Pharmacokinetics of etretinate and acitretin with special reference to treatment of psoriasis

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PREFACE

The studies upon which this thesis is based were carried out during my residency at the Department of Dermatology, Marselisborg Hospital, University of Aarhus from 1985 to 1989 and as a senior resident at the Department of Dermatology, Bispebjerg Hospital, University of Copenhagen from 1989 to 1992, and conducted in close collaboration with the Institute of Pharmacology, University of Aarhus.

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In memory of Knud Boye.

This thesis is based upon the following publications, which will be referred to by their Roman numerals:

I Jakobsen P, Larsen FG, Larsen CG. Simultaneous determination of the aromatic retinoids etretin and etretinate and their main metabolites by reversed phase liquid chromatography. J. Chromatogr. 1987; 415: 413-418.

II Larsen FG, Jakobsen P, Larsen CG, Nørgaard A, Kragballe K, Nielsen-Kudsk F. Single dose pharmacokinetics of etretin and etretinate in psoriatic patients. Pharmacol. Toxicol. 1987; 61: 85-88.

III Larsen FG, Jakobsen P, Larsen CG, Kragballe K, Nielsen-Kudsk F. Pharmacokinetics of etretin and etretinate during long-term treatment of psoriasis patients. Pharmacol. Toxicol. 1988; 62: 159-165.

IV Larsen FG, Nielsen-Kudsk F, Jakobsen P, Kragballe K. Pharmacokinetics of etretinate in psoriatic patients with liver fibrosis. Pharmacol. Toxicol. 1989; 65: 393-397.

V Larsen FG, Nielsen-Kudsk F, Jakobsen P, Schrøder H, Kragballe K. Interaction of etretinate with methotrexate pharmacokinetics in psoriatic patients. J. Clin. Pharmacol. 1990; 30: 802-807.

VI Larsen FG, Jakobsen P, Eriksen H, Grønhøj J, Kragballe K, Nielsen-Kudsk F. The Pharmacokinetics of acitretin and its 13-cis-metabolite in psoriatic patients. J. Clin. Pharmacol. 1991; 31: 477-483.

VII Larsen FG, Jakobsen P, Knudsen J, Weissman K, Kragballe K, Nielsen-Kudsk F. Conversion of acitretin to etretinate in psoriatic patients is influenced by ethanol. J. Invest. Dermatol. 1993; 100: 623-627.

ABBREVIATIONS

AUC_T:	calculated area under the plasma concentration curve corresponding to a dose interval at steady-state (which equals AUC from zero time to infinity after a single dose)
Cl:	plasma clearance of drug
C_{max}:	maximum drug concentration in plasma
$C_{min,ss}$:	plasma trough drug concentration levels at steady-state
$\bar{C}_{ss}$:	mean steady-state concentration calculated as AUC_T/T, where AUC_T is the area under the concentration curve at a dose interval (T)
HPLC:	high performance liquid chromatography
Lag-time:	time elapsing until appearance of first order drug absorption
$t_{1/2}k_a$:	half-time of the first order absorption phase
$t_{1/2}$-α:	half-time of distributive drug disposition
$t_{1/2}$-ß:	terminal half-life of drug disposition
t_{max}:	time to maximum drug concentration
V_{area}:	apparent volume of drug distribution during terminal disposition
V_c:	apparent volume of drug distribution of central compartment
V_{ss}:	apparent volume of drug distribution at steady-state

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1. GENERAL INTRODUCTION

1.1 Vitamin A

1.1.1 Definition and History. Vitamin A was isolated at the start of the twentieth century as a fat soluble substance (McCollum & Davis, 1913) and has long been known for its importance in promoting general growth, in regulating proliferation and differentiation of epithelial tissues (Olson, 1972) and in maintaining visual function (Wald, 1968) and reproduction. Its structure was determined by Karrer et al. (1931) and found to correspond to all-trans-retinol (Fig.1). Retinoids are generally defined as the natural and synthetic analogues of vitamin A. A new definition based on the biological activity rather than the chemical structure has been proposed, namely, that a retinoid is a substance which can elicit specific biological responses by binding to and activating a specific receptor or sets of receptors (Sporn & Roberts, 1985; Sporn *et al*, 1986).

1.1.2 Intestinal Absorption of Carotenoids and Retinyl Esters. Retinol is a true vitamin in the sense that it cannot be synthesized by mammals and therefore has to be obtained through the diet where it is provided in two forms: carotenoid vitamin A precursors (provitamin A), found primarily in plants; and preformed vitamin A, found naturally only in animal products. Carotenoids belong to a widely distributed family of tetraterpenes which are synthesized by a large variety of photosynthetic microorganisms and members of the plant kingdom. Some of these carotenoids have the vitamin A configuration on half of the hydrocarbon chain. Beta-Carotene is the carotenoid with the highest provitamin A biologic activity. It is attacked in the intestinal mucosa by two soluble enzymes: first, beta-carotene-15,15'-dioxygenase, and second retinaldehyde reductase. The first enzyme catalyzes the cleavage of ß-carotene at the central double bond, to yield two molecules of retinal and the second reduces it to retinol (Goodman & Huang, 1965; Goodman & Olson, 1969). The formation of retinal from ß-carotene is under tight biological control, so that excessive intake of carotenoids does not cause clinical symptoms of hypervitaminosis A (Underwood, 1985; Silvermann *et al*, 1987; Allen & Bloxham, 1989). Recent work indicates that carotene may be cleaved both centrally (as originally reported by Goodman and collaborators) and peripherally by two separate enzymes (Dmitrovski, 1991). Retinal generated by central cleavage is presumably reduced to retinol by a reductase and the apo-carotinals formed by peripheral cleavage may be further processed to retinol or retinoic acid (Blomhoff *et al*, 1992). In man carotene is also absorbed unmodified and may discolor the integuments when ingested in excessive amounts (Moore, 1957).

Dietary retinyl esters from animal sources are hydrolyzed to retinol in the intestine by a carboxylic ester hydrolase that can act on a wide variety of ester substrates (Erlandson & Borgstrom, 1968). The resulting unesterified retinol is apparently absorbed into the enterocytes by facilitated diffusion when retinol is present at physiological levels and by passive diffusion when it is present in pharmacological amounts (Hollander, 1981). Following absorption retinol is esterified with long-chain fatty acids (Goodman *et al*, 1966). Said *et al* (1989) demonstrated that retinol-binding protein (RBP) and bovine milk ß-lactoglobulin enhance the uptake of retinol from small intestinal inverted sacs of suckling rats.

1.1.3 Esterification of Retinol. The mechanism of esterification of retinol in the enterocytes appears to involve two important enzymes: acyl CoA:retinol acyltransferase (ARAT) (Helgerud *et al*, 1983) and lecithin:retinol acyltransferase (LRAT) (MacDonald & Ong, 1988). It has been suggested that LRAT esterifies retinol during absorption of a "normal" load of retinol, while ARAT esterifies excess retinol when large doses are absorbed and cellular retinol binding protein type II (CRBP-II) becomes saturated (Blomhoff *et al*, 1990).

1.1.4 Liver Handling of Chylomicron Retinyl Esters. From the intestine retinyl esters are transported bound to chylomicrons via the lymphatics into the systemic blood (Krinsky *et al*, 1958). The chylomicrons are then metabolized in extrahepatic tissues by the lipolytic removal of much of the chylomicron triglyceride (Goodman, 1984) and the chylomicron remnant thus formed is absorbed by the liver parenchymal cells. The observed rapid hydrolysis may be catalyzed by a retinyl ester hydrolase found in the plasma membrane or in early endosomes (Harrison & Gad, 1989). Retinol is subsequently transferred to the endoplasmic reticulum, where RBP is found in high concentration (Blomhoff *et al*, 1985a). Binding of retinol to RBP presumably initiates a translocation of retinol-RBP to the Golgi complex, followed by secretion from the cells (Ronne *et al*, 1983).

In the liver parenchymal cells, most of the retinol is transferred to the nonparenchymal stellate (Ito) cells and stored as retinyl esters (Wake, 1980). The transfer of retinol from parenchymal to stellate cells is quite specific, as other components of chylomicron remnants (i.e. cholesterol and vitamin D) are not transferred (Blomhoff *et al*, 1982). The intercellular traffic is probably mediated by RBP (Blomhoff *et al*, 1988). In mammals, about 50 to 80% of the total body vitamin A is normally stored in liver stellate cells as retinyl esters (Blomhoff *et al*, 1985b).

1.1.5 Retinol Mobilization from the Liver. Retinol mobilization and delivery are highly regulated processes especially controlled by processes that regulate the rates of RBP synthesis and secretion by the liver. It has been suggested that stellate cells synthesize RBP and mobilize retinol-RBP directly into the plasma without prior transfer of retinol to the parenchymal cells (Blomhoff *et al*, 1990).

Most of the retinol-RBP in plasma is reversibly bound to another protein, transthyretin, which serves to reduce the glomerular filtration and renal catabolism of RBP (Goodman, 1985). RBP is the only retinoid-specific binding protein that has been identified in plasma.

1.1.6 Cellular Uptake of Retinol. The precise mechanisms controlling uptake of retinol from plasma remain obscure at present. Retinol uptake by the cells appears to involve one or more of the following processes: (1) The small amount of unbound retinol may be in equilibrium with retinol-RBP in plas-

ma, and be available for cellular uptake without the use of a cell surface receptor (Fex & Johannesson, 1988); (2) retinol might enter cells as a result of fluid phase endocytosis (Blomhoff *et al,* 1990); (3) or may be taken up by a cell surface receptor (Heller, 1975). Blomhoff et al (1991) concluded that the first two processes may account for a small part of cellular retinol uptake *in vivo* but that a specific cell surface receptor for RBP is likely to exist and may be the most important mechanism for cellular uptake of retinol.

1.1.7 Cellular Retinol Binding Protein (CRBP) and Cellular Retinoic Acid Binding Protein (CRABP) with Special Reference to the Skin. In the cytoplasm, two main types of retinoid-binding proteins have been identified, the cellular retinol-binding proteins (CRBP type I and II)(Kanai *et al,* 1968; Li *et al,* 1986) and the cellular retinoic acid-binding proteins (CRABP type I and II) (Chytil & Ong, 1979; Takase *et al,* 1986; Baily & Siu, 1988). All of these proteins belong to the superfamily of hydrophobic ligand-binding proteins found in many tissues (Sundelin *et al,* 1985). Studies on the distribution of CRBPs and CRABPs have demonstrated that CRBP-I and CRABP-II are the predominant intracellular retinoid-binding proteins in most tissues. However, CRBP-II makes up more than 1% of the total cytosolic protein of the jejunal mucosa and may be involved in esterification of retinol (Ong, 1984; Crow & Ong, 1985).

CRBPs have been found to be present in low but equal concentrations in epidermis and dermis, and not elevated in psoriatic lesions when compared with normal or nonlesional psoriatic skin (Siegenthaler *et al*, 1986a). In contrast, CRABPs are present in much higher amounts in the epidermis than in the dermis, and are significantly elevated in psoriatic plaques as compared to nonlesional skin, or in skin of non-psoriatics (Siegenthaler *et al*, 1986b). Åstrom *et al* (1991) have demonstrated that CRABP-II, but not CRABP-I, mRNA is induced by retinoic acid in adult human skin *in vivo* and in cultured human skin fibroblasts *in vitro.*

1.1.8 Nuclear Receptors. Molecular studies of nuclear receptors have led to the identification of a superfamily of ligand-inducible regulatory proteins that includes receptors for retinoic acid (RARs)(Petkovich *et al,* 1987). Hitherto, no nuclear receptors for retinol have been identified suggesting that the conversion of retinol to retinoic acid is a necessary metabolic step in the *in vivo* action of retinoids.

Three forms of human RARs that are structurally homologous to steroid hormone, thyroid hormone and vitamin D3 receptors have been cloned (hRAR-α,-ß,-γ) (Petkovich *et al*, 1987; Giguere *et al*, 1987; Benbrook *et al*, 1988; Brand *et al*, 1988; Krust *et al*, 1989). Comparison of the amino acid sequences between human and mouse RARs show that interspecies conservation of a given member of the RAR subfamily (α, ß, or γ) is much higher than the conservation of all three receptors within a given species (Krust *et al*, 1989). Therefore, RAR-α, ß, and γ may exhibit specific functions. Studies of the distribution of mRNA for these receptors have revealed a greater tissue specificity for RAR-ß compared with RAR-α (Rees *et al*, 1989) and also a tenfold higher affinity for retinoic acid (Brand *et al*, 1988). Krust *et al* (1989) have shown that hRAR-γ mRNA is the predominant RAR-mRNA species in human skin. In addition, homologous receptors displaying low affinity for retinoic acid have been cloned, and termed RXR (Mangelsdorf *et al*, 1990).

Others found that retinoic acid is bound with the same affinity to both RAR-α and RAR-ß (Crettaz *et al*, 1990), and that only retinoid analogues with an acidic end-group are able to actively bind to both receptors. Martin *et al* (1992) described the most selective ligand for each of the three receptor subtypes detected in a screening program including more than 50 synthetic retinoids of the naphthalenic and benzoic acid family. The investigators concluded that it is possible to design biologically active analogues of retinoic acid, which selectively bind to either RAR-α, RAR-ß or RAR-γ.

1.2 Vitamin A and Vitamin A Analogues in Relation to Dermatology

1.2.1 Therapy with Vitamin A. Vitamin A deficiency in experimental animals and in humans is associated with xerosis, epithelial hyperkeratosis and with squamous metaplasia of mucosal surfaces (Wolbach & Howe, 1925; Frazier & Hu, 1931). Because these effects are reversible after vitamin A intake (Wolbach & Howe, 1933) and the similarity between certain disorders of keratinization and hypovitaminosis A, therapy with vitamin A in psoriasis, ichthyoses and Darier's disease was carried out (Peck *et al,* 1941; Rapaport *et al,* 1942; Keddie, 1948; Frey & Schoch, 1952). The results were disappointing because very high doses of vitamin A were needed in order to obtain a clearing of the cutaneous lesions and the symptoms of hypervitaminosis A predominated (fatigue, weakness, anorexia, bone pain, headache, peeling of the skin and dryness of the mucous membranes).

1.2.2 The Skin Papilloma Model. Retinoids with less side effects and more selective therapeutic activity were clearly needed, and extensive research was begun with synthetic analogues of vitamin A. To date, more than 1,500 different compounds have been synthesized and biologically tested (Bollag, 1983). Bollag and coworkers tried to predict the usefulness of a retinoid compound based on the "therapeutic index" determined for each product in animal testing. Skin papillomas of female Swiss albino mice were induced by using 7,12- dimethylbenz(a)anthracene (DMBA) as an initiating agent painted on the back skin on days 1 and 15 and croton oil twice weekly as a promoting agent from day 28 onwards (Bollag, 1972; 1974). Papillomas normally appeared within 8 months. The "therapeutic index" was then calculated as the ratio between the lowest daily dose causing a defined degree of hypervitaminosis A in a 2 weeks study, and the dose causing 50% regression of papillomas when given once a week for 2 weeks (Bollag, 1974). Whether this model could be applied for human skin diseases is questionable, although psoriasis and other disorders of keratinization are associated with both increased epidermal proliferation and increased keratinization.

1.2.3 Retinoic Acid. Retinoic acid exists not only as all-trans but also as a series of cis-isomers which are now considered to be in the pathway of vitamin A metabolism (Malathi *et al,* 1963; Emerick *et al,* 1967; Zile *et al,* 1967; Ashton *et al,* 1971; De-Luca & Zile, 1975; Zile *et al,* 1982). Attention was initially focused on all-trans-retinoic acid for local treatment of a variety of dermatoses with hyper- or dyskeratinization (Stuttgen, 1962; Beer, 1962; Kligman *et al,* 1969). It was found to possess chemopreventive as well as chemotherapeutic activity on benign and malignant epithelial tumors in animals (Bollag, 1971; 1972). However, while oral therapy appeared promising with respect to efficacy, its use was hampered by the well-known toxic effects of hypervitaminosis A.

13-cis-retinoic acid (isotretinoin) was introduced due to its low toxicity in mice (Bollag, 1971). Isotretinoin was a great leap forward as an oral agent in the treatment of severe, recalcitrant, nodulocystic acne unresponsive to conventional therapy (Peck *et al,* 1979). However, its clinical use has been hampered by a potentially teratogenic effect (Stern *et al,* 1984) and it is therefore obligatory for fertile women to use effective contraception during therapy and for at least one month after the drug is discontinued (Windhorst & Nigra, 1982).

1.2.4 Etretinate and Acitretin. In 1972 Bollag and coworkers synthesized two aromatic retinoids, etretinate (ethyl all-trans-9-(4-methoxy-2,3,6-trimethyl-phenyl)-3,7-dimethyl-2,4,6,8-nonatetraenoate) and etretin or acitretin (all-trans-9-(4-methoxy-2,3,6-trimethyl-phenyl)-3,7-dimethyl-2,4,6,8-nonatetraenoic acid) (often referred to as second generation retinoids) which gave a significant regression of chemically induced papillomas and possessed a good therapeutic index (Bollag, 1974; 1985). The chemical structure of both compounds differs from vitamin A acid in possessing a substituted aromatic ring in place of the cyclohexenyl ring (Fig.1). Etretinate was selected for clinical trials for two reasons: it was found more active in mice both after oral and parenteral administration and secondly showed a better gastrointestinal absorption (also in mice) (Bollag, 1985). It was soon found that etretinate was beneficial in the treatment of many dermatoses such as severe psoriasis vulgaris (Ott & Bollag, 1975; 1976; Ehmann & Voorhees, 1982; Kaplan *et al,* 1983) and pustular and erythrodermic psoriasis (Lorand *et al,* 1983; Wolska *et al,* 1983). During the following years it became evident that acitretin also was well absorbed in man and that it was one of the main metabolites of etretinate after chronic dosing. Both therapeutic effects and adverse effects were comparable for the two drugs (Gollnick *et al,* 1988; Kragballe *et al,* 1989).

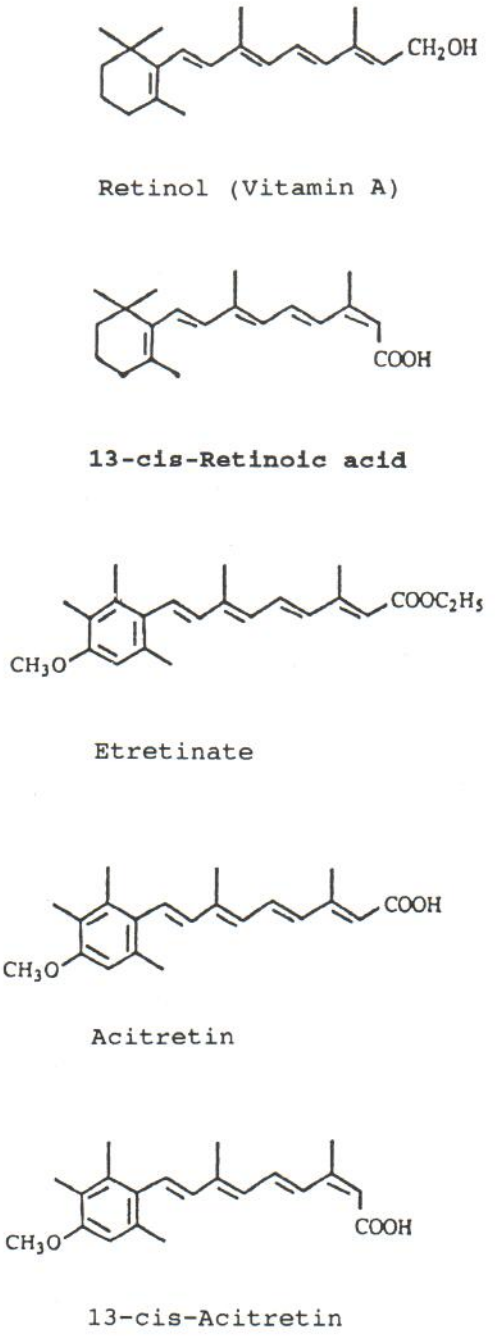

Fig.1. Chemical structures of the retinoids

1.3 Toxicology of Etretinate and Acitretin

Acute vitamin A intoxication has been known for a long time by eskimos and arctic travelers after ingestion of polar bear or seal livers. The symptoms are short-lasting with headache, lethargy, nausea and vomiting. After one day the patient experience skin desquamation (Knudson & Rothman, 1953). The first report of chronic vitamin A intoxication was in a child (Josephs, 1944) and later in an adult (Sulzberger & Lazar, 1951). Clinical symptoms of chronic hypervitaminosis A can develop after a few weeks or several years and include pruritus, headache, pseudotumor cerebri, skeletal pain, hair loss or skin desquamation (Muenter *et al,* 1971). This is relevant in relation to both etretinate and acitretin, because long-term therapy with the two compounds is associated with a distinctive adverse effect profile typical for hypervitaminosis A.

1.3.1 Hepatotoxicity. In about 10-30% of patients there will be a modest and often transitory elevation of liver enzymes

(SGOT, SGPT, or LDH) (Ellis & Voorhees 1987; Gupta *et al,* 1989; Kragballe *et al,* 1989). In the laboratory data from a multicenter trial, Orfanos *et al* (1979) found no significant difference in liver function during or after etretinate therapy. However, several investigators have reported on liver biopsy verified hepatotoxic damage following etretinate (Fredriksson, 1978; Thune & Mørk, 1980; Foged & Jacobsen, 1982; van Voorst-Vader *et al,* 1984; Gavish *et al,* 1985; Vahlquist *et al,* 1985; Weiss *et al,* 1985; Camuto *et al,* 1987) and acitretin therapy (van Ditzhuijsen *et al,* 1990). The hepatotoxicity can roughly be divided into two categories. In the first category, hepatitis developes within a few months of therapy and appears to be an idiosyncratic response. This type of toxicity may progress to chronic aggressive hepatitis (Thune & Mørk, 1980). The second category is a dose dependent (pharmacologic) toxic reaction where prospective biopsy studies have shown no significant damage to the liver during etretinate therapy (Glazer *et al,* 1982; Roenigk *et al,* 1985; Zachariae *et al,* 1985).

Both etretinate and acitretin should be viewed as potentially hepatotoxic and should not be administered to patients with severe liver disease or alcoholism.

1.3.2 Effects on Lipids. Retinoids exert an unfavorable influence on the serum lipid pattern. Both etretinate and acitretin increase the triglyceride levels predominantly, but a shift of cholesterol from the high-density lipoproteins (HDL) to the low-density lipoproteins (LDL) is also commonly observed (Michaelsson *et al,* 1981; Vahlquist *et al,* 1985; Vahlquist *et al,* 1988). The individual response to retinoid treatment with respect to the effects on serum lipoproteins is highly variable and can not be predicted (Vahlquist *et al,* 1985; Vahlquist *et al,* 1988). There are two major theoretical concerns in terms of alterations in lipoprotein levels; acute pancreatitis as a result of high concentrations of triglyceride and increased atherogenesis. Neither has yet been reported in relation to etretinate or acitretin. Although the mechanisms of retinoid induced hyperlipidemia are not known it may be due to either an increased synthesis of lipoproteins or by a decreased catabolism of circulating lipoprotein particles.

1.3.3 Ocular Adverse Effects. Adverse ocular reactions are among the most frequent side effects of etretinate and acitretin therapy and consist of blepharoconjunctivitis, dry eyes, blurred vision and contact lens intolerance. Although, dry eyes are common during retinoid therapy the tear fluid secretion rate appears normal or even increased. However, depression in the function of the Meibomian glands can result in a reduction of the lipid content of the tear film, allowing evaporation (Fraunfelder *et al,* 1985). A more serious adverse ocular reaction is pseudotumor cerebri (Bonnetblanc *et al,* 1983; Viraben *et al,* 1985), in which case retinoids should be discontinued at once (Fraunfelder *et al,* 1985). Weber *et al* (1988) found that one out of 4 patients treated with etretinate had reduced scotopic electroretinogram amplitude. In contrast, during a 1-year period of etretinate treatment, Pitts *et al* (1991) observed no evidence of progressive visual or electrophysiological dysfunction. Recently, keratoconus has been reported as a possible side-effect to acitretin therapy (Larsen *et al,* 1993).

1.3.4 Skeletal Changes. One of the major problems encountered with long-term etretinate (DiGiovanna *et al,* 1986) and acitretin therapy (Kilcoyne, 1988) is their effect on the skeleton. Radiographic monitoring during continuous etretinate or acitretin therapy has shown degenerative spondylosis, osteoporosis, ossification of ligamentous insertions, slender long bones, ossification of the anterior or posterior longitudinal ligaments of the spine, and the appearance of small spurs along the anterior margins of the cervical, thoracic and lumbar spine (DiGiovanna *et al,* 1986; Kilcoyne, 1988; Halkier-Sørensen & Andresen, 1989). The clinical implications of the reported spinal and appendicular skeletal changes are unknown, but compression of the spinal cord has been observed during etretinate therapy (Tfelt-Hansen *et al,* 1989).

Treatment in children must be cautiously evaluated with respect to the risk:benefit ratio. Although one study revealed no apparent adverse impact on growth following etretinate therapy (Shelnitz *et al*, 1987), serious adverse reactions including premature epiphyseal closure (Prendiville *et al*, 1986) and slender long bones, disc space narrowing, periosteal thickening, bone reabsorption and osteoporosis (Halkier-Sørensen *et al*, 1987) have been reported.

1.3.5 Teratogenicity. Etretinate and acitretin are potentially teratogenic in humans. The teratogenic effects probably occur within the first 3 weeks of gestation (Shalita, 1988). Grote *et al* (1985) reported on a 10-week old aborted fetus with isolated unilateral leg hypoplasia, whose mother stopped etretinate therapy 4 months before conception. Necropsy of a 23-week old fetus, whose mother was treated with etretinate 7 months before becoming pregnant, revealed abnormal return of the inferior vena cava into the left atrium, large atrial septal defect of the ostium secundum type and horseshoe kidneys (Verloes *et al,* 1990). Vahlquist & Rollman (1990) reported on a woman who became pregnant 7.5 months after stopping etretinate and delivered a healthy male child. When the fetus was 16 weeks old, concentrations of etretinate and acitretin in amniotic fluid were below 0.4 ng/ml, whereas plasma concentration of etretinate was 3.6 ng/ml.

Thus, exclusion of pregnancy before initiating retinoid therapy and use of contraceptives during treatment are required. After cessation of long-term etretinate or acitretin therapy, the generally recommended period for anticonceptives measures is at least 2 years.

1.3.6 Cutaneous Adverse Effects. Virtually all patients treated with therapeutic doses of etretinate or acitretin develop within the first 2 weeks xerosis, pruritus, peeling palms and soles, dryness of mucous membranes, and cheilitis (Goldfarb *et al,* 1987). Cheilitis occurs most of time. Another common problem is an itching dermatitis affecting up to 50 % of the patients (Ellis & Voorhees, 1987). Nail growth rates increase during etretinate therapy (Galosi *et al,* 1985). Redness and swelling of the skin fold around the dystrophic nail resembling paronychia-like changes have been reported (David *et al,* 1988). Nail fragility is seen both during etretinate (15%) and acitretin (28%) therapy (Kragballe *et al,* 1989). Diffuse

hair loss tends to occur at high doses of both retinoids, but is cosmetically significant only in a few cases (Ellis & Voorhees, 1987). However, it appears to be reversible on stopping therapy or adjusting the daily dose. In a Nordic multicenter study hair-loss was observed more frequently after acitretin (48%) than etretinate (20%) treatment at therapeutic relevant doses (Kragballe *et al*, 1989).

1.4 Potential Drug Interactions with Etretinate and Acitretin

A therapeutic drug interaction occurs when one drug significantly alters the degree of response of another drug (Rowland & Tozer, 1980). In relation to retinoids only limited information is available in the literature. However, retinoid treated patients commonly receive two or more drugs concurrently.

In healthy volunteers acitretin did not significantly affect the anticoagulant action of phenprocoumon (Hartman *et al*, 1989). A single case of warfarin-etretinate interaction was recently described (Ostlere *et al*, 1991). Berbis *et al* (1988b) found that acitretin did not interfere with the anti-ovulatory activity of oral combination contraceptives even during prolonged intake.

We found that the apparently increased risk for developing toxic hepatitis during coadministration of methotrexate and etretinate cannot be explained by drug accumulation due to pharmacokinetic interaction. However, influence on hepatotoxicity due to an increase in methotrexate C_{max} during concomitant therapy cannot be excluded (V)(see chapter 5).

Patients with severe recalcitrant psoriasis are often treated with retinoids in conjunction with photochemotherapy (Psoralen and UVA-irradiation), UVB-irradiation or other modalities. Beani *et al* (1991) found no significant difference in pharmacokinetic parameters of 8-methoxypsoralen between patients on PUVA alone or patients on etretinate-PUVA.

The high lipophilicity of etretinate seems to imply a non-specific binding. Thus, displacement of the retinoids from the adipose tissue by other drugs seems rather unlikely, because of a large capacity of adipose tissues to bind lipophilic compounds (Paravicini & Busslinger, 1984). This is in line with observations on patients with psoriasis or other disorders of keratinization in which acitretin had little influence on the elimination of etretinate that had accumulated in the adipose tissues during previous therapy (Lambert *et al*, 1990).

1.5 Introduction to Authors Own Investigations

In the light of the above raised problems related to the use of retinoids for longer periods and the possible risk of accumulation and associated toxicity, we decided to study the pharmacokinetics of etretinate and acitretin in psoriatic patients after oral administration of the drugs. A reversed-phase high performance liquid chromatography (HPLC) method was successfully applied in the determination of etretinate, acitretin and 13-cis-acitretin in plasma (I). Curve-fitting to the observed single or multiple-dose concentration-time data for etretinate and acitretin was performed by means of non-linear, iterative regression analysis using the computer-program KINON-85,2 (Nielsen-Kudsk,1983). As model functions were used expressions incorporating 2 or 3 exponential terms: $Cp = A^*e^{-k_e t} + B^*e^{-k_a t}$ and $Cp = A^*e^{-\alpha t} + B^*e^{-\beta t} + C^*e^{-k_a t}$, which are representative for drugs with one- or two-compartment characteristics and first order absorption. Cp is the drug concentration in plasma, A*, B* and C* are predose concentration constants determined corresponding the dose interval (T) investigated, and k_a, α, β or k_e are absorption, disposition or elimination rate constants, respectively. A*, B*, and C* were transformed to the corresponding single-dose constants by multiplying with $(1 - e^{-k_i T})/(1 - e^{-k_i n T})$, where k_i represents the appropriate rate constant and *n* the elapsed number of dose intervals. Based on an assumed systemic availability for etretinate or acitretin of 40% were further tentatively calculated values of the apparent total volumes of distribution at steady-state: $V_{ss} = D_o((A/\alpha^2) + (B/\beta^2) + (C/k_a^2))/AUC^2$, the apparent volume of distribution during the terminal disposition phase: $V_{area} = D_o/AUC/\beta$, the volume of the central compartment: $V_c = D_o(-k_a)/(\alpha A + \beta B + k_a C)$ and apparent plasma clearance: $Cl = D_o/AUC$, where D_o is the assumed systemic available dose. The time (t_{max}) at the maximum drug concentration in plasma (C_{max}) was obtained directly from the observed concentration/time data. The areas under the etretinate and acitretin steady-state plasma concentration curves corresponding to a dose interval were calculated by the trapezoidal method. Terminal rate constants for etretinate, acitretin and 13-cis-acitretin were obtained from plasma concentration-time data determined during a period after cessation of drug administration.

By performing single-dose studies (II) it was found that etretinate is partially hydrolysed to acitretin, possibly in the gut or gut wall and that acitretin is absorbed faster after oral ingestion than etretinate. However, the HPLC-assay detection limit did not allow us to visualize the terminal elimination phase which is of particular interest due to the potential teratogenicity by the retinoids. Following therapy with etretinate for 3 months (III) and acitretin for 3 (III) or 6 months (VI) our study revealed that acitretin is eliminated from the body considerably faster than etretinate. It has been shown that after long-term etretinate therapy the two main metabolites in plasma are acitretin and 13-cis-acitretin (III), whereas after long-term acitretin therapy the corresponding 13-cis-isomer occurs as the main metabolite (III, VI). Until recently the hydrolysis of etretinate to acitretin was considered non-reversible. However, preliminary results gave evidence of a possible metabolic formation of etretinate from acitretin (Chou *et al*, 1991; Wiegand *et al*, 1991). Thus, we performed a pharmacokinetic study in which the aim was to determine the concentration levels and terminal elimination half-life of etretinate as a metabolite of long-term acitretin therapy in psoriatics (VII). We detected etretinate in 7 of 10 psoriatics treated with acitretin for a period of 3 months. Concomitant intake of alcohol appears to be an important contributing factor for the conversion.

Because candidates for retinoid therapy may previously have been treated with hepatotoxic drugs and because retinoids themselves are potentially hepatotoxic, it was of interest to determine the pharmacokinetics in psoriatic patients

with liver damage. We conducted a pharmacokinetic study in etretinate treated psoriatics with various degrees of liver damage verified by liver biopsy (IV). Compared with previous results in psoriatics with normal liver function absorption and disposition rates of etretinate were not significantly altered. Due to ethical considerations it was not possible to determine the terminal elimination phase.

During combined treatment with methotrexate (MTX) and etretinate in resistant cases of psoriasis toxic hepatitis has been reported and occurred more frequently than when the drugs were administered separately (Zachariae, 1984; 1988). We conducted a study to assess the influence of etretinate on the pharmacokinetics of MTX in psoriatic patients receiving long-term concurrent therapy with the drugs (V). Although, the mean peak MTX level during coadministration of MTX and etretinate was elevated our results indicate that the apparently increased risk for developing toxic hepatitis cannot be explained by drug accumulation due to pharmacokinetic interaction.

This thesis reviews the seven original studies (I-VII) dealing with the subjects mentioned above, and includes a discussion of the results obtained by others.

2. DETERMINATION OF RETINOIDS IN PLASMA

2.1 Introduction

The progress in the investigation of vitamin A metabolism was for many years hampered by the lack of suitable techniques to deal with these extremely labile compounds. The introduction of synthetic retinoids as drugs in dermatology and cancer research has, however, led to a number of sensitive and specific drug assays.

The analysis of retinoids depends on several of their inherent chemical properties: (1) their intense absorption in the near-ultraviolet region; (2) the propensity of several retinoids to fluoresce; (3) their sensitivity to light and oxidation; (4) and their conversion to charged, intensely colored complexes in the presence of certain acids (Frolik & Olson, 1984). Examples of analytical methods for quantitating retinoids include conventional chromatography (alumina, silicic acid, Sephadex LH-20, thin-layer chromatography), high-performance liquid chromatography (HPLC), gas-liquid chromatography and chemical analysis (fluorescence, ultraviolet absorbancy and inactivation, colorimetry, mass spectrometry).

2.2 Sample Collection and Storage

Because retinoids are susceptible to isomerization (Mousseron-Canet, 1979; Pfoertner *et al*, 1987) and oxidation (Oyler *et al*, 1989), precautions must be taken to minimize these degradation reactions. McClean *et al* (1982) reported that after 2 hr exposure to fluorescent light only 77% of isotretinoin and 34% of etretinate could be recovered in serum. After exposure to white fluorescent light for 1 hr, blood concentrations of etretinate, acitretin and 13-cis-acitretin were not significantly different from pre-exposure values (Bugge *at al*, 1985). The authors concluded that whole blood provides better protection against photodecomposition than serum. Berne *et al* (1990) observed that when etretinate and acitretin were dissolved in ethanol and irradiated with UVB or UVA, extensive and reproducible cis-isomerization occurred at the 13-position, whereas irradiation of isotretinoin produced only moderate trans-isomerization. We observed that after 15 min exposure to normal laboratory light in transparent glass tubes, only about 20% of etretinate, 27% of acitretin (etretin), 44% of isotretinoin (13-cis-retinoic acid), and 47% of 13-cis-acitretin could be recovered from the solution (I). When kept in amberglass tubes for the same period the corresponding values were 48%, 60%, 89%, and 83%, respectively. The retinoids were partly transformed into their cis-trans isomers.

The stability of retinoids in plasma can be a problem at room temperature or at 37°C (Wyss, 1990). Paravicini & Busslinger (1983) showed that etretinate levels did not change when stored in plasma at -20°C for 8 weeks. Also, acitretin and 13-cis-acitretin were stable for at least 90 days at -20°C (Al-Mallah *et al*, 1987). In order to minimize the problem of air oxidation, plasma samples have been frozen under nitrogen (Palmskog, 1980) or some other inert atmosphere (i.e. argon). Also, ascorbate and ethylenediaminetetraacetate (EDTA) have been added to the sample (Frolik *et al*, 1978).

In conclusion, the following precautions seem sufficient when handling retinoids: all manipulations should be carried out in subdued or yellow light and in amberized glassware; blood samples should be immediately centrifuged; the headspace above the plasma or serum sample should be as small as possible or the tubes should be flushed with an inert gas (nitrogen or argon).

2.3 Extraction Procedures

Most procedures for extraction of retinoids are based on a liquid-liquid extraction. One approach is an initial protein precipitation with an organic solvent. Methanol was used for the plasma extraction of 13-cis-retinoic acid (recovery 40-80%) (Kerr *et al*, 1982). However, one way to optimize the recovery is to liberate the retinoid from the plasma proteins by precipitation with a water miscible solvent. Deproteination with methanol, followed by addition of acetate buffer and hexane-methylene chloride-isopropanol resulted in a phase separation and, therefore, a concentration effect with recoveries of retinol, 13-cis-retinoic acid, and all-trans retinyl acetate in the order of 85 $\pm$ 10% (Besner *et al*, 1980). A phase separation was also established for the serum extraction of retinol, 13-cis-retinoic acid, and etretinate, following addition of butanol-acetonitrile and dipotassium monohydrogen phosphate (McClean *et al*, 1982). Recoveries were about 100%. Butanol was combined with acetonitrile to improve the reproducibility of analytical recovery. With addition of dipotassium monohydrogen phosphate, the interface was more defined, the organic phase was optically clearer, partitioning was faster, and the phase volume ratios were consistent.

Alternatively, direct extraction methods are also used. Using phosphate buffer (pH 6) and diethyl ether, 13-cis- and all-trans-retinoic acid were extracted from whole blood or urine (Puglisi & De Silva, 1978). Etretinate and acitretin were

extracted from whole blood or plasma following addition of phosphate buffer (pH 7) and ethyl acetate. Overall recoveries were about 90% (Puglisi & De Silva, 1978). Also, diethyl ether-ethyl acetate mixtures have been successfully used for the extraction of 13-cis-retinoic acid (Al-Mallah *et al*, 1988), etretinate (Besner *et al*, 1982) and acitretin (Besner *et al*, 1982; Al-Mallah *et al*, 1988); and diethyl ether and phoshate buffer for the extraction of 13-cis-retinoic acid (Vane *et al*, 1982), and etretinate and acitretin (Palmskog, 1980; Vane *et al*, 1989b). In our study, addition to plasma of ammonium acetate (pH 6) followed by diethyl ether, yielded mean percentage recoveries for etretinate, acitretin, and 13-cis-acitretin of about 85, 97 and 101, respectively (I).

2.4 Analytical Methods for Detection of Retinoids

2.4.1 Conventional Chromatography, Gas-Liquid Chromatography, Mass Spectrometry, Fluorescence, UV Detection, and Colorimetry. Conventional chromatography was one of the earliest techniques employed to separate retinoids. Alumina chromatography has been used to separate (1) retinol from its esters (Vahlquist, 1974); (2) 13-cis from all-trans retinol (Barnholdt, 1956); and retinaldehyde from retinoic acid (Olson, 1961). A disadvantage of this technique is its inability to separate the various retinyl esters as well as the metabolites of the natural retinoids (Mahadevan *et al*, 1959, Vahlquist, 1974). Silica acid chromatography has been employed to separate retinol, retinaldehyde and retinoic acids and its polar metabolites (Zile & DeLuca 1965; Pollack *et al*, 1973). Drawbacks to this method of chromatography include a low recovery of retinyl esters, various oxidation products, and isomerization of some retinoids chromatographed (Pollack *et al*, 1973; Zile & DeLuca, 1968). Sephadex LH-20 (liquid-gel partition chromatography) yields a quantitative recovery of retinol, retinaldehyde, retinyl esters, and retinoic acid but lack of capability to separate the various retinyl esters from each other and from retinaldehyd limits its applicability (Ito *et al*, 1974). Thin-layer chromatography on silica gel G has been employed to separate retinol, retinoic acid, retinaldehyd (and isomers), and retinyl esters (Zile & DeLuca, 1968; Zimmerman, 1974) and retinoic acid metabolites (Dunagin *et al*, 1965). Recoveries are usually higher than 70% but the method suffers from instability of the retinoids.

Gas-liquid chromatography has a limited use in the analysis of retinoids due to the instability of the polyene system at elevated temperatures. Napoli (1986) used gas chromatography in combination with mass spectrometry (GC-MS) for the determination of physiological levels of retinoic acid. A detection limit of 75 pg could be achieved using a deuterated retinoic acid as internal standard. A disadvantage of the technique was the inability to resolve the all-trans and 13-cis-isomers of the methyl retinoates and the need of a HPLC preseparation step. GC-MS is not the method of choice for the determination of retinoids due to the need for extensive sample work-up and problems associated with distinguishing between geometric isomers of first and second generation retinoids (Wyss, 1990).

Retinol and its esters are the only naturally occurring retinoids that fluoresce appreciably under normal conditions. From the observation that binding of retinol to specific proteins such as RBP enhances fluorescence, very sensitive techniques for plasma determination of both retinol (Futterman *et al*, 1975) and of holo-RBP (Glover *et al*, 1974) were developed. Although fluorescent measurements could be useful in specific instances the assay reliability is influenced by: (1) other natural fluorescent compounds; (2) solvent fluorescent contaminants; (3) and quenching of the fluorescence produced.

Owing to the high absorption maxima (>300 nm) and high extinction coefficients ($>10^4$) of the retinoids, UV-detection is useful when the absorbancy due to retinoids is great compared to other compounds in the sample. Also, UV-detection is widely used in connection with HPLC.

A large number of colorimetric methods for the naturally occurring retinoids have been developed (for review see Frolik & Olson (1984)). The determination of retinol and its esters are based on the formation of an intense transient blue color when mixed with a Lewis acid under anhydrous conditions. The method is rapid and inexpensive but suffers from several disadvantages: (1) accurate measurement of the transient blue color complex; (2) most polyenes react with the reagents to form colored complexes; and (3) not all batches of Lewis acids prove to be suitable for color development (Frolik & Olson, 1984).

All of the above mentioned techniques have been of great importance in the study of vitamin A metabolism. However, the development of HPLC in the past decades with its ability to handle small amounts of material has revolutionized retinoid research and it has become the technique of choice in the investigation of vitamin A metabolism and in pharmacokinetic studies of the first and second generation retinoids.

2.4.2 High Performance Liquid Chromatography (HPLC). Normal-phase HPLC is older than reversed-phase HPLC and therefore first used for retinoid separation. The method selected depends on several factors including sensitivity aspects, the availability of an internal standard, and the desired extent of sample pretreatment.

Normal-Phase HPLC. In normal-phase HPLC, the more apolar compounds elute from the support first, whereas in its reversed-phase counterpart these compounds bind tightly to the column. The solvent systems most often used in normal-phase HPLC are hexane or dichloromethane with minor amounts of a more polar organic modifier. Normal-phase HPLC, employing pure silica gel as stationary phase is especially suitable for retinoids that are not highly polar, such as retinol and retinyl esters (De Leenheer *et al*, 1988), and separation of geometrical isomers is easy under these conditions. Esterification of more polar compounds prior to chromatography can be advantageous in order to reduce the polarity of these retinoids. Also, the extract can be directly injected onto the analytical column without the need for evaporation of the extraction solvent. The lack of concentration step might, however, sacrifies some sensitivity.

The technique has been used for plasma determinations of

Table I. *Examples of Reversed-Phase HPLC Methods for the Determination of Acitretin, 13-cis-Acitretin and Etretinate*

Reference	Extraction	Column	Mobile Phase	DL or QL (ng/ml)
*Palmskog, 1980	0.1 M Phosphate buffer (pH 6), diethyl ether	Hibar LiChrosorb RP-18	Acetonitrile-water (80:20, v/v) containing 1% acetic acid	10
*Besner, *et al*, 1982	1 M Phosphate buffer (pH 6), diethyl ether-ethyl acetate (75:25, v/v)	Spherisorb ODS	Acetonitrile-water (76:24) containing 1% ammonium acetate	25
#Bugge *et al*, 1985	Butanol-acetonitrile (1:1), dipotassium hydrogen phosphate	Zorbax ODS	Gradient acetonitrile-water-acetic acid (1000:1000:10 to 1900:100:0.8, v/v) containing 0.77 g/l ammonium acetate	10
#Jakobsen *et al*, 1987	1% Ammonium acetate (pH 6), diethyl ether	Nucleosil C18	Acetonitrile-0.1 M ammonium acetate (80:20, v/v)	1-2
+Al-Mallah *et al*, 1987	Phosphate buffer (pH 7), diethyl ether-ethyl acetate (50:50, v/v)	Nucleosil C18	Methanol-1% acetic acid (85:15, v/v)	1-2

Abbreviations:

* Simultaneous determination of acitretin and etretinate
\+ Simultaneous determination of acitretin and 13-cis-acitretin
\# Simultaneous determination of acitretin, 13-cis-acitretin and etretinate
DL Detection Limit
QL Quantification Lilmit

acitretin and etretinate with detection limits in the range of 3 to 20 ng/ml (Hanni *et al*, 1979; Puglisi & de Silva, 1978; Paravicini & Busslinger, 1983; De Leenheer *et al*, 1990).

Reversed-phase HPLC. Reversed-phase HPLC is the technique most often used for retinoids because it is rapid, has a high sensitivity and can cope with compounds over a wide polarity range. Mixtures of acetonitrile or methanol and water are often used in the mobile phase, usually in combination with ammonium acetate to eliminate tailing of compounds containing a free carboxylic acid moiety. As mentioned earlier, polar compounds elute from the column first, which is of importance when investigating retinoic acid metabolism, because most metabolites of retinoic acid are more polar than the mother substance. For a more comprehensive description of the several methods published for the determination of retinoic acid and its metabolites in both plasma and various tissue samples the reader is referred to the review by Wyss (1990).

Several reversed-phase HPLC methods for determination of the synthetic, aromatic retinoids etretinate, acitretin or 13-cis-acitretin have been published (see Table I for comparison). We developed a simple and sensitive reversed-phase HPLC method which was applied for pharmacokinetic studies (I). The isocratic solvent was acetonitrile-0.1 M ammonium acetate (80:20, v/v) (pH 7) recycled through the chromatographic system. Later we observed that when the chromatographic eluent was changed to 75% acetonitrile, pH 4, the separation of the chromatographic peak of the cis- and trans isomers of acitretin was improved (VI,VII). Under the above mentioned conditions the chromatographic performance for etretinate was poor. A shorter column (12 cm) with Nucleosil 300-5μC18 and an eluent consisting of 82% acetonitrile in 0.1 M ammonium acetate, pH 7, with a flow rate of 1.8 ml/min sharpened the chromatographic peak and decreased the retention time but the cis- and trans isomers of acitretin eluted with the solvent front. Furthermore, the internal standard was changed from isotretinoin to the isopropyl ester of acitretin, a compound with chemical properties very similar to those of etretinate. The internal standard was completely separated from etretinate. We concluded, that the method was simple and sensitive and applied it in the performance of single and multiple dose pharmacokinetic studies in psoriatic patients receiving treatment with etretinate or acitretin.

3. PHARMACOKINETICS OF ETRETINATE

3.1 Introduction

Etretinate is a highly lipophilic compound extensively bound in plasma to lipoproteins (Vahlquist *et al*, 1981; Carillet *et al*, 1990; Urien *et al*, 1992) and mainly metabolized in the liver (Hanni *et al*, 1977; Hanni 1978; Paravicini *et al*, 1981). The initial metabolic step probably takes place in the gut or gut wall

and appears to be a hydrolysis to the corresponding free acid (acitretin) or its 13-cis-isomer (13-cis-acitretin), respectively (II;III)(Fig.2). The subsequent metabolic pathway which probably occurs in the liver is apparently a demethylation of the methoxy group at the aromatic ring and subsequent an elimination as the ß-glucuronide in the bile if the metabolites contain intact side chain, or by renal excretion if the metabolites have shortened side chain (Hanni, 1978; Brindley, 1989; Vane *et al*, 1989b). From human urine 14 metabolites characterized by a shortening of the tetraene side chain were isolated (Hanni *et al*, 1977). The metabolites in the urine accounted for 11% of the total dose. From human bile treated with ß-glucuronidase, the two major metabolites were identified as the 11,12-dihydro-13,14,15,20-tetranor phenolic derivative of acitretin and the 11,12,13,14-tetrahydro-15-nor phenolic derivative of acitretin (Vane *et al*, 1989a).

A study on Sprague-Dawley rats revealed that the maximum fraction of etretinate disappearing from the intestinal lumen was approximately 0.35 and that there was no evidence that the uptake of etretinate by the gastro-intestinal membrane involved an active transport system (Zimmerman & Johnson, 1991).

3.2 Single Dose Studies

Hanni (1978) was the first to report a pharmacokinetic study on etretinate. After a single oral dose of 100 mg etretinate administrated in a beadlet formulation to a healthy volunteer, a plasma C_{max} of 600 µg/l was reached after 4 hr. For the corresponding metabolite acitretin, C_{max} was higher than 1000 µg/l and occurred about 3 hr after drug administration. Radioactivity from oral tritiated etretinate 2 mg/kg was measured in urine and feces within the first 8 days and accounted for 12 and 75% of the dose, respectively. Paravicini (1981) and Paravicini *et al* (1981) fitted concentration-time profiles following both intravenous and oral doses of etretinate in 5 healthy volunteers. Three phases of decline of the drug concentration following intravenous tritiated etretinate 10 mg were apparent (half-life of the phases: 5 to 10 min, 30 to 60 min, and 6 to 12 hr). There were indications of at least one other longer phase, but the sensitivity limit of the HPLC assay did not allow visualization (Paravicini *et al*, 1981). In studies (II,III) on the pharmacokinetics of oral etretinate, the plasma concentration data of the "unmarked" drug showed only two phases during disposition. It is well-known, however, that early distributive disposition phases may be concealed by an initial absorption phase, especially when this takes place at a slow rate (Rowland & Tozer, 1980). The pharmacokinetics of etretinate following a single oral dose of 75 mg have been studied in healthy volunteers and psoriatics (Vahlquist *et al*, 1981). t_{max} was observed 3 to 6 hr after dosing and was markedly lower in the psoriatics than in the volunteers. Whether or not this reflects a poorer absorption of etretinate in psoriatics as compared with normals is unknown. The metabolite acitretin appeared in plasma shortly after etretinate and its concentrations paralleled that of the parent drug. Ray *et al.* (1981) studied the pharmacokinetics in 8 patients with Darier's disease receiving a single oral dose of etretinate (on average 69 mg). For the parent drug and its metabolite, acitretin, t_{max}-values were 3.2 and 3.3 hr, respectively, and apparent mean elimination half-lives were 4.5 and 4.8 hr, respectively.

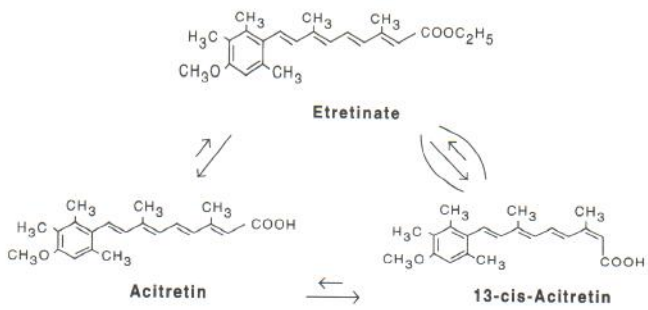

Fig.2. Proposed *in vivo* metabolism of etretinate, acitretin and 13-cis-acitretin (from VII).

We have performed a pharmacokinetic study in 3 psoriatics after a single oral dose of 40 mg (4 x 10 mg) etretinate in a gelatine capsule formulation (II). Patients fasted overnight prior to drug administration and for 3 hr after. The longer lag-time (mean 1.24 and 0.69 hr, respectively), t_{max} (mean 3.3 and 2.3 hr, respectively) and half-life of the absorption process ($t_{1/2}k_a$) (mean 0.86 and 0.55 hr, respectively) for etretinate as compared with the metabolite acitretin seem to suggest a presystemic hydrolysis, probably in the gut wall. This corroborates results by Paravicini *et al* (1981), who observed significant plasma levels of acitretin after oral administration of etretinate, whereas metabolite concentrations after an i.v. bolus were relatively low. Also, in one autopsy case the jejunal mucosa contained high levels of both etretinate and acitretin (Vahlquist *et al*, 1986). Assuming equal drug clearances, the fractional part of absorbable etretinate being hydrolysed (AUCacit/(AUCacit + AUCetret)) was in the range of 0.19-0.42. The extent of the presystemic hydrolysis does not seem to relate to the concentration of solubilizing bile salts in the duodenum (Lucek *et al*, 1988). Although patients with biliary T-tube drainage compared to those of healthy volunteers showed substantially reduced blood concentrations of etretinate and acitretin (probably due to a lack of absorption), the fractional contents of acitretin were comparable in the two groups.

After distribution, between 55 and 85% of etretinate resided outside the initial volume of distribution (II). The apparent mean terminal disposition half-lives for etretinate and the metabolite acitretin of 6.6 hr (range 4.3-8.5 hr) and 5.2 hr (range 3.8-7.5 hr), respectively, corroborates earlier findings (se Table II for review). However, it is well-known that etretinate accumulates in the subcutis from where it is only very slowly released (Rollman & Vahlquist, 1983). By performing single dose studies the terminal plasma concentrations fall below the HPLC-assay detection limit precluding the estimation of this longer elimination phase.

3.2.1 Influence of Milk or Food on the Absorption of Etretinate. Vahlquist *et al* (1981) observed in 6 psoriatics that the mean

Table II. *Pharmacokinetics of Etretinate Reported in Single Dose Studies*

		Etretinate				Acitretin (metabolite)		
Reference	No of Pat.	Dose (mg)	C_{max} (ng/ml)	t_{max} (hr)	$t_{1/2}$-ß (hr)	C_{max} (ng/ml)	t_{max} (hr)	$t_{1/2}$-ß (hr)
Hanni 1978	(n) 1 (He)	100	600	4	3	1000	3	0.75
Paravicini *et al.*, 1981	(n) 5 (He)	10 (i.v.)			(6-12)			
	(n) 5 (He)	100	(400-1600)	(2-3)	7.3		3	
Ray *et. al*, 1981	(n) 8 (Da)	68.8	124	3.2	4.5	187	3.3	4.8
Vahlquist *et al*, 1981	(a) 5 (He)	75	930 (855-1030)	(3-6)	8.3	583 (397-764)	(3-6)	8.9
	(a) 6 (Pso)	75	545 (294-788)	(3-6)	8.3	470 (271-573)	(3-6)	8.9
Brazzell *et al*, 1982	(b) (n) 6 (He)	100	200 (116-780)	4 (2.5-5)		525	3	
Colburn *et al*, 1984	(c) 8 (He)	100	418	3.6	9.4	410	3.1	2.9
	(d) 8 (He)	100	1322	6.3	7.8	168	7.1	8.5
	(e) 8 (He)	100	476	8.0	6.4	331	6.0	3.9
	(f) 8 (He)	100	1425	3.4	10.5	359	3.1	6.2
Massarella *et al*, 1985	(g) 14 (Pso)	100		(1.5-8)	7.3 (3.6-25)			
Larsen *et al*, 1987	(h) 3 (Pso)	40	238 (180-353)	3.3 (3-3.5)	6.6 (4.3-8.5)	106 (77-136)	2.3 (1.8-2.9)	5.2 (3.8-7.5)
Lucek *et al*, 1988	(i) 6 (He)	100	267	3	6.5	394	3	4.2
	(i) 2 (Bi-T)	100	(30-47)	(4-6)	(6-12)	(42-54)	(2-8)	(3-6)
Gollnick *et al*, 1990	(j) 8 (Pso)	0.8 mg/kg	578 (241-1366)	4		161 (100-200)	4	

Abbreviations:

(He) Healthy volunteers
(Da) Mb. Darier
(Pso) Psoriatics
(Bi-T) Patients with biliary T-tube drainage.
(n) No information about concomitant water or food intake.
(a) Capsules (3 × 25 mg) ingested with 200 ml of water. A light breakfast was taken half an hour after dosing.
(b) Date obtained from Hoffmann-La Roche Inc., Nutley, USA.
(c) Capsules (4 × 25 mg) ingested with 120 ml of water.
(d) Capsules (4 × 25 mg) ingested with a high fat meal.
(e) Capsules (4 × 25 mg) ingested with a high carbohydrate meal.
(f) Capsules (4 × 25 mg) ingested with whole milk.
(g) Capsules (4 × 25 mg) ingested with 200 ml of water. Food was allowed 4 hr after dosing.
(h) Capsules (4 × 10 mg) ingested with 250 ml of water. A standard breakfast was allowed 3 hr after dosing.
(i) Administered immediately after dissolving the pure drug in 25 ml of absolute alcohol and mixing with 100 ml of water.
(j) Capsules ingested together with a low fat breakfast.

Table III. *Pharmacokinetics of Etretinate Reported in Multiple Dose Studies*

Reference	No of Pat.	Etretinate				Acitretin (metabolite)		
		Dose (mg per day/time)	C_{max} (ng/ml)	t_{max} (hr)	$t_{½}$-ß (time)	C_{max} (ng/ml)	t_{max} (ng/ml)	$t_{½}$-ß (time)
Paravicini *et al*, 1981	5 (He)	50/100 d			(a) 4-8 d			
		10-25/>1 yr			80-100 d			
Ray *et al*, 1981	8 (Da)	48/6 wk	319	3.5	8 hr	226	3.5	9.9 hr
Vahlquist *et al*, 1981	5 (Pso)	75/10 d	(400-600)		(b) 17 hr	(200-300)		
	2 (Pso)	50/6-12 mo			76-136 d			
Massarella *et al*, 1985	14 (Pso)	25-100/6 mo			121 d (84-168 d)			
Larsen *et al*, 1988	3 (Pso)	50-70/3 mo	(237-1403)	(3-3.3)	(9.9-41.1 d)	(135-164)	(1.8-2.8)	(4.4-8.5 d)
Vahlquist *et al*, 1987	4 (Pso)	.72 mg per kg/>6 mo	650	4	(c) 10 hr (7-11 hr)		4	9 hr
	4 (CRF)	.69 mg per kg/.3-2 mo	1400	4	10 hr (7-11 hr)		4	9 hr

Abbreviations:

(CRF) Chronic renal failure. Other abbreviations as stated in Table II.
(a) Calculated 24 hr after a 10 mg i.v. radioactive-labelled bolus on day 11.
(b) Value obtained from 1 patient studied "long enough to permit a prediction of the final slope".
(c) Blood samples drawn for a period of 12-24 hr.
d Days
mo Months
wk Weeks
yr Years

C_{max}(etret) was significantly higher than that of the mean C_{max}(acit). In contrast, other studies have shown higher C_{max} of the metabolite as compared with the parent drug (Table II). This discrepancy might be related to whether or not etretinate administration is followed by concomitant water, milk or food intake. Thus, an increase of C_{max} of about 260% following etretinate administration with milk was found compared with the water intake (DiGiovanna *et al*, 1984). Comparison between the serum levels of etretinate and acitretin revealed that at each time point coadministration of etretinate with milk resulted in higher serum levels of parent drug than those of the metabolite, whereas the opposite was seen in relation to water administration. We found that when etretinate was ingested with water, the ratios AUC(etret)/AUC(acit) and C_{max}(etret)/C_{max}(acit) exceeded 1 indicating that the parent drug constituted the main proportion of drug reaching the systemic circulation (II). However, the above mentioned findings are not necessary in contradiction to those of ours, because it is well known that during repeated oral administration of etretinate the 13-cis-metabolite of acitretin constitutes the main metabolite in plasma (III) and in the study by DiGiovanna *et al* (1984)(repeated oral dosing) the HPLC-assay did not separate acitretin and 13-cis-acitretin and thereby presumably allowing a too high estimation of acitretin in serum.

A food-induced increase in AUC (oral) are generally thought to be related to a reduction in first-pass hepatic elimination, reduction in the rate of gastric emptying, improvement of drug dissolution, activation of intestinal transport mechanisms, change in pH of the gastric juice, or increased drug solubility of very lipophilic drugs (Melander, 1978; Morgan & Smallwood, 1990). Colburn *et al* (1985) found that plasma concentrations of etretinate were increased when administered with a high fat meal and whole milk compared to ingestion with a high carbohydrate meal or during a complete fast. In contrast, plasma levels of the 2 main metabolites remained quantitatively unaffected following any of the meals. The authors proposed that the mechanism related hereto is an incorporation of etretinate in the lipid portion of the bile acid micelles and that these micelles pass through the

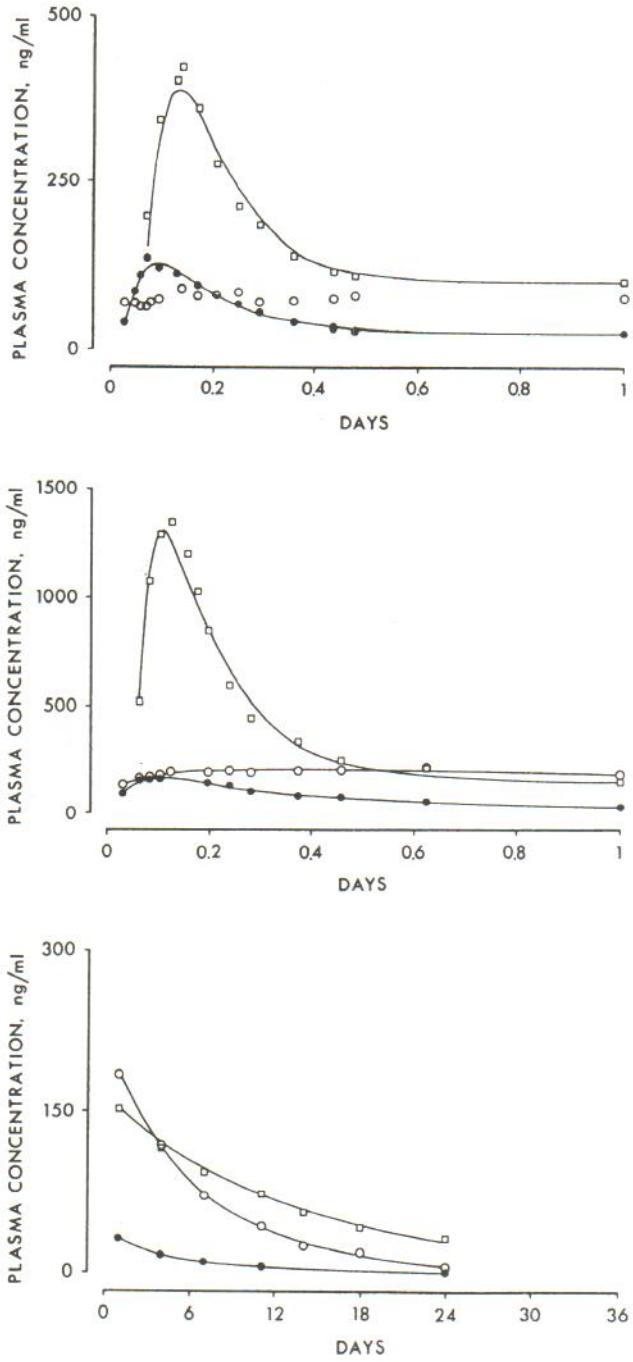

Fig.3. Time course of plasma concentrations of etretinate (-□-) and its metabolites acitretin (-●-) and 13-cis-acitretin (-○-) in a psoriatic patient after oral etretinate treatment for 1 (upper figure) and 3 (middle figure) months. Etretinate 40 mg daily was administered for the first 4 weeks, then the dosage was adjusted to 70 mg daily. The lower figure shows the disappearance of drug and metabolites from plasma after cessation of therapy. The curves represent the computer-generated curves of best fit obtained by iterative nonlinear regression analysis. The concentration-time data obtained for the 13-cis metabolite after 1 month of treatment showed irregular variations and could not be analysed pharmacokinetically (from III).

lymphatic system to the systemic blood and escape first-pass metabolism in the gut or liver.

In comparison to etretinate, spironolactone is a highly lipophilic drug that is only partially absorbed after oral administration. There is evidence suggesting that the food induced increase in AUC (oral) of spironolactone is caused by delayed gastric emptying, promoting disintegration of the tablets and improving dissolution of the drug (Sutter & Lan, 1975; Melander, 1978). It is possible that the same mechanisms may play some role to the increased bioavailability of etretinate following a meal.

3.3 Multiple Dose Studies

Paravicini *et al* (1981) reported on a study in psoriatic patients undergoing chronic therapy with etretinate 10 to 25 mg/day for more than a year (see Table III for review). The minimum plasma concentrations at steady-state were between 100 and 150 ng/ml, which was roughly an order of magnitude higher than that observed 24 hr after a single oral dose of 100 mg. The concentration-time profiles were significantly different from those was predicted from the single-dose data. The investigators concluded that the nonpredictability might be either a result of the limits of the HPLC method used or a result of a nonlinear process involved in the distribution or elimination of etretinate. Plasma concentrations in the range of 20 to 50 ng/ml was still observed 140 days after cessation of therapy. Massarella *et al* (1985) studied the pharmacokinetics of etretinate in 14 psoriatics before, during, and after 6 months of therapy. A rise in AUC and C_{max} occurred after multiple doses. The dose-normalized etretinate AUC-values after 6 months of therapy were much the same as those calculated by curve-fitting the first dose. An apparent terminal $t_{1/2}$ of about 7 hr occurred after the first dose, whereas after the last dose the $t_{1/2}$ was about 120 days. DiGiovanna *et al* (1989) followed serum etretinate levels after cessation of therapy in 47 patients with various disorders of keratinization. Detectable serum concentrations (0.05 to 1.2 mg/l) were observed up to 150 weeks after discontinuation of therapy.

Our 3 months multiple dose study (III) on etretinate was a follow-up of the patients who participated in the single dose study. Plasma samples were obtained during a 24-hr period after 1 and 3 months of treatment and finally twice weekly for a 5-week period after cessation of therapy in order to determine the terminal elimination half-life (Fig.3). In contrast to the single dose study it became evident that the 13-cis-isomer (13-cis-acitretin) appeared at levels, which allowed it to be pharmacokinetically analyzed. For etretinate the mean lag-time of 0.26 hr was only about half of that found in the single dose study, whereas the apparent mean $t_{1/2}k_a$ of 0.74 was about twice the value found in this study. Our results substantiated previously findings (II) in which acitretin as a metabolite was absorbed faster than the parent drug etretinate. The existence of a "deep compartment" is reflected in our 3 months pharmacokinetic parameters as compared to those of the single dose study by a pronounced increase in V_{area} and a much lower plasma clearance. The considerably lower clearance of etretinate as compared to acitretin (both as a parent drug and me-

tabolite) also results in $C_{min,ss}$-values which are significantly higher for the former compound (Lauharanta & Paravicini, 1982; Colburn *et al*, 1985). The mean terminal elimination half-life of etretinate of 25 days (9-41 days) was significantly longer than that derived from the single dose study, but the very high values of about 120 days reported by Massarella *et al* (1985) were not attained. The considerable variation in terminal $t_{1/2}$ corroborates previous findings from psoriatic patients after cessation of long-term etretinate therapy (DiGiovanna *et al*, 1989). The mean terminal elimination half-lives for the 2 metabolites acitretin and 13-cis-acitretin were found to be 6.5 days (4.4-8.5 days) and 15.9 days (4.5-22.8 days), respectively. However, multiple dose studies with acitretin have revealed that the terminal elimination half-life of parent drug and its 13-cis-metabolite is in the order of about 2 and 5 days, respectively (III;VI). These findings indicate that the metabolism and subsequent elimination of the 2 metabolites appears to be limited by the formation rate (Paravicini & Busslinger, 1984; Brindley, 1989). The higher degree of accumulation after 3 months of therapy of 13-cis-acitretin as compared to acitretin were also evidenced by higher terminal interval levels for the former compound.

During the initial elimination phase the plasma concentrations tend to be higher in patients who have received higher cumulative drug doses (Lauharanta *et al*, 1982). There is no correlation between drug dosage and rate of elimination (DiGiovanna *et al*, 1989), which also seems to be the case from our study (III) although only 3 patients were investigated. A definite correlation between plasma levels and therapeutic effects of etretinate have not been demonstrated.

3.4 Pharmacokinetics of Etretinate in Psoriatic Patients with Liver Fibrosis

For many drugs that are mainly metabolized in the liver plasma clearance is reduced and $t_{1/2}$ increased in patients with severely impaired hepatic function when compared with normals (Klotz *et al*, 1975; Souich & Erill, 1977). However, there are examples in the literature in which this is not true (Meredith *et al*, 1984). The likelihood of a drug showing altered pharmacokinetics in patients with impaired hepatic function may depend on several factors, such as intrinsic hepatic clearance, shunting of portal blood into the systemic circulation through intra-or extrahepatic shunts, protein binding as well as the metabolic pathway involved.

Although the liver function is generally regarded as normal in psoriatics, liver biopsies studies from psoriatics have revealed the presence of a higher incidence of pathological changes in comparison to a nonpsoriatic group (Zachariae & Søgaard, 1973; Nyfors & Poulsen, 1976). The abnormalities include fatty change, mild lobular and portal inflammation and focal hepatocellular necrosis. Mild fibrosis was seen rarely. Because no correlation has been observed between pathological liver abnormalities and severity or duration of psoriasis, they may be more dependent upon other factors such as abnormal alcohol intake, nutritional factors and concomitant diseases.

A pharmacokinetic study has been conducted in 7 etretinate treated psoriatic patients with various degrees of liver damage (fibrosis or cirrhosis) verified by liver biopsy (IV). All possible histological changes (fatty infiltration, nuclear variability, periportal inflammation, focal necrosis, fibrosis and cholestasis) were graded according to a four step scale as follows: 1 = not present; 2 = slight; 3 = moderate and 4 = severe. Cirrhosis was judged as present or absent. The liver damage was probably caused by either previous treatment with methotrexate (3 patients), history of excessive alcohol consumption (3 patients) or both (1 patient). Five of the patients were pharmacokinetically investigated during 24 hr after both 2 and 6 weeks of treatment, whereas the remaining 2 were only investigated pharmacokinetically during a 24-hr period after chronic dosing (2-3 yr) at steady-state. Other conditions were kept similar to those in our previous studies (II,III). We found no significant changes in determined absorption and disposition pharmacokinetic parameters of etretinate in comparison to our previous studies in psoriatic patients with normal liver function (II,III). Furthermore, there were no statistically significant differences in the average pharmacokinetic parameters of absorption and disposition in patients studied 2 and 6 weeks after initiation of therapy. The average AUCcor-values after 2 and 6 weeks of treatment were comparable and in the order of 6064 and 6181 ng hr/ml, respectively. In the 2 patients studied during long-term therapy (2-3 yr) a trend towards an increase in AUCcor was observed, whereas t_{max} was relatively unaffected. There appeared to be a considerable inter-individual variation with regard to the systemic availability of etretinate, whereas the intra-individual variation was less pronounced. Furthermore, it appeared that the central compartment constituted about 8% (3-13%) and 13% (5-24%) of the calculated total volume of distribution at steady-state after 2 and 6 weeks of treatment, respectively. The mean value of 12.4 days of the apparent $t_{1/2}$-ß in patients studied during 6 weeks and 20 days in patients studied after long-term therapy are within the range previously found by us (III). However, our experimental design did not allow a definite determination of terminal disposition rates for parent drug or its two metabolites.

The metabolite acitretin showed definitely shorter lag-times and half-lives of appearance in all experiments as compared with etretinate. This is in accordance with our previous findings (II;III) and appears to support the assumption that a presystemic biotransformation occurs in the gut or gut wall, even though a first-pass biotransformation cannot be excluded. The AUCcor-values after 2 and 6 weeks of therapy were comparable (mean 3147 and 2655 ng hr/ml, respectively). Also, after both 2 and 6 weeks of therapy and in the two patients investigated during long-term etretinate therapy the AUCcor-values for the 13-cis-metabolite were significantly higher than those of acitretin. This is in agreement with our previous findings (III) that 13-cis-acitretin constitutes the main metabolite during long-term etretinate therapy.

The patients selected for our study varied widely in severity of liver damage, but it was not possible to perform any correlation between degree of liver damage and absorption and disposition pharmacokinetic parameters.

Table IV. *Pharmacokinetics of Acitretin Reported in Single Dose Studies*

Reference	No of Pat.	Acitretin				13-cis-Acitretin (metabolite)		
		Dose (mg)	C_{max} (ng/ml)	t_{max} (hr)	$t_{1/2}$-ß (hr)	C_{max} (ng/ml)	t_{max} (hr)	$t_{1/2}$-ß (hr)
Larsen *et al*, 1987	(a) 6 (Pso)	40	241 (98-526)	1.9 (0.6-3)	5.5 (3.3-8.0)			
McNamara & Jewell 1987	(b) 18 (He)	50	245 (87-480)	2.8 (2-5)	6.7	21	22	60
McNamara *et al*, 1988	(c) 18 (He)	50	416 (196-728)	2.7 (2-5)	2.8	45	10	50
Stuck *et al*, 1989	(d) 6 (He)	50	418	3.3	2.6	52	4.0	(f)
	(e) 6 (CRF)	50	248	1.8	(f) 2.3	50	7.3	(f)

Abbreviations:

(CRF) Patient with chronic renal failure on hemodialysis. Other abbreviations as stated in Table II.

(a) Capsules (4 × 10 mg) ingested with 250 ml of water.

(b) Capsules (2 × 25 mg) ingested with 240 ml of water.

(c) Capsules (2 × 25 mg) ingested with a standard breakfast.

(d) Capsules (2 × 25 mg) ingested with a glass of water immediately followed by a breakfast.

(e) Capsules (2 × 25 mg) ingested with a glass of water and breakfast at 30 min.

(f) $t_{1/2}$ represents the initial decline in plasma concentrations of acitretin. The elimination half-life could not be calculated because the plasma levels after 24 hr were below or close to the assay limit.

3.5 Storage of Etretinate in a "Deep Compartment"

As previously mentioned, etretinate is sequestered into adipose tissue. During treatment with etretinate for 1 month the drug concentrations in subcutis increased rapidly reaching values several times higher than those in plasma (Rollman & Vahlquist, 1983). Following long-term treatment (several years) the lipid depots apparently become saturated since the levels of etretinate did not exceed 16 µg/g. Assuming a total mass of body fat of 10 kg and an uniform distribution of the drug in the tissue, the amount of etretinate stored in adipose tissues would be about 100 mg after a few months of treatment (Rollmann & Vahlquist, 1983). Thus, even though an increased fat catabolism occurred for one reason or another it would be rather unlikely to expect toxic levels of etretinate in plasma, even if the drug is released from the lipid depots in a very short time.

In contrast to the subcutis, the epidermis does not accumulate etretinate progressively and cessation of therapy is followed by a rapid disappearance (Rollman & Vahlquist, 1983). From an autopsy study it was concluded that etretinate accumulated in at least three compartments, namely fat, adrenals and liver (intermediate concentrations to that in other tissues) (Vahlquist *et al*, 1986). Liver biopsy samples obtained at the end of a 6 months daily dosing revealed concentrations comparable to those in plasma (Paravicini & Busslinger, 1984). Concentrations in suction blister fluid (Kiistala, 1968) determined after 6 weeks of therapy showed significantly lower levels (but equal) of both etretinate and the metabolite acitretin as compared to plasma (Lauharanta & Paravicini, 1982).

4. PHARMACOKINETICS OF ACITRETIN

4.1 Introduction

Acitretin is also a highly lipophilic compound and is mainly bound to albumin in plasma (Vahlquist *et al*, 1981; Carillet *et al*, 1990; Urien *et al*, 1992). Because it contains a comparatively polar carboxylic acid group (pK_a 3.7) acitretin is less likely to be sequestered in adipose tissue and one might therefore expect it to be cleared from the body more rapidly. The metabolic pathway has been previously described (see "Pharmacokinetics of Etretinate"). Following intravenous administration of acitretin to healthy volunteers, the mean recovery in excreta accounted for 93% of the dose within 18 days; 53% was recovered in urine and 40% in feces, respectively. Approximately 84% of an oral dose was recovered within 18 days, 37% in the urine and 47% in the feces (Brindley, 1989; Williams, 1990). Acitretin has been found to exhibit a considerable shorter terminal half-life than etretinate. Several clinical trials has revealed a comparable clinical efficacy of the two compounds. Acitretin has, therefore, recently replaced etretinate in some European countries.

4.2 Single Dose Studies

Single-dose pharmacokinetics were examined following intravenous infusion of acitretin 19 mg administered as a mixed micelle solution (Brindley, 1989; Brindley *et al* (Hoffmann La-Roche, data on file 1987)). The same 12 healthy volunteers were later also given single 50 mg oral capsule formulation and an aqueous microsuspension. Following the intravenous dose, peak concentrations of parent drug (694 to 1258 ng/ml) appeared to decline biexponentially and fell below the 4 ng/ml detection limit of the HPLC assay within 14 hr. By contrast, unchanged drug could be detected for at least 14 hr after oral dosing. From patients with chronic end-stage renal failure in hemodialysis, the mean AUC of acitretin and its metabolite were about 50% lower as compared to healthy volunteers (Stuck *et al*, 1989). Since patients in hemodialysis were taking other medications (aluminum hydroxide, antihypertensives, antithrombotic drugs), a decreased bioavailability caused by an impaired absorption of acitretin might account for lower plasma levels.

We have performed a pharmacokinetic study in 6 psoriatics after a single oral ingestion of 40 mg (4 x 10 mg) acitretin in a gelatine capsule formulation (II). Patients fasted overnight prior to drug administration and up to 3 hr after. As judged from pharmacokinetic parameters related to the absorption phase (lag-time, t_{max} and $t_{1/2}k_a$), acitretin seemed to be absorbed faster than etretinate in the fasting state (Table IV). The AUCcor-values revealed that a 5-fold interindividual variation existed with regard to the systemic availability of acitretin. Incomplete absorption is probably the most compelling explanation for the large variation in bioavailability (Brindley, 1989). After distribution, about 45% of acitretin resided outside the initial distribution volume. In case of etretinate a tendency towards a higher fraction residing outside the initial distribution volume probably coincides the higher lipophilicity of this compound. Assuming a systemic availability of 40%, a considerably variation in plasma clearance was observed (58-303 ml/kg/hr). However, our apparent mean value of 176 ml/kg/hr was in accordance with previously results obtained after intravenous administration indicating that acitretin is a "low clearance drug" and that hepatic first-pass metabolism does not make a significant contribution to the decreased bioavailability of acitretin (Brindley, 1989).

The apparent mean terminal elimination half-life for acitretin in the 24 hr study was 5.5 hr (3.3-8.0 hr). The terminal elimination half-life could not be determined from the single dose study because of low terminal plasma concentrations precluding this phase to be visualized. The 13-cis-metabolite in our study could only be demonstrated at levels of 1-2 ng/ml. However, this metabolite has been found at higher levels both in healthy volunteers and in patients with chronic end-stage renal failure after a single oral dose (McNamara *et al*, 1988; Stuck *et al*, 1989).

4.2.1 Influence of Food on the Absorption of Acitretin. Eighteen healthy volunteers received 50 mg oral doses of acitretin during a complete fast or following a moderate breakfast (McNamara , 1988). A mean increase of 90% of AUC for acitretin was observed when administered with food. The apparent t_{max} for acitretin was unaffected by food suggesting that the rate of absorption was unaffected. Furthermore, the rate of absorption and formation of 13-cis-acitretin appeared to be constant in the presence of food. Importantly, concomitant intake of food reduced the interpatient variation in AUC from 40% in the fasting state to 28%. It is currently recommended that acitretin should be administered with food.

4.3 Multiple Dose Studies

Paravicini *et al* (1985) was the first to report a multiple-dose pharmacokinetic study of acitretin in 11 psoriatics (Table V). The disposition of acitretin involved an initial rapid distributive phase ($t_{1/2}$ range 1.4 to 4.3 hr) followed by a slower elimination phase ($t_{1/2}$-values between 33 and 60 hr). The 13-cis-metabolite declined monoexponentially ($t_{1/2}$-values between 53 and 99 hr) almost in parallel with the parent drug.

Multiple dose pharmacokinetics has also been studied in healthy volunteers receiving acitretin for 2 weeks (Brindley, 1989). The plasma disposition appeared to be biexponential with an observed initial $t_{1/2}$ of 2 to 6 hr and a terminal elimination $t_{1/2}$ of about 50 and 60 hr for the parent compound and the 13-cis-metabolite, respectively. In accordance to the elimination $t_{1/2}$-values for drug and metabolite, approximate steady-state trough plasma concentrations were reached within about 7 and 10 days. No significant correlation was found between steady-state plasma levels of acitretin or 13-cis-acitretin and the body weight of patients treated with acitretin. Furthermore, comparison of the concentration-time data course for young and elderly subjects indicated that plasma concentrations were higher in the elderly group. However, similar terminal elimination half-lives for drug and metabolite were found in both groups (Williams, 1990).

The more rapid clearing of acitretin from plasma, as compared to etretinate, has been substantiated by Berbis *et al* (1988a). Thirty days after cessation of a 2-7.5 months treatment period, the residual plasma levels were in all cases below the detection limit of the HPLC assay (2 ng/ml). In 2 patients a residual concentration of 13-cis-acitretin was still present.

Following administration of 13-cis-acitretin to 3 healthy volunteers appreciable levels of acitretin were detected in the plasma (Geiger & Brindley, 1988). The AUC-ratio of cis to trans isomers was approximately 10. Plasma levels of both compound declined in parallel, resulting in similar half-lives of about 66 hr. This study demonstrated that 13-cis-acitretin and acitretin are interconverted following oral administration. Glutathione, the most prevalent intracellular thiol, might play an important role in the interconversion of acitretin and 13-cis-acitretin (Jewell & McNamara, 1990). Studies in etretinate and acitretin treated irradiated (UVB and UVA) hairless mice gave evidence for a 13-cis-isomerization occuring in epidermis (Berne *et al*, 1990).

We have performed 3 multiple dose pharmacokinetic studies on acitretin and its main metabolite, 13-cis-acitretin (III;VI;VII). The concentration-time data for the metabolite 13-cis-acitretin exhibited a slow, minor and postponed con-

Table V. *Pharmacokinetics of Acitretin Reported in Multiple Dose Studies*

Reference	No of Pat.	Acitretin				13-cis-Acitretin (metabolite)		
		Dose (mg per day/time)	C_{max} (ng/ml)	t_{max} (hr)	$t_{1/2}$-ß (hr)	C_{max} (ng/ml)	t_{max} (hr)	$t_{1/2}$-ß (hr)
Paravicini *et al*, 1985	(a) 11 (Pso)	50/2 mo	306	3.5	50 (33-60)	168	4.3	75 (53-99)
Larsen *et al*, 1988	(b) 4 (Pso)	40-70/3 mo	(229-408)	2.0 (1-2.8)	58 (36-94)			90 (28-123)
Larsen *et al*, 1990	(c) 12 (Pso)	30/6 mo		2.3 (1.0-3.5)	47 (17-111)			119 (37-249)
Larsen *et al*, 1993	(d) 10 (Pso)	30/3 mo	240 (149-366)	3.1 (1.4-4.1)	192 (24-610)			185 (36-617)

Abbreviations:

(a) No information about concomitant intake of water or food. In 5 patients, plasma concentrations were determined only on cessation of therapy and for a 19-day period to cover the entire elimination phase.

(b) Capsules ingested together with 250 ml of water.

(c) Capsules ingested together with breakfast.

(d) Capsules ingested together with breakfast. In 7 out of 10 patients etretinate was found to be a metabolite of acitretin.

centration increase in dose intervals after acitretin administration. Pharmacokinetic analysis was only appropriate from the concentration-time data obtained during the terminal disposition phase.

The first study was a follow-up of the patients who participated in our single dose study (II). The experimental conditions were similar to those reported for etretinate (section 3.3). The mean apparent absorption half-time ($t_{1/2}k_a$) of acitretin after 1 and 3 months of treatment (0.77 and 0.74 hr, respectively) appeared to be twice the value found in the single dose study whereas the mean lag-time of 0.26 hr after 3 months was only half of the value obtained in this study (Fig.4). The maximum plasma concentrations occurred within about 0.8-2.8 hr and the mean value after 3 months was similar to that found in the single dose study. During therapy for 3 months, the mean half-time of the distributive α-phase of disposition seemed to be increased by about 40-60%. This could, however, be caused by the determination of too short terminal elimination half-lives involving the distributory phase. The percentual part of acitretin residing outside the central compartment (V_c) constituted about 68-88% of the calculated total volume of distribution at steady-state (V_{ss}), which is markedly increased compared to our single dose study. The mean terminal elimination half-life of 3.8 days for 13-cis-acitretin was about 1.5 times higher than for the parent drug (mean 2.4 days).

Our second multiple dose study was designed to evaluate the pharmacokinetics of acitretin and its 13-cis-metabolite during treatment for 6 months in 12 psoriatic patients (VI). The main purpose was to describe the terminal elimination phase for both parent drug and its 13-cis-metabolite. Acitretin (3 x 10 mg) in a gelatine capsule formulation was ingested together with breakfast. The patients were investigated pharmacokinetically during 24 hr after 1 and 6 months of treatment. Finally, a decline in plasma concentrations of both mother substance and its 13-cis-metabolite were followed for 56 days after cessation of therapy. Compared to our previous study (III) in which acitretin was taken only together with water, we observed no significant changes in mean values of t_{max} and $t_{1/2}k_a$ when acitretin was ingested with food. This suggests that the rate of absorption was unaffected by food and corroborates findings by McNamara *et al* (1988). As judged from t-lag, t_{max}, and $t_{1/2}k_a$ there were no statistically differences in the average absorption rate after 1 and 6 months of treatment. However, a considerable inter- and intraindividual variation existed. The mean AUCcor and the mean $\bar{C}_{ss}$ for both acitretin and 13-cis-acitretin showed a slight, but insignificant increases during therapy. Both after 1 and 6 months of therapy the mean values of AUCcor and $\bar{C}_{ss}$ for 13-cis-acitretin were higher than those for the parent compound. The mean terminal elimination half-lives for acitretin and 13-cis-acitretin were 47 hr (17-111 hr) and 119 hr (37-249 hr), respectively. Taken together, these findings suggest a higher degree of accumulation of 13-cis-acitretin as compared with acitretin. However, both acitretin and 13-cis-acitretin accumulate to a lesser degree in adipose tissue than does etretinate (Rollman & Vahlquist, 1983; Larsen *et al*, 1992).

Until recently the hydrolysis of etretinate to acitretin was considered non-reversible (Fig.2). However, preliminary results from a European Multicenter Trial gave evidence of a possible metabolic formation of etretinate from acitretin. Of 59 acitretin treated patients, 12 had etretinate concentrations higher than 5 ng/ml, 24 showed etretinate traces and 23 patients had no detectable etretinate concentrations (Wiegand

et al, 1991). Thus, we undertook a pharmacokinetic study with the aim of determining the concentration levels of etretinate as a metabolite in 10 psoriatic patients after long-term acitretin therapy (VII). Patients were receiving 30 mg (3 x 10 mg) acitretin daily for a period of 3 months. The gelatine capsules were ingested together with breakfast. The patients were investigated pharmacokinetically during 48 hr at cessation of therapy and decline in plasma concentrations of both mother substance and its metabolites were followed for 64 days after cessation of therapy (Fig.5). We detected etretinate in 7 out of the 10 patients ($\bar{C}_{ss}$ range 3 to 57 ng/ml). The metabolic formation and the pharmacokinetic parameters of etretinate is discussed in section 4.4.

Compared to our earlier studies (III,VI) the absorption process for acitretin appeared to proceed at a slower rate as evidenced by a longer mean t_{max} and $t_{1/2}k_a$ (3.1 and 1.9 hr, respectively). However, a considerable inter-individual variation existed. The distributive α-phase of acitretin showed half-lives in accordance to those obtained in previously studies (III,VI). The AUCcor-values for acitretin and 13-cis-acitretin were similar (mean 4354 ng hr/ml and 4720 ng hr/ml, respectively) and did not differ significantly from those reported earlier (VI). After cessation of therapy, the elimination rate of both acitretin and 13-cis-acitretin seemed to be related to the observed mean steady-state level of etretinate. This was evidenced by longer terminal $t_{1/2}$ in patients with high levels of etretinate in plasma.

Similar to etretinate, a definite correlation between plasma levels and therapeutic effects of acitretin have not been demonstrated. We have suggested, that during long-term administration of etretinate or acitretin monitoring of drug and metabolite plasma concentrations should be performed in conjunction with the routine control of therapeutic effects and possible toxic side effects, with the aim of obtaining a better basis for correlating these measures (Larsen *et al*, 1992).

4.4 Metabolic Formation of Etretinate from Acitretin

Chou *et al* (1991;1992) observed that simultaneous administration to rats of a single oral dose of acitretin, with varying amounts of ethanol, showed a dose-dependent formation of etretinate. Furthermore, etretinate was formed *in vitro* from acitretin in both rat and human liver supernatant. Etretinate was detected after incubation with and without ethanol addition, but not when enzymes were degraded by heating to 100°C for 10 min. Comparison of acitretin and etretinate levels in rat portal and jugular vein plasma following ethanol administration indicated that etretinate was formed mainly systemically, rather than during absorption (Chou *et al*, 1992).

As previously mentioned, we detected etretinate in 7 out of 10 psoriatics treated with acitretin for a period of 3 months (VII) (Fig.5). The consumption of alcohol appears to be an important contributing factor for the formation of etretinate, as evidenced by a relatively high $\bar{C}_{ss}$-values of etretinate (51 and 57 ng/ml) in 2 patients with an excessive alcohol intake during the course of therapy. The terminal elimination half-lives of etretinate were in the range of 27 to 59 days (mean 46 days), which is in accordance to what we previously found

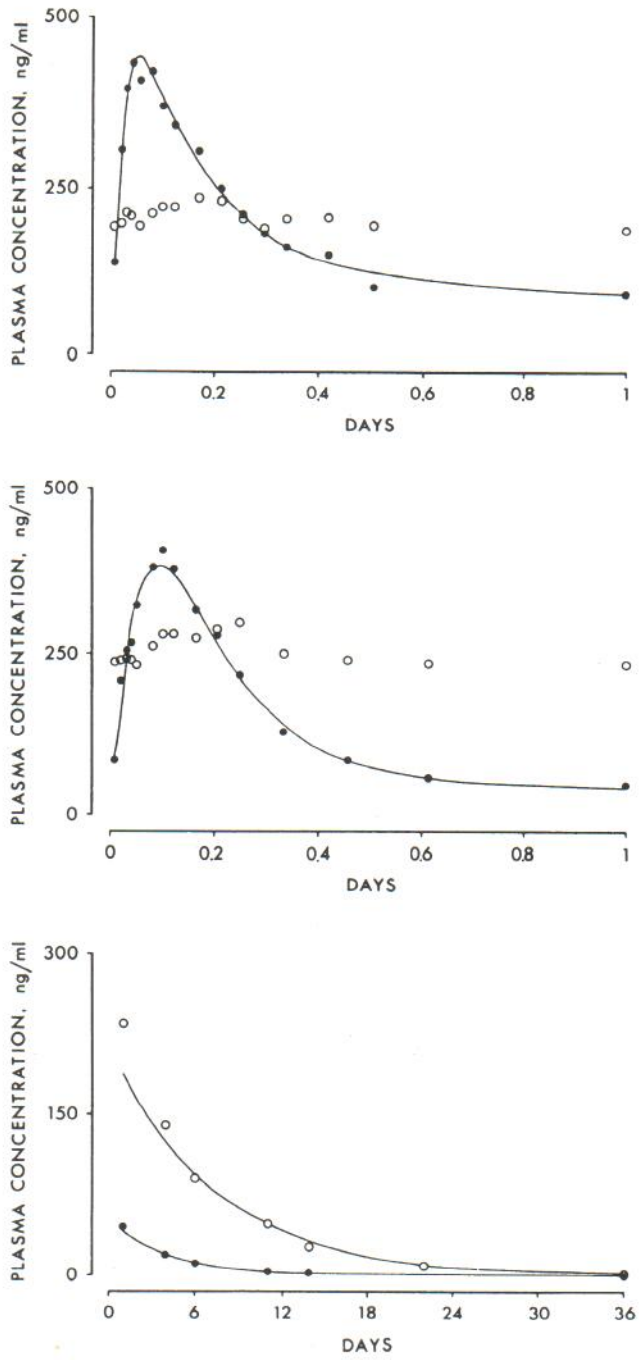

Fig.4. Time course of plasma concentrations of acitretin (–●–) and its metabolite 13-cis acitretin (–○–) in a psoriatic patient after oral acitretin treatment for 1 (upper figure) and 3 (middle figure) months. Acitretin 40 mg daily was administered for the first 4 weeks, then the dosage was adjusted to 60 mg daily. The lower figure shows the disappearance of drug and metabolite from plasma after cessation of therapy. The curves represent the computer-generated curves of best fit obtained by iterative nonlinear regression analysis. The concentration-time data obtained for the 13-cis metabolite after 1 and 3 months of treatment showed irregular variations and could not be analysed pharmacokinetically (from III).

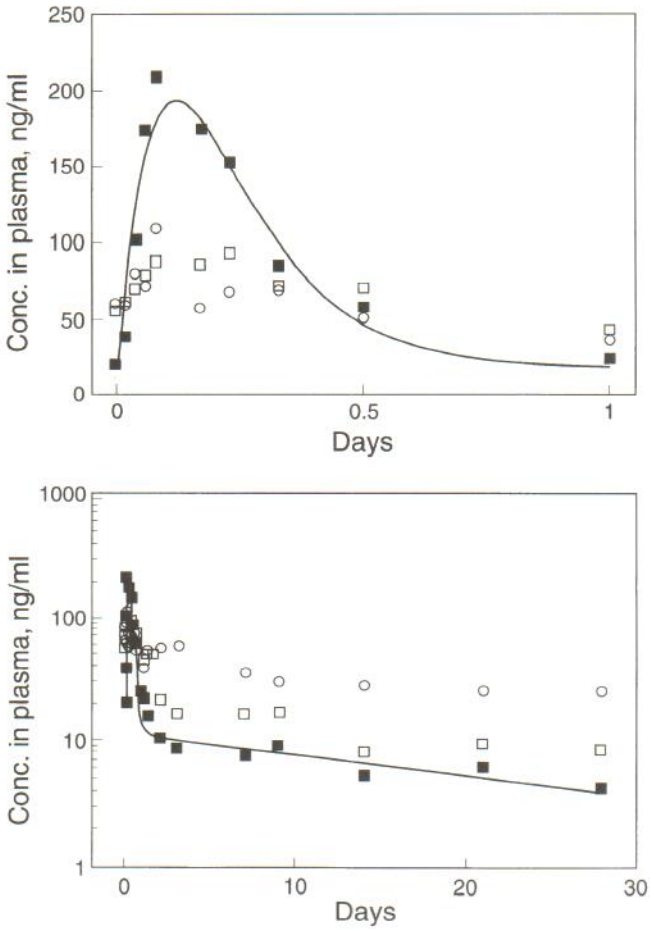

Fig 5. Time course of plasma concentrations for 24 hr of acitretin (-■-) and its metabolites 13-cis-acitretin (-□-) and etretinate (-○-) in a psoriatic patient after treatment with a daily oral dose of acitretin 30 mg for 3 months (upper figure). The lower figure shows the concentration courses of drug and metabolites from plasma after cessation of therapy. The curves represent the computer-generated curves of best fit obtained by iterative nonlinear regression analysis. The concentration-time data found for etretinate and 13-cis-acitretin showed irregular variations and could not be analysed pharmacokinetically (from VII).

(III), but somewhat less than the about 120 days reported by Massarella *et al* (1985).

When etretinate was administered by intragastric infusion throughout organogenesis in the mouse (day 8-15) mean etretinate concentrations of 6.5 ng/ml in maternal plasma resulted in retinoid-specific defects (Löfberg *et al*, 1990). In our study, 4 of 10 patients showed "teratogenic levels" of plasma etretinate and 2 patients had levels just below (4.4 and 5.9 ng/ml).

Although the enzyme(s) involved in the esterification of acitretin to etretinate remain(s) obscure at present a possible mechanism can be suggested. Miller & DeLuca (1985) showed that all-trans-retinoic acid is metabolized to ethyl retinoate in rat liver microsomes and that the production is greatly stimulated by the addition of acetyl coenzyme A (CoA), suggesting the formation of a retinoic acid-coenzyme A intermediate (retinoyl-coenzyme A). Ethanol metabolism includes formation of acetate and may increase the concentration of CoA. Thus, if a low concentration of CoA is rate-limiting in the metabolic formation of etretinate, ethanol metabolism could increase the velocity by enhancing CoA formation. A similar way of ethanol-induced increase in acetylation is known to take place with other drugs (i.e. sulphadimidine). (Olsen & Morland, 1978).

Lange *et al* (1981) and Laposata *et al* (1987) described the formation of ethyl fatty acid esters in various organs in response to ethanol. From post-mortem samples these esters were also found in human tissues, including adipose tissue (Laposata & Lange, 1986). The observation is important because this product is specific for ethanol (Bjorntorp *et al*, 1990). Also, adipose tissue contains the highest concentration of free fatty acids compared to any other organ, providing a high substrate level for the formation of ethyl fatty acid esters. In rabbit myocardium two soluble enzymes were discovered that catalyzed the formation of fatty acid ethyl esters from free fatty acid and from ethanol at physiological concentrations of both (Mogelson & Lange, 1984).

4.5 Tissue Levels of Acitretin

Recently, epidermal shave biopsies and subcutaneous fat samples have been obtained from psoriatics undergoing therapy with acitretin. The acitretin concentrations in subcutis varied from 15 to 1437 ng/ml, but the mean values at 1 and 6 months of therapy showed only a minor and insignificant increase in the values of mean concentrations (177 and 227 ng/g) (Larsen *et al*, 1992).

Similar to etretinate (Lauharanta & Paravicini, 1982), relatively low levels of both parent drug and metabolite were detected in suction blister fluid at steady-state (VI). The explanation for this finding might be that acitretin and 13-cis-acitretin are extensively bound to serum albumin and only minor proportions ($<0.1\%$) are free to diffuse outside the vascular space (Brindley, 1989). With regard to 13-cis-acitretin, we observed a highly significant correlation between plasma and blister fluid levels, whereas this was not the case with acitretin.

Importantly, acitretin and 13-cis-acitretin are transferred into breast milk (Rollman & Pihl-Lundin, 1990). Although, the estimated amount of the drug consumed by the suckling infant corresponded only to about 1.5% of the maternal dose, the authors warned against the use of acitretin in breast-feeding women.

There is evidence that many organic compounds enter saliva by a passive diffusion process, for which the lipid solubility and the degree of ionization are important factors (Danhof & Breimer, 1978). For many drugs saliva concentrations appear to be similar to the unbound concentrations in plasma. This might explain our findings that drug and metabolite levels at steady-state in saliva were below 1 ng/ml (VI).

5. METHOTREXATE AND ETRETINATE IN COMBINATION

5.1 Introduction

Since its introduction by Farber *et al* (1948) for the treatment of acute leukemia and demonstration of the dramatic resolution of uterine trophoblastic tumors (Li *et al*, 1956), methotrexate (MTX) has been employed in cancer chemotherapy. Gubner *et al* (1951) first used the closely related compound aminopterin (differs from MTX by not being methylated at the N-10 position) to treat psoriasis. Since then sodium methotrexate has been established as a potent anti-psoriatic agent (Roenigk *et al*, 1969; Nyfors & Brodthagen, 1970).

Although, studies with cancer patients have shown complete absorption of small oral MTX doses (<30 mg/m^2) (Henderson *et al*, 1965; Huffman *et al*, 1973), the absorption in psoriatics of even smaller MTX doses is significantly impaired and a great inter-patient variation exists (Hendel *et al*, 1982). There is also an increased incidence of malabsorptive enteropathy in psoriatic patients (Hendel *et al*, 1982). At doses used in psoriasis, intramuscular administration results in rapid and complete absorption (Halprin *et al*, 1971; Edelman *et al*, 1984). Usually higher and more prolonged serum levels are observed compared to oral administration of the same dose (Freeman-Narrod *et al*, 1975). MTX metabolism occurs probably mainly in the gastrointestinal tract or enterohepatic circuit. MTX is excreted primarily as the unchanged drug by the kidneys through a combination of glomerular filtration and active tubular transport (Bleyer, 1978). Only 1-2% of an intravenously administered dose is excreted in the stool as the parent compound and metabolites (Huffman *et al*, 1973).

The principal toxicities with MTX are hepatitis, myelosuppression and gastrointestinal mucositis. The MTX-induced liver damage, will in some patients lead to fibrosis or cirrhosis following chronic therapy (Weinstein *et al*, 1973, Nyfors, 1980; Zachariae *et al*, 1980). Toxicity leading to cirrhosis appears to be the result of total cumulative dose of the drug and not the duration of therapy (Weinstein *et al*, 1973). The biochemical basis for the potential hepatotoxicity is unknown, but depletion of erythrocyte folate has been proposed (Hendel *et al*, 1985). However, Zachariae *et al* (1987) found no significant differences of erythrocyte folate between patients with progression in liver fibrosis or with cirrhosis and patients with no progression.

5.2 Combined Therapy with Methotrexate and Etretinate in Relation to Psoriasis

Several investigators have described the benefits of combined MTX and etretinate therapy in resistent cases of psoriasis vulgaris (van der Veen, 1982; Adams, 1983; Tuyp & MacKie, 1986). It also appears as pustular psoriasis (Rosenbaum & Roenigk, 1984) and Reiter's disease (Zachariae, 1987) respond to this modality. However, Zachariae (1984, 1988) has warned against the combination due to the development of a severe toxic hepatitis in 2 out of 10 treated patients, whereas this condition has not been demonstrated in any of 531 patients on MTX alone (Zachariae, 1988). This clinical observation lead to assumption of a potentially dangerous drug interaction.

5.3 Methotrexate and Etretinate Pharmacokinetics During Combined Treatment

In a pharmacokinetic study, Harrison *et al* (1987) studied the metabolism of MTX in a single psoriatic patient before and during etretinate therapy. Following intravenous infusion plasma MTX rose during combined therapy from 0.05 - 0.07 mmol/l to 0.11 mmol/l, a concentration which might be potentially toxic.

We studied the influence of etretinate on the pharmacokinetics of MTX in 6 psoriatic patients treated for more than 2 years with combined therapy (V). The control patients comprised psoriatics receiving only MTX therapy and were matched with regard to gender, age, weight, duration of MTX therapy, and creatinine clearance. Before entering the study patients from both groups received methotrexate weekly in divided oral doses of 5 to 7.5 mg three times with 12-hr intervals. In addition, the absorption and disposition pharmacokinetic parameters of etretinate were calculated. Following intramuscular administration of 0.2 mg/kg MTX shorter t_{max} (mean 0.7 and 1.3 hr, respectively), higher C_{max}-values (mean 992 and 721 nmol/l, respectively), and shorter half-lives of pharmacokinetic parameters related to the absorption (mean 0.22 and 0.58 hr, respectively), and distributory (mean 1.5 and 1.8 hr, respectively) process were obtained for patients during combined treatment with etretinate as compared to controls (Fig.6). The differences between C_{max} were statistically significant. Mean values of the distributive α-phase, apparent volume of distribution at steady-state (V_{ss}), total body clearance, and terminal elimination half-life ($t_{1/2}$-ß) were lower during combined treatment with etretinate

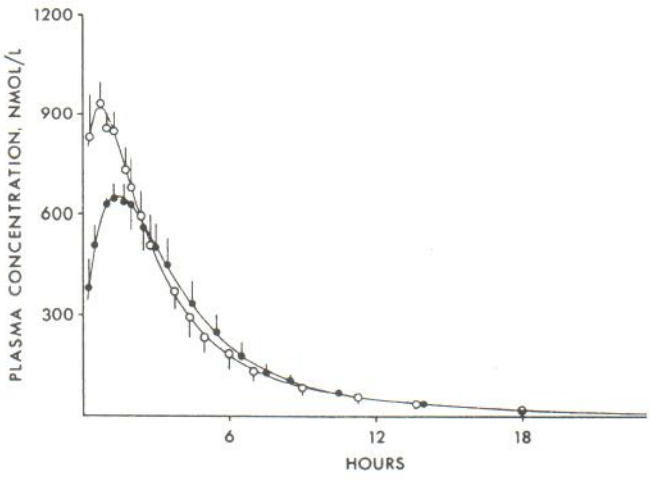

Fig.6. Mean plasma methotrexate concentrations over 24 hr in 6 psoriatics treated with both methotrexate and etretinate (–○–) and in 6 control patients treated only with methotrexate (–●–). The curves represent the computer-generated curves of best fit obtained by iterative nonlinear regression analysis (from V).

compared to controls, but this difference was statistically insignificant. Our mean values of $t_{1/2}$-ß of 5.3 and 7.5 hr, respectively, appeared slightly longer than the 4.7 hr observed by Kamel *et al* (1988) also after intramuscular administration to psoriatics.

Following intraveneous administration 58-92% of the MTX dose (0.1-10 mg/kg) was excreted in the urine within the first 24 hr (Henderson *et al*, 1965), which is in good accordance to the about 63-97% found in our study after intramuscular administration. Drugs that interfere with renal excretion of MTX may be potentially dangerous. Salicylate, probenecid and other weak organic acids diminish renal tubular transport of MTX (Liegler *et al*, 1969; Bourke *et al*, 1975), and an increased incidence of leucopenia has been reported during combined aspirin and MTX therapy (Mandel, 1976). Also, MTX might precipitate in acidic urine. As judged from the molar percent recovery of MTX in the urine, renal interference between MTX and etretinate seems unlikely. Drug interactions with MTX may possible also take place through displacement from binding sites on plasma proteins (Liegler *et al*, 1969) or by interference with the enterohepatic circulation of MTX by inhibition of bowel bacteria and suppression of MTX metabolism (Shen & Azarnoff, 1978). There is no evidence of such interactions between MTX and etretinate.

We found no differences in the average absorption and disposition pharmacokinetic parameters of etretinate and its two metabolites acitretin and 13-cis-acitretin as compared to psoriatics undergoing long-term etretinate therapy (III). However, the mean terminal elimination half-life of 108 days was prolonged compared to previously results (III,V) but in accordance with the about 120 days found by Massarella *et al* (1985).

In conclusion, our findings of increased C_{max} and unchanged total body clearance and terminal elimination half-life of MTX during combined treatment with etretinate indicate that the reported increased risk for developing hepatotoxicity cannot be related to drug accumulation due to pharmacokinetic interaction. However, a potentially hepatotoxicity due to elevated peak MTX level cannot be ruled out.

6. CONCLUSIONS AND FUTURE STUDIES

6.1 Conclusions

The pharmacokinetics of etretinate and acitretin have been discussed in this thesis. Our data combined with the results of others provide the basis for the following conclusions:

1. The reversed-phase HPLC method is a simple and sensitive method for determining plasma levels of etretinate, acitretin and 13-cis-acitretin.
2. Acitretin is absorbed faster after oral ingestion than etretinate, which is partially hydrolysed to acitretin, possibly in the gut or gut wall. Considerable inter-individual variations exist with regard to the systemic availability of the two drugs.
3. Following long-term etretinate therapy the two main metabolites in plasma are acitretin and 13-cis-acitretin. After long-term acitretin therapy the corresponding 13-cis-metabolite occurs as the main metabolite.
4. Etretinate accumulates in adipose tissues from where it is only slowly released following cessation of therapy.
5. The terminal elimination half-life of etretinate (up to 120 days or more) is considerable longer than for acitretin (2-3 days). The metabolism and subsequent elimination of acitretin and 13-cis-acitretin as metabolites of etretinate, appears to be limited by the formation rate.
6. Following acitretin therapy suction blister fluid concentrations of both parent drug and metabolite are lower than plasma concentrations. Saliva concentrations were below 1 ng/ml at steady-state.
7. Absorption and disposition rates of etretinate in patients with hepatic fibrosis or cirrhosis were not significantly altered compared to results obtained from psoriatics with normal liver function.
8. Etretinate has been shown to be a metabolite of acitretin, and consumption of alcohol appears to be an important contributing factor for the formation of etretinate.
9. The metabolic formation of etretinate makes it impossible to draw any definite conclusion about the actual period during which pregnancy must be avoided following cessation of acitretin therapy. However, if acitretin therapy is undertaken, fertile women should be informed of potential teratogenicity.
10. The increased risk for developing hepatotoxic reactions during coadministration of methotrexate and etretinate cannot be explained by drug accumulation due to pharmacokinetic interaction. Influence on hepatotoxicity by the demonstrated increase in methotrexate C_{max} cannot be excluded.

6.2 Future Studies

Acitretin is metabolized to etretinate in humans and ethanol appears to be an important contributing factor (VII). Therefore, it would be of interest to describe the metabolic pathway and the organ(s) involved in the ethyl esterification of acitretin. Furthermore, it would be of importance to investigate if a similar esterification takes place with 13-cis-retinoic acid (isotretinoin). If so, the corresponding ethyl ester might have a considerable longer terminal $t_{1/2}$ than the parent drug which could influence the recommended anticonceptive period following therapy.

Etretinate and acitretin should be considered as potentially hepatotoxic drugs and further studies need to be done in order to evaluate if a potential hepatotoxicity is related to the plasma drug level.

We were unable to demonstrate any altered pharmacokinetics of etretinate and its metabolites in patients with varying degree of liver damage, as compared to patients with normal liver function (IV). However, this can not be excluded. If psoriatics with liver fibrosis or cirrhosis exhibit a reduced hepatic clearance and a longer terminal elimination phase this might possible be disclosed by following decline in plasma levels after long-term retinoid therapy. Also, pharma-

cokinetic studies with acitretin in patients with liver disease are lacking.

At present, retinoids with fewer adverse effects and more selective therapeutic activity are clearly needed. Progress during the last few years in identification of specific nuclear retinoid receptors opens new avenues of investigation of the pharmacology as well as the molecular mechanism of retinoid activity. This may lead to a more rational use of retinoids and discovery of new compounds which could be of benefit in the treatment of various skin diseases.

7. DANISH SUMMARY

Afhandlingen sammenfatter farmakokinetiske undersøgelser af 2 syntetiske retinoider etretinat og acitretin (etretin).

Vitamin A har betydning for udvikling af normal vækst, regulering af celledeling og differentiering af epithelielt væv, opretholdelse af synsfunktion samt gonadefunktionen. Mangel på vitamin A kan bl.a. medføre hudforandringer, som simulerer visse hudsygdomme, hvorfor man tidligere forsøgte at introducere stoffet som et peroralt farmakon. Ved høje doser opnåedes effekt, men subjektive (f.eks. utilpashed, hovedpine og kvalme) og objektive (f.eks. leverpåvirkning) bivirkninger umuliggjorde peroral anvendelse i terapeutiske doser. Siden er der syntetiseret retinoider med et bedre terapeutisk indeks. To af disse, etretinat og acitretin, har vist sig anvendelige i behandlingen af patienter med svær psoriasis. Begge farmaka er fedtopløselige. Under langvarig behandling ophobes etretinat i fedtvæv, hvorfra det kun langsomt frigives efter endt behandling. Da begge farmaka er potentielt fosterbeskadigende kræves idag, at kvinder i den fødedygtige alder benytter sikker svangerskabs forebyggende midler under behandlingen og mindst 2 år efter. For acitretin blev den svangerskabs forebyggende periode for nylig udvidet fra 2 måneder til 2 år, efter fund af etretinat i plasma hos acitretin behandlede patienter.

Vi har udviklet en følsom og specifik højtryks vædske kromatografisk metode (HPLC) til bestemmelse af etretinat, acitretin samt af metaboliten 13-cis-acitretin i plasma. Patienter med svær traktabel psoriasis blev behandlet med enten etretinat eller acitretin i varierende perioder (3-6 måneder). Farmakokinetiske parametre blev opnået både efter eengangs- og multipel farmakonindgift. Bedømt ud fra lag-time, tid for maximum plasma koncentration samt absorptionshastighedskonstant absorberes acitretin hurtigere end etretinat fra mave-tarm kanalen. Efter indgift af enkelt-dosis etretinat måltes metaboliten acitretin i plasma før modersubstansen tydende på en præsystemisk hydrolyse, omend omdannelse i leveren (»first pass metabolism«) ikke kan udelukkes. For begge stoffer observeredes en markant inter- og intraindividuel variation i systemisk tilgængelighed efter peroral indgift. Etretinat ($t_{1/2}$ op til 120 dage eller mere) udskiltes væsentligt langsommere end acitretin ($t_{1/2}$ = 2-3 dage) fra organismen.

Hos 7 ud af 10 psoriasis patienter behandlet med acitretin gennem 3 måneder fandtes etretinat i plasma. Ingen af patienterne havde tidligere modtaget retinoid behandling, og analytiske fejlkilder for tilstedeværelse af etretinat kunne udelukkes. Patienter med høj etretinat koncentration i plasma, havde samtidig et stort dagligt alkoholforbrug. Omdannelsesvejen fra acitretin til etretinat er hidtil ikke endeligt klarlagt. Patienter bør oplyses om risikoen ved at indtage alkohol i behandlingsperioden.

Både etretinat og acitretin udskilles overvejende gennem leveren. Vi gennemførte en farmakokinetisk undersøgelse hos 7 etretinat behandlede psoriasis patienter med varierende grad af leverskade (fibrose, cirrhose). Bedømt ud fra absorptions- og fordelings relaterede farmakokinetiske parametre fandtes ingen ændring sammenholdt med tidligere studier gennemført på psoriasis patienter med normal leverfunktion. Dog var det ikke muligt at bestemme terminale eliminationshastighed p.gr.a. etiske overvejelser, hvorfor en endelig konklusion vedrørende farmakokinetikken af etretinat hos psoriasis patienter med leverfibrose eller cirrhose ikke er mulig.

Hos patienter med svær traktabel psoriasis har etretinat eller acitretin behandling været kombineret med methotrexate (MTX). Efter flere rapporter om levertoksiske reaktioner under kombinationsbehandling gennemførte vi en farmakokinetisk undersøgelse hos psorisis patienter i langtidsbehandling med MTX og etretinat. Sammenholdt med en tilsvarende kontrolgruppe, som kun fik MTX behandling, observeredes ingen signifikant ændring i fordelings- og eliminations relaterede farmakokinetiske parametre for MTX efter intramuskulær injektion. Dog påvistes en signifikant stigning i maximale MTX koncentration (C_{max}) under kombinationsbehandling. Hvorvidt en potentiel levertoksisitet kan relateres hertil er uvist.

Inden for de sidste få år er det lykkedes at identificere intranukleære receptorer for vitamin A syre. Fremtidig forskning inden for dette område åbner mulighed for syntese af nye mere selektive og mindre toksiske retinoider i behandlingen af forskellige hudsygdomme.

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