Permeability studies of alkylamides and caffeic acid conjugates from Echinacea using a Caco-2 cell monolayer model

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INTRODUCTION

Alkylamides and caffeic acid conjugates, the principal compound classes in Echinacea ethanolic extracts, are considered to be responsible for stimulation of the immune system. Despite the long history of use of echinacea and the supposed immunological activity of these two groups of compounds, very little is known about their bioavailability.

The Caco-2 cell monolayer system is used to identify compounds with potential absorption problems as well as to select compounds with optimal passive absorption characteristics from a series of pharmacologically active molecules [1]. Consequently, these systems are potentially useful in investigating potentially active phytochemicals.

In this study, we have investigated the potential bioavailability of several alkylamides and caffeic acid conjugates from an ethanolic echinacea preparation (MediHerb Echinacea Premium Liquid) as well as two isolated synthetic alkylamides.

Methods

Caco-2 cells were obtained from the American Type Culture Collection (Rockville, MD, USA). Cells (passage 45) were seeded onto polycarbonate cell culture inserts (Transwell, mean pore size 0.45 µm, 6.5mm diameter) and allowed to grow and differentiate for 27 days. Transepithelial electrical resistance was measured using the Millicel-ERS system (Millipore Corp., Bedford, MA, USA).

Permeability experiments were performed in Hank's balanced salt solution containing 25mM HEPES in air at 37C. The plates were shaken in a Heifolf Titramax 1000 at 400 rpm at 37C throughout the experiment. At the indicated time points, the basolateral volume was replaced with fresh Hank's. The apical solution was only sampled at the conclusion of the experiment. Samples were analysed using HPLC and LC-MS. A C18, 3µ, 100x2.0mm Phenomonex Luna column was used with a solvent flow rate of 0.3 ml/min. An acetonitrile/water mobile phase was used.

The amides **(2)** and **(7)** were synthesised via adaptation of the method described by Bohlmann and Miethe [2]. The products were purified by column chromatography and were homogeneous by GCMS and 1H and 13C NMR, with data consistent with the structure and stereochemistry as drawn in Figure 3.

Results

Compound (see Figure 3 for names)	apical Conc. (µg/mL) (n=2)	% of EPL	% Uptake at 90 min. (n=3-5)	Apparent Permeability (x 10^{-6} cm.s^{-1}) (n=3-5)
2,4-Dienes				
1 m/z = 229	23.2 ± 3.3	12.5	102 ± 20	319 ± 221
3 m/z = 243	24.0 ± 6.4	12.9	81 ± 24	176 ± 89
4 m/z = 245	14.9 ± 0.9	8.1	62 ± 15	100 ± 35
5 m/z = 247	52.9 ± 4.3	28.5	74 ± 22	138 ± 60
6 m/z = 249	7.0 ± 2.0	3.8	16 ± 7	20 ± 9
7 m/z = 251	14.2 ± 1.4	7.7	3 ± 1	3 ± 0
8 m/z = 257	14.7 ± 1.9	7.9	49 ± 16	75 ± 31
2-enes				
2 m/z = 231	16.9 ± 0.9	9.1	83 ± 14	167 ± 54
9 m/z = 259	4.7 ± 0.4	2.6	41 ± 16	59 ± 26
10 m/z = 271	0.6 ± 0.2	0.3	53 ± 24	8 ± 4
11 m/z = 285	10.7 ± 2.0	5.8	6 ± 2	6 ± 3
12 m/z = 299	1.5 ± 0.3	0.8	31 ± 26	37 ± 34
Total AA	185.2 ± 24.1	100		
Caffeoylquinics				
Caftaric Acid	12.8 ± 3.3	18.7	1 ± 3	2 ± 3
Echinacoside	8.4 ± 1.6	12.2	0 ± 0	0 ± 0
Cichoric Acid	47.4 ± 18.2	69.1	3 ± 5	4 ± 5
Tartaric Acid	152.1		0 ± 0	0 ± 0
Cinnamic acid	212.1		83 ± 4	147 ± 15
Total CA	68.6 ± 23.1	100		
Synthetic **7**	5.3		5 ± 1	4 ± 1
Synthetic **2**	5.0		62 ± 7	89 ± 17
Mannitol			2 ± 1	0.8 ± 0.3

Table 1: Alkylamide and caffeic acid conjugates permeability across Caco-2 monolayers after 90 minutes. Data are means ± SD

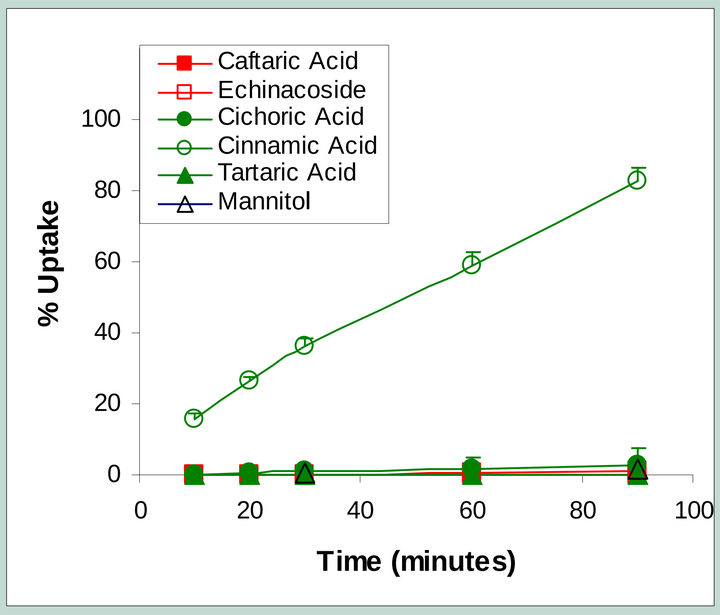
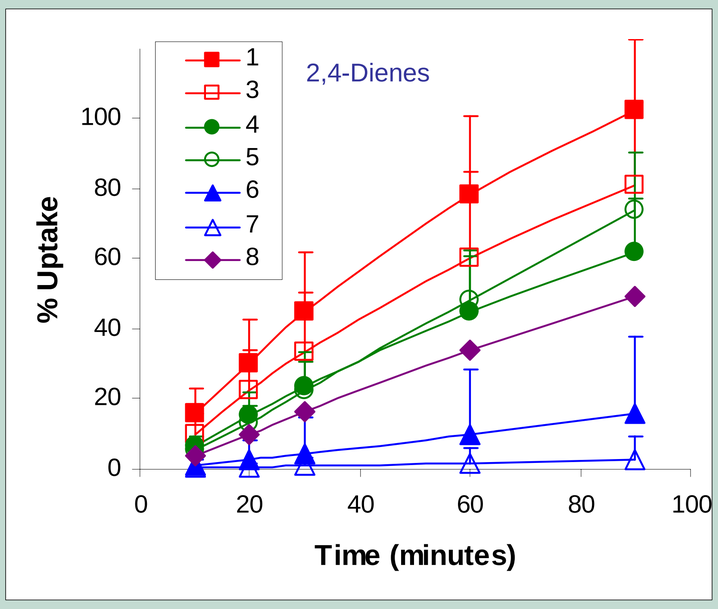


Figure 1: Caffeic acid conjugate transport kinetics in Caco-2 monolayers. Mannitol was used as a poor intestinal uptake control. Cinnamic and tartaric acids are potential metabolites.
Values are means ± SD for 3-5 monolayers

- **Caffeic acid conjugates don't readily cross the intact monolayer**
- **Cinnamic acid readily diffuses across the intact monolayer**



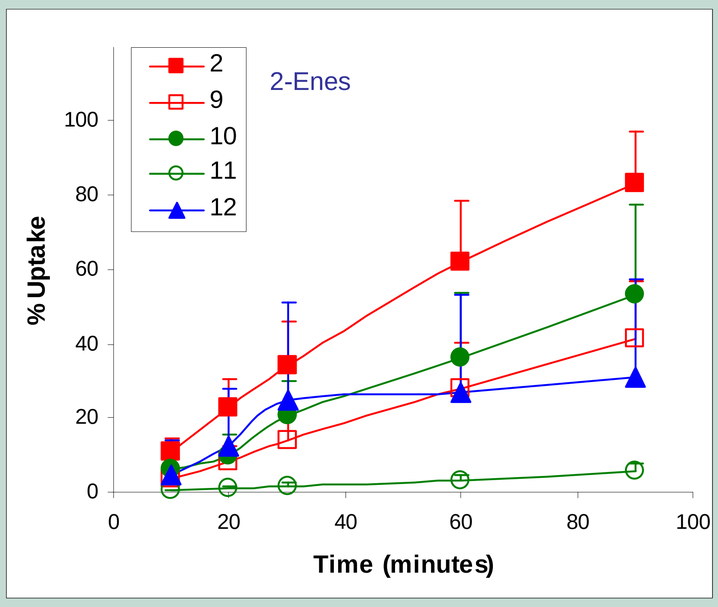


Figure 2: Alkylamide transport kinetics in Caco-2 monolayers.
Values are means ± SD for 5 monolayers

- **Alkylamides readily diffuse across the monolayer P_{app}>10^{-6}**
- **Permeabilities vary greatly for different alkylamides**
- **There is no apparent link between alkylamide concentration and P_{app}**
- **Permeability differences correlate with variations in alkylamide structure**
- **Synthetic alkylamides have similar P_{app} to those found for the same alkylamides from the Echinacea mix**

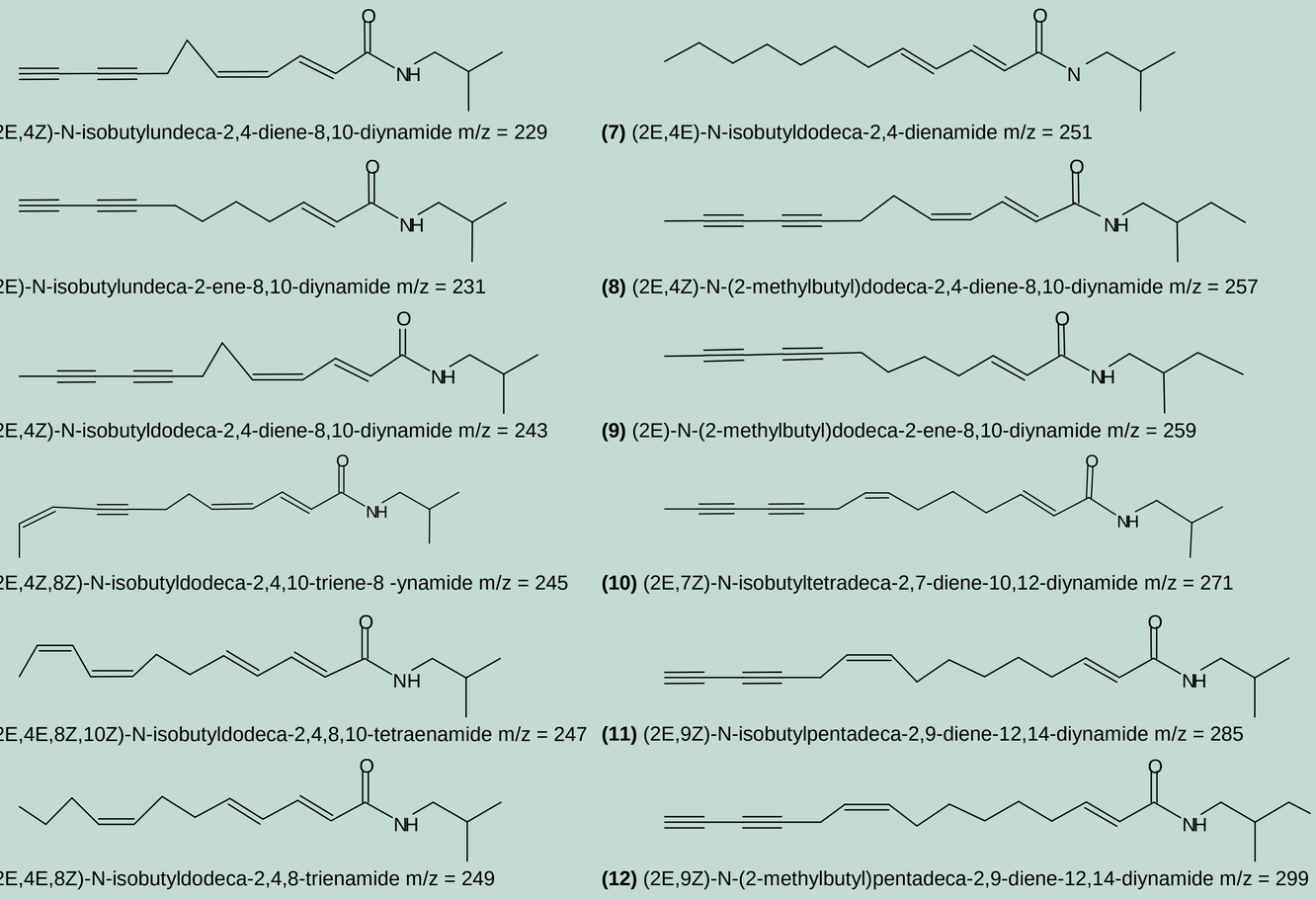


Figure 3: Structures of isobutylamides and methylbutylamides.

Summary

Alkylamides readily cross the Caco-2 cell monolayer.

2,4-dienes are more readily transported than the equivalent 2-ene alkylamides (compare 1 and 2, 8 and 9).

Increases in unsaturation correlate with increases in P_{app} (compare 1 and 7).

Methylation reduces P_{app} (Compare 1 and 3, 3 and 8, 2 and 9).

Caffeic acid conjugates have very poor uptake.

- **Alkylamides but not caffeic acid conjugates are likely to cross the intestinal barrier and thus be available to elicit pharmacological effects.**

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References:
[1] Artursson P, Palm K and Luthman K. Adv. Drug Deliv. Rev. (2001) 46: 27-43

[2] Bohlmann F and Miethe R. Chemische Berichte (1967) 100: 3861-3868

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