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Relative absorption of tryptophan ethyl ester amide derivatives with various fatty acid chains in the dog

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Sandoz compound 57-118 is a mixture of tryptophan ethyl ester amide derivatives (analogues I–V) possessing one of five fatty acid chains which differ in chain length, configuration, or the degree of unsaturation. The relative absorption of each of the five analogues was investigated in the dog following single oral doses of the homologous mixture containing one ^{14}C labelled analogue. It was shown that the extent of absorption of analogue I and its *trans*-isomer, II, both with a mono-unsaturated fatty acid chain, were similar. An additional double bond in the fatty acid moiety (analogue III) facilitated gastrointestinal absorption. On the other hand, saturated fatty acid chains appeared to render the molecule less efficiently absorbed; the extent of absorption being dependent on the chain length. Thus, analogue V with 16 carbon atoms on the fatty acid chain was better absorbed than IV with 18.

The gastrointestinal absorption of lipids is a relatively complicated process. It is generally believed that the intestinal mucosal uptake of lipids is governed by two potentially rate-limiting factors, i.e. an unstirred water (diffusion) layer adjacent to the mucosa and the lipid membrane of the microvillus surface (Wilson et al 1971; Sallee et al 1972; Sallee & Dietschy 1973). As the chain length of fatty acids and alcohols increases, the aqueous solubility decreases while the passive permeability coefficient increases and, eventually, the permeability of the lipoidal pathway of the intestinal membrane becomes sufficiently greater than that of the aqueous diffusion layer so that the total transport rate becomes effectively aqueous diffusion-controlled (Sallee & Dietschy 1973; Ho et al 1977). Using excised rabbit jejunum tissue preparations, Westergaard & Dietschy (1976) have shown that the maximum rate of uptake of a series of fatty acids into the intestinal mucosal cell decreases with each carbon atom added to the fatty acid chain. However, little information is available on the overall effect of homologue size on the absorption of long-chain fatty acids in-vivo.

Sandoz compound 57-118 is a mixture of tryptophan ethyl ester amide derivatives possessing one of five fatty acid chains which differ in chain length, configuration, or the degree of unsaturation (Table 1). In the present study, the absorption of each of the analogues, administered in the homologous mixture, was investigated in the dog. The absorption was measured after single oral doses of the mixture containing one ^{14}C labelled analogue. This experimental design was preferred to dosing each analogue individually in order to avoid other potential variables such as the slowing of gastric emptying which also was shown to be related to the chain length of fatty acids (Hunt & Knox 1968).

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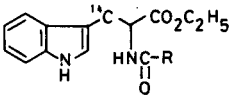
Table 1. Composition of [^{14}C] 57-118 used.



Analogue	Chemical name	R	Fatty acid moiety	% w/w
I	Ethyl α-(1-oxo-*cis*-9-octadecenylamino)-1H-indole-3-propanoate	$(CH_2)_7C(H)=C(H)(CH_2)_7CH_3$	Oleic	85
II	Ethyl α-(1-oxo-*trans*-9-octadecenylamino)-1H-indole-3-propanoate	$(CH_2)_7C(H)=C(CH_2)_7CH_3$	Elaidic	6
III	Ethyl α-(1-oxo-*cis*-9,cis-12-octadecadienyl-amino)-1H-indole-3-propanoate	$(CH_2)_7C(H)=C(H)CH_2C(H)=C(H)(CH_2)_4CH_3$	Linoleic	6
IV	Ethyl α-(1-oxooctadecylamino)-1H-indole-3-propanoate	$(CH_2)_{16}CH_3$	Stearic	2
V	Ethyl α-(1-oxohexadecylamino)-1H-indole-3-propanoate	$(CH_2)_{14}CH_3$	Palmitic	1

Table 2. Mean blood concentrations of radioactivity and relevant pharmacokinetic parameters after single oral doses of [^{14}C] 57-118 in the dog.

	Radiolabelled analogue					
	I [18:1][1]	II [18:1]	III [18:2]	IV [18:0]	V [16:0]	*t*-test[2]
Mean blood concn (F) at (h):						
1	0	0	0·019 (0·017)[3]	0·009 (0·015)	0·011 (0·011)	NS[4]
2	0·017 (0·015)	0	0·065 (0·018)	0·012 (0·020)	0·021 (0·012)	III > I, II, IV, V
4	0·045 (0·012)	0·017 (0·029)	0·109 (0·028)	0·035 (0·019)	0·046 (0·012)	III > I, II, IV, V
6	0·060 (0·018)	0·031 (0·032)	0·099 (0·032)	0·044 (0·018)	0·060 (0·013)	III > II, IV
8	0·079 (0·020)	0·062 (0·021)	0·091 (0·022)	0·036 (0·035)	(·061 (0·004)	I, III > IV
24	0·107 (0·030)	0·110 (0·023)	0·089 (0·021)	0·059 (0·051)	0·089 (0·044)	NS
48	0·096 (0·022)	0·095 (0·017)	0·077 (0·020)	0·057 (0·033)	0·084 (0·046)	NS
72	0·100 (0·024)	0·091 (0·015)	0·079 (0·025)	0·062 (0·034)	0·085 (0·043)	NS
96	0·105 (0·026)	0·101 (0·011)	0·074 (0·021)	0·056 (0·050)	0·081 (0·042)	NS
144	0·097 (0·016)	0·075 (0·004)	0·069 (0·020)	0·051 (0·029)	0·071 (0·032)	I > IV
Mean peak blood concn (F)	0·112 (0·027)	0·113 (0·018)	0·117 (0·016)	0·072 (0·033)	0·093 (0·039)	NS
Mean time of peak concn (h)	50·7 (24·4)	48·0 (41·6)	4·7 (1·2)	88·0 (60·4)	18·7 (9·2)	IV > III, V
Mean area under concn curve, 0–144 h (F·h)	13·91 (3·14)	12·76 (1·86)	11·19 (2·94)	7·80 (5·27)	11·35 (5·43)	NS

[1] Number of carbon atoms: number of double bonds on the fatty acid chain.
[2] Significant at $P < 0·05$.
[3] Standard deviation, n = 3.
[4] Not significant.

Method

The ^{14}C-labelled analogues were synthesized by the Synthetic Tracer Laboratory of Sandoz, Inc., East Hanover, New Jersey, USA. During the synthetic process, the ^{14}C-label was incorporated into the tryptophan ethyl ester moiety at the α-carbon of the side chain. Five dilution batches of [^{14}C]57-118 (specific activities 0·008–0·013 μCi mg^{-1}) were then prepared by adding non-radioactive components to the different ^{14}C-labelled analogues. Each batch was studied in three adult male beagle dogs, average weight ca 10 kg. The dose (500 mg kg^{-1}) was prepared as a 400 mg ml^{-1} suspension in corn oil and each dog received the suspension (1·25 ml kg^{-1}) administered in two gelatin capsules. Venous blood samples were collected in heparinized syringes immediately before and at 1, 2, 4, 6, 8, 24, 72, 96 and 144 h after drug administration, and aliquots were pipetted for assay. Urine was collected quantitatively from each dog at 24 h intervals for 6 days. After the final collection, each cage was rinsed with ethanol and water. All urine and cage wash samples were stored frozen until analysis.

Radioactivity was measured in a liquid scintillation spectrometer (Model 460, Packard). The urine and cage

Table 3. Urinary excretion of radioactivity after single oral doses of [^{14}C]57-118 in the dog.

	Radiolabelled analogue					
	I	II	III	IV	V	*t*-test
Urinary excretion (% of dose) during (h):						
0–24	5·09 (3·40)[1]	5·73 (0·76)	12·91 (2·14)	3·35 (1·56)	4·28 (0·56)	III > I, II, IV, V
24–48	1·96 (0·87)	1·76 (0·63)	1·11 (0·52)	1·34 (0·88)	1·57 (1·48)	NS
48–72	0·55 (0·23)	0·40 (0·06)	0·27 (0·12)	0·28 (0·18)	0·38 (0·26)	NS
72–96	0·21 (0·08)	0·21 (0·02)	0·13 (0·01)	0·18 (0·09)	0·20 (0·12)	NS
96–120	0·13 (0·01)	0·15 (0·02)	0·07 (0·06)	0·11 (0·06)	0·12 (0·04)	NS
120–144	0·09 (0·08)	0·09 (0·08)	0·07 (0·06)	0·07 (0·06)	0·28 (0·31)	NS
0–144	8·02 (2·48)	8·34 (1·29)	14·57 (2·41)	5·33 (2·74)	6·83 (2·09)	III > I, II, IV, V
Cage wash (% of dose)	0·12 (0·21)	0·05 (0·09)	0·19 (0·32)	0·14 (0·24)	0·48 (0·41)	NS

[1] Standard deviation, n = 3.

wash were assayed directly by counting aliquots in a xylene based scintillator solution (Scint-A, Packard). Aliquots of blood were air-dried and combusted in a sample oxidizer (Model 306, Packard), using 9 ml of absorption medium (Carbosorb II, Packard) and 12 ml of scintillation cocktail (Permafluor V, Packard). Dose preparations were assayed by both the direct and combustion methods. Concentrations of radioactivity in blood were calculated in terms of F-values, where F = (d min^{-1} ml^{-1} of blood)/(d min^{-1} g^{-1} of body weight). Pairwise comparisons between the analogues were performed using Student's *t*-test.

Results and discussion

Mean blood concentrations of radioactivity, together with pertinent pharmacokinetic parameters, are given in Table 2. Following a 500 mg kg^{-1} oral dose of the homologous mixture, the onset and rate of absorption of I and its *trans*-isomer, II, both with a monounsaturated fatty acid chain, were slow, with the first measurable radioactivity levels at 2–4 h post dose. The mean peak blood concentrations and peak times of these two analogues were virtually identical, F = 0·113 at approximately 50 h. Analogue III, with two double bonds on the fatty acid chain, showed a relatively rapid onset and rate of absorption, achieving peak concentration (F = 0·117) at 4–5 h after dosing. In contrast, the peak time was significantly longer, and the peak concentration lower, for analogue IV which had a saturated fatty acid chain. Compared with IV, analogue V, which also had no double bond but had two less carbon atoms on the fatty acid chain, showed slightly enhanced peak blood concentrations and significantly shorter peak times. The blood levels of all five analogues declined slowly after reaching their peaks, with substantial radioactivity concentrations being detected at 144 h post dosing.

The excretion of radioactivity in the urine is summarized in Table 3. After each dose, urinary excretion was most rapid during the 0–24 h interval; the rate varied from 0·14% of dose h^{-1} for analogue IV to 0·54% of dose h^{-1} for analogue III. At 144 h post dosing, the total recovery of radioactivity in the urine ranged from 5·3% of the dose of IV to 14·6% of the dose of III, with III being significantly different from the other analogues.

The biotransformation of [^{14}C]57-118 involves hydrolysis of the ethyl ester and amide bonds, resulting in the formation of ethanol, [^{14}C]tryptophan, and a fatty acid. Tryptophan can be utilized for protein synthesis, thus forming ^{14}C-labelled proteins. Individual proteins have markedly different turnover rates. The half-life of serum proteins can be measured in days, that of muscle myosin in months, while labelled amino acids incorporated into collagen in young growing animals do not disappear from the body. Therefore, absorbed [^{14}C]57-118 in the present study gave rise to a pseudoconstant level of radioactivity in blood (24–144 h data, Table 2) which could be used as a measure of absorption. Additionally, the area under blood level curve from 0 to 144 h, calculated by trapezoidal rule, and the cumulative urinary excretion were also used as absorption indices.

As shown in Table 2, the area under curve and the pseudo-constant blood level (at 144 h) yielded the same rank order in absorption efficiency of the five analogues. The urinary excretion results (Table 3) also agreed with the blood data, although a discrepancy was observed in the data for III. The relatively rapid absorption of this analogue (peak time = 4·7 h) resulted in enhanced excretion during the early post-dosing phase (0–24 h, Table 3), but was not supported by proportionately larger area under curve values, although the initial blood concentrations (2–8 h) of III were significantly higher. While the exact cause of the discrepancy is unknown, it is certain that the amount of radioactivity recovered in the urine must represent absorbed drug, whereas the blood radioactivity concentration may vary for a number of reasons, including a possible difference in metabolic fate as suggested by the marked difference in blood concentration profile between III and the other analogues. Therefore, the urinary excretion data are believed to be a more accurate indication of the amount absorbed of compound III. Accordingly, a general trend in the absorption efficiency of these compounds can be derived, although statistical significance was shown only for certain parameters for the best absorbed and most poorly absorbed analogues, namely III and IV (Tables 2 and 3), probably due to the relatively small number of dogs used. Thus, the extent of absorption of I is similar to its *trans*-isomer, II. An additional double bond in the fatty acid moiety facilitates gastrointestinal absorption, as demonstrated by analogue III. On the other hand, saturated fatty acid chains would render the molecule less efficiently absorbed; the extent of absorption appears to be dependent on the chain length. Hence, analogue V with 16 carbon atoms on the fatty acid chain is better absorbed than analogue IV with 18.

The results of the present study tend to support the notion that an inverse relationship exists between the absorption efficiency and chain length of long-chain fatty acids (Westergaard & Dietschy 1976). Furthermore, the data also suggest a direct relationship between the degree of unsaturation of the fatty acid chain and its absorption. This characteristic is probably related to the increased aqueous solubility with increasing degrees of unsaturation of hydrocarbon chains of equal length (Tanford 1980). For the stereoisomers with identical chain length and degree of unsaturation, the configuration appears to have little effect on their intestinal absorption.

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Plasma concentrations of clobazam and its *N*-desmethyl metabolite; protection against pentetrazol-induced convulsions in mice

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The anticonvulsant effects of acute administration of clobazam and its principal metabolite *N*-desmethylclobazam were studied in mice. Pentetrazol, given by slow intravenous infusion 1 or 2 h after the anticonvulsant dose, was used as the convulsant stimulus. Log dose response relationships for both clobazam and *N*-desmethylclobazam appeared linear, but there was no correlation between plasma concentrations of clobazam and protection. However, correlation between plasma concentrations of *N*-desmethylclobazam and protection was significant in both cases.

Monitoring plasma concentrations of benzodiazepines in order to predict pharmacological response is often considered impracticable (Mandelli et al 1978), as this relationship is complicated by such factors as uneven distribution, tolerance to the benzodiazepine effects and the occurrence of active metabolites, this latter especially with those benzodiazepines that undergo demethylation, e.g. diazepam and clobazam.

The principal metabolite of clobazam in man and certain animal species is *N*-desmethylclobazam (Volz et al 1979). During chronic treatment in man, this accumulates to steady-state levels some eight times higher than those of the parent compound (Rupp et al 1979) as a result of its much longer half-life. As it also has an affinity for the benzodiazepine receptor, which appears to be similar to that of clobazam itself (Hunt 1979), it is likely that desmethylclobazam makes a significant contribution to the overall effect of clobazam treatment.

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In this study, we show that the anticonvulsant effects of clobazam in mice, when measured 2 h after acute administration, show a significant correlation with the plasma levels of desmethylclobazam but not those of the parent compound itself.

Methods

Clobazam (Hoechst UK) was dissolved in a vehicle having the composition: propylene glycol 0·4 ml, ethanol 0·1 ml, benzyl alcohol 0·015 ml, sodium benzoate 50·0 mg, benzoic acid 2·25 mg, distilled water to 1·0 ml. *N*-Desmethylclobazam (Hoechst UK) was dissolved in dimethylsulphoxide (DMSO).

Adult male mice (80) (Tuck No. 1), 25–40 g, were randomly assigned to two groups, one group for the clobazam study and the other for the desmethylclobazam study. The first group, comprising 50 mice, was subdivided into groups of 10. Four of these subgroups received a subcutaneous dose of clobazam, either 1, 2·5, 5 or 10 mg kg^{-1}; the remaining 10 mice were given vehicle alone. All mice received the same injection volume of 2·5 ml kg^{-1}. In the metabolite study, 30 mice were subdivided into groups of 5. Five of these subgroups received a subcutaneous dose of desmethylclobazam, either 2·5, 5, 10, 20 or 40 mg kg^{-1}; the remaining 5 mice were given DMSO alone. In this study, all mice received an injection volume of 1·25 ml kg^{-1}.

One hour after the desmethylclobazam dose and 2 h after the dose of clobazam, the mice were tested with pentetrazol (leptazol, metrazol, pentylenetetrazole). Pentetrazol (Sigma, London; 10 mg kg^{-1} in 165 mM