and reliable activity of dietary supplements.

Product	Petasin+Isopetasin per unit* as specified by manufacturer (mg)	Petasin+Isopetasin per unit* as determined by HPLC-DAD/MS (mg)
Petadolex®, Enzymatic Therapy (softgels)		
Lot: 40242	7.5	10.51
Lot: 42241	7.5	14.89
MigraSolve Petadolex®, Rainbow Light (softgels)		
Lot: K5093A	7.5	13.22
Lot: I5154A	7.5	12.22
Continence, Solaray® (capsules)		
Lot: 091901	7.5	0.0058
Lot: 091512	7.5	0.0254
Butterbur, Solaray® (vegetarian capsules)		
Lot: 101402	7.5	0.0123
Lot: 092609	7.5	0.0258
Butterbur, NOW Foods® (Vcaps®)		
Lot: 742589 1804	11.25	0.0179
Lot: 722027 0906	11.25	0.0182
MigraDefense, KAL® (RapidSolv® tablets)		
Lot: 101503	7.5	0.0185
Lot: 091009	7.5	0.0285
Lot: 091807	7.5	0.0156

TABLE 1. Quantitative analysis of petasin and isopetasin content in various commercially available butterbur products [25].
*Unit is one tablet, capsule or softgel.

Contact address
Prof. Dr. Rudolf Bauer
Institute of Pharmaceutical Sciences
Karl-Franzens-Universität Graz
Universitätsplatz 4
A-8010 Graz (Austria)
E-mail: rudolf.bauer@uni-graz.at

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