EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions
C2 - Management of scientific committees; scientific co-operation and networks

REPORT OF THE SCIENTIFIC COMMITTEE ON ANIMAL NUTRITION ON THE SAFETY OF FUMARIC ACID

(adopted on 22 January 2003)

1. BACKGROUND

At the Standing Committee for Feedingstuffs, Germany drew the attention of the Commission on a recent scientific publication[1] relating to the toxicity of fumaric acid (E 297) when used as feed additive (preservative) in feedingstuffs for calves. The article reported about dose dependent clinical adverse effects (heart, kidney), however also death occurred.

Similar nephrotoxic signs of fumaric acid were described when used in human therapy against psoriasis (see attached literature)

Fumaric acid is currently authorised for all animal categories in all feedingstuffs without maximum level (see table below)

No. (or EC No.)	Additive	Chemical formula, description	Species or category of animal	Maximum age	Minimum content	Maximum content	Other provisions	Period of authorisation
					CFU/kg of complete feedingstuff			
E 297	Fumaric acid	$C_4H_4O_4$	All species or animal categories	-	-	-	All feedingstuffs	Without a time limit

2. TERMS OF REFERENCE

The Scientific Committee for Animal Nutrition (SCAN) is requested to consider this scientific publication and to advise the Commission on whether, in the light of that publication, fumaric acid can still be considered safe for calves, for bovines, for mammals?

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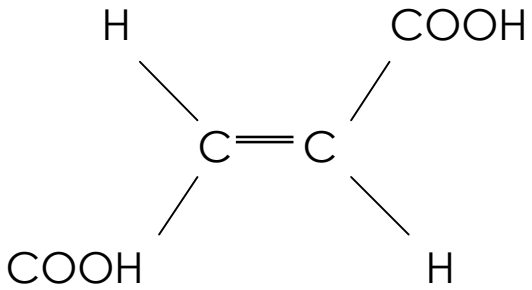
[1] Schmack K-H, 2001: Typical clinical signs associated with the addition of fumaric acid to milk substitutes. Tierärztliche Umschau 56, 411-413

3. INTRODUCTION

Fumaric acid was firstly detected in the fungus *Boletus pseudoignarius* (RUDY, 1967), isolated in 1832 by WINKLER from *Fumaria officinalis L.* and called Acide boletique. Higher amounts of fumaric acid occur in *papaveraceae* (poppy plants). *Fumaria officinalis* has played a role in human therpay since the ancient world. MESSÉGUÉ emphasizes still in 1975 the „liver healing advantages“ of *fumaria* plants.

3.1. Fumaric acid and its metabolic roles

Fumaric acid is an organic dicarboxylic acid ($C_4H_4O_4$). Fumaric acid occurs naturally in the metabolism. It plays a role in the tricarboxylic cycle (TCA cycle) and in the carry over of the amino-N from aspartate.



Succinate (acetyl-CoA and oxaloacetate are synthetised to citrate, which is - by multiple steps - converted to succinate) is oxidised to fumarate. Succinate also enters as succinyl-CoA the TCA-cycle as a conversion product of the glucogenic amino acids methionine, isoleucine and valine. The glucogenic amino acids phenylalanine and tyrosine enter the TCA cycle at the stage of fumarate.

Fumarate is further converted to malate (by saturation of the double bond). Oxidation of malate produces oxaloacetate. By transamination oxaloacetate is in a reversible equilibirum with aspartate.

Malate is also important outside the TCA cycle ((i) as a shuttle of reducing equivalents (NADH) from cytoplasm into mitochondria, (ii) it can be converted in the cytoplasm to pyruvate (NADPH source by malic enzyme) and (iii) as a precursor of cytoplasmic synthesis of fatty acids).

Aspartate is used for protein synthesis and as a precursor of the pyrimidine bases (the carbon skeleton is used for Uracil, Thymine and Cytosine) and the purine bases (Adenosine and Guanine contain the N_1 from amino-N-aspartate and another amino group from aspartate on the C_6 of Adenosine). Fumarate results from the the carry over of the aspartate-amino-N via succinate.

By the same kind of reaction fumarate also results from the urea cycle in the (periportal) hepatocytes. Citrulline and aspartate form arginino-succinate, which is in turn converted to arginine and fumarate. This reaction is reversible. Aspartate can again be resynthesised from fumarate via malate and oxaloacetate.

The urea cycle leads also to binding and excretion of bicarbonate and takes therefore part in the regulation of the acid-base-equilibrium. Urea formation is reduced if pH and/or the HCO_3^- concentration in the extracellular space decrease. The resulting increase in ammonia concentration is compensated by ammonia fixation as glutamin (in perivenous hepatocytes).

Fumarate results also from the degradation of the amino acid tyrosine (hydroxyphenylalanine). Tyrosine serves as a precursor of several syntheses (melanine (a pigment in skin and hair), dopamine and the catecholamines (hormones of the perineal glands), thyroid hormones and probably ubiquinone).

3.2. Fumaric acid as feed additive

Fumaric acid is as other organic acids (formic acid, propionic acid, citric acid) authorised for all animals in all feedingstuffs without maximum level. The organic acids are used as feed preservatives, however they show particularly in pigs also a growth promoting effect.

The first extended information on fumarid acid as feed additive was published in 1979 (Fumarsäure in der Tierernährung). KIRCHGESSNER AND ROTH refer herein to their publications from 1976 and 1978. The authors attribute a growth promoting effect to fumaric acid in piglets and fattening pigs, the optimum dosage is given with 1.5 to 2.0 % in feed. The same authors describe later on (1988, 1991) the ergotropic effects of dietary inclusion of organic acids (citric acid, fumaric acid and formic acid as well as Ca- and Na-formate) as significant improvements of growth rate and efficiency of feed utilization of young animals (piglets). The merely dietary pH lowering with an inorganic acid (ortho-phosphoric acid) failed to show a nutritive efficacy. Studies on the mode of action of organic acids indicated a higher protein and energy digestibility, a lower stomach pH and reduced levels of NH_3 and lactic acid in the stomach and duodenal digesta. Furthermore, the duodenum mainly contained a significant lower bacterial population of *E. coli* and enterococci. By this way the burden of metabolism of the host may be reduced which results in a higher overall performance.

BRUNE AND PALLAUF reported 1979 trials on calves. They added 0.3 % fumaric acid, 0.3% fumaric acid plus 0.2 % Mg-fumarate and 0.3 % formic acid, respectively, to the milk replacer amd fed these cold diets for 15 days to groups of 6 calves (51 kg bw). Effects on the health status of the calves were not observed. The digestibility of crude protein seemed to be more improved by fumaric acid and the combination of fumaric acid and fumarate compared to formic acid.

BUNTENKÖTTER (1979) classified fumaric acid as „practically non toxic“, based on LD_{50} data of 6 g/kg rat and 10 g/kg chicken.

Any adverse effects of fumaric acid in animal nutrition were not reported until 2000 (see also KIRCHGESSNER's textbook, 1997).

More recent data on fumaric acid are scarce in animal physiology or nutrition. WOLFFRAM ET AL. (1992) investigated the transport of citrate and fumarate across the pig intestinal brush-border membrane (BBM) using isolated BBM vesicles. Citrate and fumarate uptake was stimulated by an inwardly directed Na+ gradient consistent with Na+/citrate (fumarate) co-transport. Cis-inhibition and trans-stimulation experiments strongly suggest the existence of a common transport site for tri- and dicarboxylates. Uptake of tri- and dicarboxylates seems to be potential-sensitive since citrate and in particular fumarate transport was enhanced by an inside negative potential difference.

BLANK ET AL. (1999) conducted experiments to determine the effect of dietary fumaric acid supplementation (1, 2 and 3 %) and buffering capacity of the diet on ileal and fecal digestibilities of crude protein (CP), gross energy (GE), and amino acids in early-weaned pigs. The inclusion of fumaric acid to the diet with a low buffering capacity increased ($p < 0.05$) the ileal digestibilities of CP, GE, and the majority of amino acids. The highest responses were found at an inclusion level of 2 % fumaric acid. The improvements in apparent ileal amino acid digestibilities ranged from 4.9 to 12.8 percentage units. Supplementation of fumaric acid to a diet with a high buffering capacity led only to numerical increases in ileal digestibilities of CP, GE, and amino acids. In both studies, fumaric acid supplementation had no effect on the fecal digestibilities of CP, GE, and amino acids, except histidine. The authors conclude that supplementation of fumaric acid to starter diets during the first 3 to 4 weeks after weaning augments the ileal digestibilities of GE, CP, and amino acids.

3.3. Organic acids in milk replacer

The use of organic acids in calf rearing operations developed when milk replacer should be offered ad libitum at room temperatures („cold diet“) and freshly prepared only once daily or even in longer intervals. Organic acids especially formic acid were used to prevent the artificial milk from microbial or fungal spoilage. This total procedure proved to be helpful in calf operations with *E.coli* problems. Therefore the use of acidified milk replacers is widly spread in calf rearing operations in Germany.

The dosage of organic acids is adjusted so that a pH of the artificial milk between 4.8 and 4.2 is reached. For this purpose formic acid is mostly used still at present.

4. FUMARIC ACID IN THE THERAPY OF PSORIASIS

Already in 1959 the German chemist WALTER SCHWECKENDIECK detected the antiinflammatory properties of fumaric acid occuring in plants. Based on these findings the physician GUENTHER SCHAEFER developed in the 70s a psoriasis therapy and fumaric acid dosages by experience. Because fumaric acid itself is poorly absorbed after oral intake, esters (FAE) are used for treatment. Mixtures of esters are registered as drugs (Fumaderm®) in Germany (the low strength tablets contain 30 mg dimethylfumarate, 76 mg Ca-ethylhydrogen-fumarate, 5 mg Mg-ethylhydrogenfumarate and 3 mg Zn-ethylhydrogenfumarate, the high strength tablets 120 mg dimethylfumarate, 87 mg Ca-ethylhydrogenfumarate, 5 mg Mg-

ethylhydrogenfumarate and 3 mg Zn-ethylhydrogenfumarate). FAEs are almost completely absorbed in the small intestine. Dimethylfumarate is rapidly hydrolysed by esterases to monomethylfumarate, which is regarded as the active metabolite.

4.1. Clinical experience

NIEBOER ET AL. (1990) were the first who investigated the clinical effect of FAE in a double blind study. They found that a combination of monoethylfumarate and dimethylfumarate was superior to dimethylfumarate alone, whereas monoethylfumarate alone was ineffective. In a double-blind, placebo controlled study with fumarate preparations on 12 females and 27 males NUGTEREN-HUYING ET AL. (1990) could show an antipsoriatic effect of a combination of dimethylfumarate and monoethylfumarate, but not for octylhydrogenfumarate and monoehtylfumarate. In a further clinical study KOLBACH AND NIEBOER (1992) treated 196 patients with formulations as described above. In these studies the preparation of FAEs was clearly superior to dimethylfumarate alone. THIO ET AL. (1995) confirmed the results obtained in previous studies in 83 psoriasis patients treated with the commercial mixture up to 3 years. 2 further studies (ALTMEYER ET AL, 1994, MROWIETZ ET AL., 1998) with 100 and 101 patients lasting 4 months the efficacy of FAEs in psoriasis treatment was also confirmed. AMEEN AND RUSSELL-JONES (1999) reported the successful clearance of severe chronic plaque psoriasis following treatment with fumaric acid esters (FAE) in two patients who had failed previous systemic therapy. In the studies of CHRIST (1999) on 54 patients which where treated in addition with intravenous thymus extract and selenium, a faster healing rate with fumaric acid alone was shown. From these results it was postulated that the above treatments (fumaric acid, thymus extract and selenium) have a synergystic effect.

From the guidelines for therapeutic use, published in 1999 by MROWIETZ ET AL. for the German Fumaric Acid Ester Consensus Conference, it can be calculated that the fumaric acid equivalent oral intake of human patients may amount - after a period (about 8 weeks) of increasing doses - to 900 mg fumaric acid equivalent/day for another 8 week period.

4.2. Adverse effects of fumaric acid ester therapy

NUGTEREN-HUYING ET AL. (1990) reported from a double-blind, placebo controlled study with fumarate preparations on 34 patients who completed the study flushing, diarrhoea, a reversible elevation of transaminases, lymphocytopenia and eosinophilia as side effects. One patient developed a disturbance of the kidney function which normalised after discontinuation of the therapy.

Undesired adverse effects consist of gastrointestinal complaints, which occur in more than two-thirds of patients (MROWIETZ ET AL., 1998; ALTMEYER ET AL., 1996A). The symptoms vary from mild stomach upsets, increased frequency of defecation and tenesmus, to stomach cramps, tympanites and diarrhoea. These become most frequent between week 4 and 12 (ALTMEYER ET AL., 1996). Flushing is seen in about one-third of patients. Symptoms include a sudden redness of the skin and a sensation of heat lasting between a

few minutes and a few hours. Headache may be associated. Frequency of flushing is greatest at the onset of therapy and decreases with prolonged treatment time. Both adverse effects lead to drug withdrawal in about 4 % of patients (MROWIETZ ET AL., 1998).

A significant decline in lymphocyte number is observed in nearly all (94 %) psoriasis patients under FAE treatment (ALTMEYER ET AL., 1996B). Both T cells and B cells are decreased during FAE treatment (ALTMEYER ET AL., 1996B). But after drug withdrawal a steady rise in lymphocyte number up to baseline level is observed. Transient eosinophilia occurs in about 50 % of patients (MROWIETZ ET AL., 1998; ALTMEYER ET AL., 1996A).

In psoriasis patients treated with FAE up to 3 years, liver enzymes (40 % of patients), cholesterol (17 %), triglycerides (8 %), and serum creatinine (4 %) as well as proteinuria (11 %) were found increased (THIO ET AL., 1995). In other studies - but lasting (only) 4 months - significantly elevated liver enzymes have not been found (ALTMEYER E T AL., 1994; MROWIETZ ET AL., 1998).

4.2.1. Nephrotoxic properties of fumaric acid esters

HOHENEGGER ET AL. (1989) investigated the nephrotoxic actions of high single oral doses of fumaric acid monoethylester (FAME) in the rat. Fifty mg of this substance produced morphologic lesions of the glomeruli without reducing glomerular filtration rate (GFR). Following 100 mg, the lesions were more pronounced and GFR was diminished by about 40%. Despite of hemorrhages in kidney cortex the urine did not contain erythrocytes. Urinary protein was augmented in single cases only. Fifty to 100 mg FAME induced a marked concentration defect after water deprivation. In parallel FA ME reduced lactate production from glucose by kidney inner medulla in vitro. After in vivo application, however, no morphologic lesions were found in this zone of the kidney. FAME had no effect on oxygen consumption of kidney slices despite of proximal tubular lesions observed histologically after 100 mg orally. Thus, 100 mg of FAME have distinct nephrotoxic effects in the rat.

STUHLINGER ET AL. (1990) reported a case of two sisters, aged 25 and 29 years, with generalized psoriasis guttata since childhood, which developed nausea, upper-abdominal pain, loss of appetite, palpitations and flushes in the course of local and oral administration of fumaric acid esters. Because of these side effects the treatment was discontinued after about two weeks, and the symptoms disappeared. But proteinuria and haematuria were subsequently noted, creatinine concentration rose to 2.2 and 2.5 mg/dl, respectively, while creatinine clearance fell to 44 and 27 ml/min, respectively. Examination of urinary sediments and analysis of urinary proteins gave results compatible with tubular-interstitial renal damage. The abnormal renal functions and urinary findings proved reversible within three weeks.

FLIEGNER AND SPIEGEL (1992) reported a case of fully reversible tubular toxicity with consecutive metabolic osteopathy following systemic fumaric acid therapy. This secondary effect of oral fumaric acid therapy obviously occurs very rarely, never having been described before. A 46-year-old female patient with a long history of recurrent palmoplantar psoriasis underwent oral treatment with fumaric acid and its esters. Two months later, the patient started to experience arthralgia, back pain in the early hours of the morning and myalgia with increasing frequency, progressing to disablement in moving and walking and, finally, to total immobility. Not until 9 months later was the reason for these severe disabilities found: they stemmed from hypophosphataemic osteomalacia as a result of a complex disturbance of the renal tubular system. The clinical symptoms and the results of laboratory chemistry tests returned to normal as soon as fumaric acid medication was discontinued. Two attempts at reexposure confirmed the causal relationship. The authors recommend that oral fumaric acid medication should never be administered without clinical and chemical controls.

RASCHKA AND KOCH reported in 1999 a case of a 38 year old woman who was treated with fumaric acid for 5 years before she complained of fatigue and weakness. According to clinical laboratory she had developed severe proximal tubular damage. Hypophosphataemia, glycosuria and proteinuria persisted although medication was stopped immediately.

BOESKEN ET AL. (1998) focused their studies on potential adverse renal effects. 42/47 patients were observed between 3 and 70 months (mean: 16.5 months) without showing inreased kidney retention values, but 21/42 patients showed alterations in urin proteins. These alterations were transient and persisted only in 2 patients for more than 6 months. The authors conclude from their studies, that the adverse effects of fumaric acid esters are limited to transient alterations of the tubulus function without measurable restriction of the glomerular filration rate. But in case of additional tubulus poisoning (e.g. by exsiccosis, infections, treatment with drugs) an acute renal failure could result.

MROWIETZ (2000) summarized the German experience on renal dysfunctions as a result of FAE treatment: In the period between 1995 and 1999, a total of 14 reports were made concerning tubular dysfunction of the kidneys, which were described predominantly as proteinuria. In 7 cases these findings were associated with elevated serum creatinine. For the same period (1995-2000) about 10 million daily doses of commercial FAE tablets have been applied, at a mean daily dose of 2,5 tablets (corresponding to about 375 mg fumaric acid equivalent).

MROWIETZ cites BOESKEN ET AL. (1998) and repeats their conclusion, that the tublular dysfunction (proteinuria) has a transient character. MROWIETZ ET AL. (1999) point out: „older case reports documented

acute renal failure linked with FAE therapy for psoriasis. However, in subsequent studies und multicentre trials, there has been no evidence for an impairment of renal function“. The authors conclude that „FAEs do not possess nephrotoxic potential when used as recommended.”

4.2.2. Mode of Action

PETRES ET AL. (1975) found inhibition of the incorporation of ^{14}C-Thymidin, ^{14}C-Uridin, ^{14}C-Alanin and ^{14}C-Leucin into acid-insoluble biopolymers of cultivated PHA-stimulated human lymphocytes by FAME. At high concentrations of FAME (500 µg/ml culture medium) the inhibition of nucleic acid synthesis was 6 times higher on the average than the inhibition of protein synthesis. However, the application of the cis-isomer, maleic acid monoethylester (MAME), resulted in an increase of the incorporation rate of the labelled precursors into the RNA and DNA. This was 3.5 - 9.3 times higher than after application of FAME. The results demonstrate the specific inhibition by FAME. The rate of labelling of nucleic acids was decreased above 10 µg FAME/ml culture medium and in the case of MAME above 50 µg/ml medium. As an explanation of the specific action of FAME its influence on the enzymes of the nucleic acid synthesis, the citric acid cycle or a faulty synthesis of enzymes is discussed by the authors.

In psoriatic patients, purine nucleotide concentrations in skin- and blood cells are abnormal: the increase in the steady state level of cGMP and the decrease in cAMP concentrations are associated with an enhanced rate of cellular proliferation. A defective purin nucleotide synthesis pathway is suggested. KIEHL AND IONESCU (1992) found decreased ADP and ATP concentrations in blood cells ($p < 0.0001$). The stimulation of the TCA-cycle with fumaric acid (raised ATP ($p < 0.0001$) and) slowed down the purine nucleotide synthesis through end product inhibition. Both effects can inhibit DNA and protein synthesis activity which results in inhibition of cellular proliferation (KIEHL AND IONESCU, 1992). It is concluded that fumaric acid seems, therefore, a useful treatment for psoriatic lesions if liver and kidney functions (purine nucleotide and urea cycle) are controlled during treatment.

Most studies on the antipsoriatic mode of action of dimethylfumarate (DMF) focused on its antiproliferative effects in keratinocytes. Because inflammatory skin diseases are associated with an upregulation of endothelial cell adhesion molecules and because the presence of inflammatory cells in dermis and epidermis is considered an important feature in psoriasis, VANDERMEREN ET AL. (1997) tested the effect of DMF on cytokine-induced adhesion molecule expression in HUVEC. DMF inhibited ICAM-1, VCAM-1, and E-selectin expression and reduced adhesion of U937 cells to stimulated HUVEC. Monoethylfumarate and fumaric acid had no effect. Similar inhibitory effects for DMF on VCAM-1 expression were observed

after stimulation of HUVEC with LPS, PMA, IL-4, and IL-1 alpha or in combinations with TNF alpha. The authors conclude that the inhibitory effect on cytokine-induced endothelial adhesion molecule expression may represent another target of dimethylfumarate in psoriasis.

The aim of a study of HOXTERMANN ET AL. (1998) was to examine the systemic, particularly the immunological changes in patients suffering from psoriasis treated with FAE over a long period of time. The study was based on continuously recorded clinical data and laboratory parameters of 10 patients, who were treated over a period of 12 months with FAE. Leukopenia and particularly lymphopenia were found in all patients. Within the T cell fraction a stronger suppression of CD8+ lymphocytes was observed. The authors conclude from their investigations that the systemic effects of FAE demonstrate the suppressive character of this medication.

Interactions between infiltrating T cells and keratinocytes via the secretion of the TH1 cytokines interleukin (IL) 2 and interferon gamma (INF-gamma), the keratinocyte transforming growth factor alpha (TGF-alpha) and the cytokines IL-6 and IL-8 are thought to be the predominant mechanisms inducing skin lesions in psoriatic patients.

ASADULLAH ET AL. (1997) investigated the effect of monomethylfumarate (MMF) on proinflammatory (TNF-alpha, IL-12) and antiinflammatory (IL-10, IL-1RA) cytokine production by peripheral blood mononuclear cells (PBMC) and separated monocytes. In 24-h PBMC cultures from both psoriatic patients (n = 6-13) and healthy volunteers (n = 7-9), MMF at 100 µM induced secretion of TNF-alpha, IL-10, and IL-1RA. Kinetics of IL-10 protein and mRNA expression indicated de novo production. Moreover, MMF significantly augmented endotoxin-induced synthesis of TNF-alpha, IL-10 and IL-1RA. In contrast, no influence on IL-12 secretion was found. Similar effects of MMF in purified monocytes indicated these cells to be responsible for aberrant cytokine formation. Furthermore, enhanced expression of co-stimulatory molecules after MMF stimulation confirmed monocyte activation. Multiple restimulation with fumaric acid esters in vitro, however, and immunomonitoring in a patient during Fumaderm initial therapy suggested that initial monocyte activation is followed by subsequent deactivation associated with an antiinflammatory response.

The results may explain the well-known effects of therapy with fumaric acid esters. Thus, initial treatment is often accompanied by adverse effects which may be caused by MMF-induced TNF-alpha formation. The change in the IL-10/IL-12 balance as a result of elective induction of IL-10, however, may have antipsoriatic activity by diminishing type-1/proinflammatory cytokine over-expression and the antigen-presenting capacity of monocytes/macrophages, and by upregulation of IL-1RA.

The study of NIBBERING ET AL. (1997) focused on the intracellular signal transduction pathway which links interaction between MMF and granulocytes with increases in $[Ca^{2+}]i$ and the cAMP concentration. In summary, MMF binds to specific sites on the plasma membrane of cells. This interaction activates pertussis toxin-sensitive G proteins which then stimulate an increase in PTK and PKH4 activity. These protein kinases may regulate the rise in $[Ca^{2+}]i$ and the intracellular cAMP concentration. Elevated $[Ca^{2+}]i$ and intracellular cAMP concentration stimulate protein kinases that regulate transcription factors. Activation of these factors results in induction of downstream gene expression and thus controls cell functions, e.g. cell proliferation and production of inflammatory mediators, as has been found for cells incubated with MMF.

OCKENFELS ET AL. (1998) mono- and cocultured keratinocytes from psoriatic patients as well as from healthy volunteers with HUT 78 T cells with/without the addition of FAEs. Only dimethylfumarate (DMF) diminished IL-6 and TGF-alpha secretion in the psoriatic cocultures. However, it did not have this effect on cocultures from control subjects or on monocultures. DMF suppressed EGF-induced TGF-alpha mRNA induction in psoriatic keratinocytes. DMF inhibited INF-gamma secretion in all cultures but stimulated the IL-10 secretion. This immunomodulation away from the TH1 cytokine IFN-gamma to the TH2 cytokine IL-10 was confirmed in HUT 78 T cells by Northern blot analysis. An increased number of eosinophils is a known side-effect in patients treated with this drug, suggesting a clinical relevance of this immunomodulation in vivo.

The authors conclude that immunomodulation and the suppression of cytokines from the psoriatic cytokine network could be responsible for the beneficial effect of DMF in the treatment of a hyperproliferative and TH1 cytokine-mediated skin disease.

SEBOK ET AL. (1998) studied the effect of fumaric acid, dimethyl-fumarate, Zn-, Ca- and Mg-monoethyl-fumarate on the interferon-gamma (IFN-gamma)-induced expression of ICAM-1 and HLA-DR molecules on keratinocytes. The semiquantitative evaluation revealed that in the micromolar dose range investigated only dimethyl-fumarate demonstrated substantial growth inhibition and down-regulation of the cell surface markers. In the quantitative evaluation, dimethyl-fumarate significantly suppressed the expression of ICAM-1 (84%) and HLA-DR (67%) on HaCaT keratinocytes at a subtoxic concentration of 4.0 µM as compared to untreated controls (100%). In contrast, concentrations of 4.0, 12 and 35 µM dimethyl-fumarate had no influence on the ICAM-1 and HLA-DR expression on IFN-gamma-exposed normal human epidermal keratinocytes in primary cultures. The authors gave experimental evidence that dimethyl-fumarate may exert its antipsoriatic effect not only as an antiproliferative agent but also by down-regulation of ICAM-1 and HLA-DR molecules on hyperproliferative keratinocytes.

STOOF ET AL. (2001) studied the effect of dimethylfumarate (DMF) on the production of the chemokines CXCL1, CXCL8, CXCL9, CXCL10 and CXCL11, formerly known as GROalpha, interleukin-8, Mig, IP-10 and IP-9/I-TAC, respectively, in human keratinocytes and peripheral blood mononuclear cells (PBMC). Northern blot analysis on isolated keratinocyte RNA preparations showed a dose-dependent inhibition of CXCL1, CXCL8, CXCL9, CXCL10 and CXCL11 transcription by DMF. At 45 micromol L(-1) the inhibition was almost complete. In addition, keratinocytes and PBMC showed in the presence of DMF a dose-dependent inhibition of CXCL8, CXCL9 and CXCL10 protein production. The authors conclude, that these results show the ability of DMF to inhibit the production of chemokines that may be critically involved in the development and perpetuation of psoriatic lesions. This might explain, at least in part, the beneficial effects of treatment with fumaric acid esters in psoriasis patients.

ZHU AND MROWIETZ (2001) investigated the effect of fumaric acid esters on granulocyte-macrophage colony-stimulating factor/interleukin-4-induced differentiation of monocyte-derived dendritic cells. The results show that dimethylfumarate as well as methylhydrogenfumarate-calcium-salt (0.01-100 µg/ml) concentration-dependently inhibit monocyte-derived dendritic cell differentiation. This was reflected by an inhibition of CD1a, CD40, CD80, CD86, and HLA-DR expression as well as by a reduced capacity of dimethylfumarate-treated monocyte-derived dendritic cells to stimulate lymphocytes in the allogeneic mixed lymphocyte reaction. Other fumaric acid esters showed no effect on monocyte-derived dendritic cell-differentiation. At higher concentrations (30-100 µg/ml) dimethylfumarate, but not methylhydrogenfumarate calcium-salt induced apoptosis in monocyte-derived dendritic cells. These data point to a high susceptibility of the monocyte/dendritic cell system to dimethylfumarate and its main metabolite methylhydrogenfumarate. Other fumaric acid esters investigated were without effect. As the effects of fumarates on monocyte-derived dendritic cells observed occur at concentrations 20-fold lower compared with lymphocytes, the findings of ZHU AND MROWITZ seem to be of relevance in explaining the possible mode of action of these compounds in psoriasis.

4.3. Conclusion

The fumarate esters especially the dimethyl-fumarate by its metabolite monomethylfumarate are effective in immunomodulation in psoriatric patients, but obviously not in healthy humans. There is no evidence that fumaric acid shows a comparable immunomodulating effect.

5. OTHER PHARMACOLOGICAL EFFECTS OF FUMARIC ACID

The cardioprotective effects of fumarate have been linked to its metabolism to succinate.

PEARL ET AL. (1994) studied if fumarate-enriched cardioplegic solutions would improve the functional recovery of the neonatal piglet heart. A model of isolated hearts, which were placed on a blood-perfused working heart circuit, was used. The functional recovery at left atrial pressures of 3, 6, 9, and 12 mm Hg was 70%, 66%, 66%, and 65%, respectively, in the control group, versus 102%, 106%, 105%, and 109%, respectively, in the fumarate group ($p < 0.05$). The tissue creatinine phosphate levels after reperfusion were significantly higher in fumarate hearts, although the adenosine triphosphate levels were not significantly different.

LAPLANTE ET AL. (1997) perfused rat hearts with 11 mM glucose, 1 mM lactate, 0.2 mM pyruvate, 0.2 mM octanoate, and 0.04 or 0.4 mM fumarate. On reoxygenation after 40 min of severe hypoxia, hearts perfused with 0.4 mM fumarate showed a better recovery of contractile function and released less lactate dehydrogenase (an index of cellular necrosis) than those perfused with 0.04 mM fumarate. The data showed that, in hypoxic hearts, fumarate conversion to succinate occurred only through reduction, although it accounted for only 16% of total succinate release. Most of the succinate was formed through the oxidation of alpha-ketoglutarate or its precursors (50 ± 5%) and by another yet-unidentified pathway (34 ± 4%). These data show that, in a model of hypoxia-reoxygenation, the cardioprotective effects of fumarate were associated with its predominant metabolism to succinate through the reductive pathway.

The Chemoprevention Branch (National Cancer Institute, National Institutes of Health) is testing dozens of candidate chemopreventive compounds in the rodent model carcinogenesis systems. Significant chemopreventive (i.e., anticancer) effects have been produced among others with fumaric acid (BOONE ET AL., 1992).

KURODA ANS AKAO (1977) found the production of gastric ulcerations in rats by pyloric ligation to be inhibited by either intraperitoneal or oral administration of fumaric acid in a dose of 50 mg/kg bw. The antiulcer action was also exhibited by the four-carbon dicarboxylic acids, maleic acid, oxalacetic acid, succinic acid and malic acid. The studies on fumaric acid indicated that the acid's antiulcer action was based on its ability to inhibit the gastric juice secretion and to dilate the stomach muscle.

6. PUBLICATION OF K.-H. SCHMACK

The publication was provided by the German authorities to the European Commission and the SCAN in its original version, together with a translation in English. The Summary is presented hereunder.

"Summary"

"*A 12-year observational study in an East Westphalia veterinary practice servicing cattle and pig farms indicated that fumaric acid, used as an acidifier in milk substitute powder and milk supplements, leads to specific clinical signs. These were*

chronotropic and inotropic cardiac dysrhythmias and were dependent on the dose and age of the calf. In some cases a fatal peritoneal dropsy occurred, together with specific histopathological renal disturbances, mainly a non-purulent nephrosis. Bradycardiac dysrhythmias were detected after a daily dosage of 3 g of fumaric acid within 3-7 days. The lethal syndrome develops after 7-10 days with daily dosage of 4.3 g from the day of birth. The pathology is characterised by acute non-inflammatory peritoneal dropsy, watery mucours swelling of the retroperitoneal tissue around the kidneys, and enlarged ochre-coloured pale kidneys with confluence of the cortical kidney lobes.

The physiological effects of fumaric acid are twofold: firstly, inhibition of the decomposition of the amino acid tyrosine leads to an increase in adrenaline and noradrenaline, resulting in relaxation of the intestinal muscels together with cardiac rhythm disturbances; secondly, the influence on the urea cycle leads to an increase in the level of blood ammonia and to a disturbance of nucleic acid synthesis. This is a result of the separation of the N-group from the aspartic acid and results in necrotic cell damage of the glomeruli and tubules".

7. SCAN COMMENTS

The publication shows several serious shortcomings. No exact time course of the occurrence of the findings („a 12-year observational study") is given nor is the number of animals (suffering, in a dose titration experiment, total herd size) stated. It is only known that in the early phase of examination „12 calves died in both this and another herd, and 3 in another herd".

The author tries to establish a dose dependent occurence of clinical and pathological signs (2.1-2.4 g fumaric acid per day and calf: no cardiac damage audible; 3 g: cardiac arrhythmia after 5-7 days; 3.6 g: cardiac arrhythmia after 2-3 days and 4.3 g given to newborn calves: fatal syndrome within 7-10 days). Neither the age (except in case of the newborn) nor the body weight of the calves is given. But in another paragraph, the author writes, the syndromes were identified in 8 to 14 day old calves „with increasing frequency in following years (after 1984) irrespective of the quantity of milk powder or milk substitute containing fumaric acid which was used". This is in clear contradiction to the statement „The clinical and pathological effects of fumaric acid pathogenesis are dose-dependent".

SCAN has to accept that the syndrome could not be attributed to any (other) known cause, and excluded suspected etiologies (poisoning, infection, heredity), however a proof is not given except the statement that fumaric acid poisoning „could be pinpointed by means of reproducibility".

The author deduces his findings to a metabolic overload by fumaric acid, by which the physiological functions are just the reverse by disturbed flow balance: (i) tyrosine can not be broken down to fumaric acid, therefore adrenaline snythesis (from tyrosine) is stimulated, „activity of N. parasympathicus may also be paralysed", (ii) the urea cycle is stopped, ammonia poisoning results, primary urine is not longer reabsorbed, (iii) from (i) and (ii) renal failure results, „the glomeruli continue to excrete primary urine, which presents perinephritically and intraperitoneally after tissue filtration (protein deficient ascites, perinephritic oedema)."

SCAN criticises this interpretation, because it is speculative and based on a too simple and unsupported understanding of metabolic processes and their regulations. SCAN gives to attention that the herds observed by the author obviously suffered from disease(s), that all other (routine?) medical treatments, which were presumably also made, are not described in the article.

In his conclusions, the author states that „fumaric acid is toxic regardless of dosage". He continues "the apparently positive effect of reducing diarrhoea is a positive interpretation of a negative pathophysiological influence." In fact the reduction of diarrhoea as a follow up of oral administration of organic acids is described several times, mainly in piglets and predominantly by formic acid and its salts and is discussed in relation with the microbial findings in the gastrointestinal flora.

It should be noted, that one of the mostly occurring side effects of psoriasis treatment with FAE, diarrhoea, was not observed by SCHMACK, in contrary, he wrote „in the further observation phase following increased fumaric acid dosage, the calves produced obstipatory dung following atonic intestinal lethargy".

8. CONCLUSIONS

Fumaric acid is studied in piglets in concentrations up to 4 % in the diet for 4 weeks. It obviously stimulates feed intake. The recommended concentration is between 1.5 and 2 %. No side effects have been observed. An improvement of ileal digestibilities of GE, CP, and amino acids is plausible. The safe dose corresponds to about 1,000 mg/kg bw (field level) up to 2,000 mg/kg bw (experimental level).

Information on (veal) calves is rather scarce. Comparable statements as for piglets can not be made for calves. There is no evidence for the safety of a certain dosage for a longer time (4 or more weeks), but also not for the opposite. The recent publication given to the attention of SCAN is not convincing.

Comparisons with the human use of fumaric acid esters are problematic. Fumaric acid is said to be poorly absorbed in humans, the esters will probably be quantitatively absorbed in the gut. Nothing is known on the absorption rate of fumaric acid in calves, particularly in newborn calves. Different absorption rates can not be excluded with a desired margin of safety.

Doses which are considered as safe for psoriatic patients are expressed primarily in fumaric acid esters. It could be assumed by conversion that about 0,9 g fumaric acid equivalent/d are safe for humans. Doses for young calves are between 2 and 3 g fumaric acid/d. Converted to body weight, the difference increases from 15 mg/kg for humans to 44 - 67 mg/kg calf.

Higher doses of fumaric acid esters than described above are probably nephrotoxic after long term use. Tubular dysfunctions in calves, even transient, can not be excluded even if regarding a theoretical lower absorption rate of fumaric acid in the light of the higher calf doses and its neonatal stage.

But an upper dietary limit can not be proposed by SCAN due to the lack of knowledge in the absorption rate and in the nephrotoxic potential and nature of fumaric acid in calves.

Studies on veal calves would be helpful in answering the question of the Commission. These should be done as tolerance studies with several doses, different periods of administration and with special regard to kidney function.

For bovines no specific requirement for data is seen because of the infrequent use of fumaric acid in ruminants. The main reason for using fumaric acid is due to its cost not preservation of feed but prevention of diarrhoea. Ruminating bovines are quite less susceptible to diarrhoea as young bovines in the suckling status.

An immunomodulating effect of fumaric acid on mammals is not expected because this effect is only described for fumaric acid esters and mainly in (cell cultures of) psoriatic patients.

9. ANNOTATION

SCAN wishes to draw to the attention of the Commission that all organic acids authorised are approved without any dosage limit. This has been done with the assumption that the market forces would regulate application rates. In the light of the recent understanding of animal and consumer protection it seems doubtful if such an approval should be maintained. SCAN could envisage that maximum concentrations are introduced for all organic acids. This would result in submitting at least target animal tolerance studies for all animal species and categories for which the relevant organic acids are approved.

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