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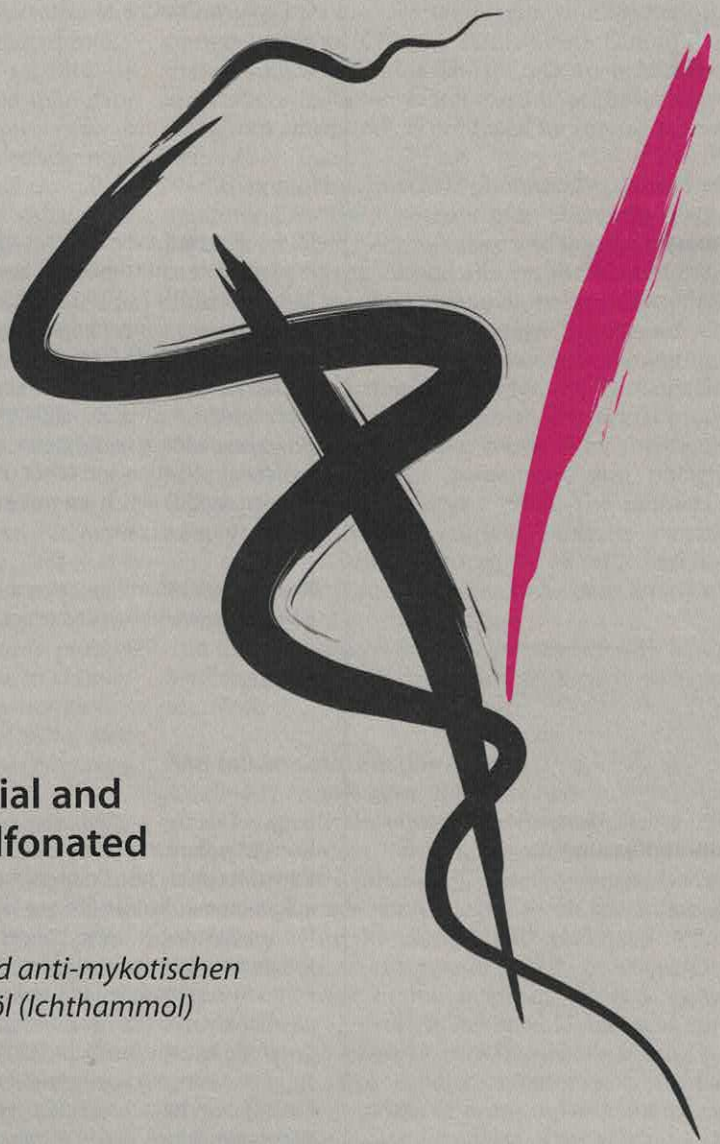

SONDERDRUCK

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Zur anti-entzündlichen, anti-bakteriellen und anti-mykotischen Wirkung von dunklem sulfonierten Schieferöl (Ichthammol)

G. Gayko, W. Cholcha, M. Kietzmann[1)]

Summary

Dark sulfonated shale oil (Ichthammol, ammonium bituminosulfonate) is an active ingredient of natural origin that was included in Annex II of European Council Regulation (EEC) 2377/90. Therefore, this well-tolerated substance is available further on as a dermatological agent for the application to animals from which foodstuffs are made for human consumption. The manifold pharmacological actions could be substantiated in numerous *in vitro* studies using Ichthammol from Seefeld, Austria. The anti-inflammatory action which is well-known from clinical experience could be traced back to an influence of the substance on the formation, secretion and effect of inflammation mediators. Anti-bacterial and anti-mycotic actions which are well-known from clinical use could be confirmed in *in vitro* studies as well.

Keywords: Ichthammol, ammonium bituminosulfonate, anti-inflammatory, anti-bacterial, anti-mycotic effects.

Zusammenfassung

Dunkles sulfoniertes Schieferöl (Ichthammol, Ammoniumbituminosulfonat) ist ein Wirkstoff natürlichen Ursprungs, der in Annex II der Verordnung EWG Nr. 2377/90 aufgenommen wurde. Die gut verträgliche Substanz steht damit auch weiterhin für den Einsatz bei Tieren, die der Lebensmittelgewinnung dienen, als Dermatotherapeutikum zur Verfügung. Die vielfältigen pharmakologischen Wirkungen konnten in zahlreichen *In-vitro*-Untersuchungen mit dem in Seefeld, Österreich gewonnenen Ichthammol belegt werden, wobei die aus klinischer Erfahrung bekannte anti-entzündliche Wirkung auf eine Beeinflussung der Bildung, Freisetzung und Wirkung von Entzündungsmediatoren zurückgeführt werden konnte. Die aus dem klinischen Einsatz bekannten anti-bakteriellen und anti-mykotischen Wirkungen finden in den *in-vitro*-Studien ebenfalls Bestätigung.

Schlüsselwörter: Ichthammol, Ammoniumbituminosulfonat, Entzündungshemmung, anti-bakterielle Wirkung, anti-mykotische Wirkung.

Introduction

Ichthammol[1] (ICHTHYOL-GESELLSCHAFT, Hamburg), a multi-component mixture of substances of natural origin, has a long tradition as a veterinary medicinal product because of its anti-inflammatory, anti-bacterial and anti-mycotic actions. The dark sulfonated shale oil is produced in Seefeld (Austria) according to the guidelines of Good Manufacturing Practice (GMP). In the course of the procedure for establishing maximum residue limits of veterinary medicinal products in foodstuffs of animal origin (MRL procedure according to European Council Regulation (EEC) No. 2377/90), Ichthammol (ammonium bituminosulfonate) was recently included in Annex II after evaluation of the Committee for Medicinal Products for Veterinary Use (CVMP). In Annex II, substances are listed which are found safe so that it is not necessary to fix maximum residue limits for the consumers' protection. Especially for a multi-component mixture as Ichthammol, the origin as well as controlled manufacturing process (GMP) are important prerequisites for a constant good quality and, thereby, also a decisive evaluation base for the substance. This refers above all to purity which is shown in the absence of risky substances (e.g. polycyclic aromatic hydrocarbons [PAH]).

All studies described hereafter were carried out with Ichthammol from Seefeld, Austria. In particular, the studies on toxicology the results of which are crucial for inclusion in Annex II presupposed that the content of benzo[a]pyrene, the best known substance among the PAH, is negligible and therefore harmless. Similar studies with other Ichthammol qualities are not available in literature.

The inclusion in Annex II is a basic requirement for the future use of the substance which is known for more than 100 years in veterinary medicine. With regard to the prohibition of many substances which are pharmacologically active and considering its broad spectrum of efficacy, different areas of indication consequently present themselves for Ichthammol. This also applies to animals from which food is produced for human consumption.

The starting material for the production of sulfonated shale oil is sulfur-rich oil shale, a kerogen-containing sedimentary rock from the mesozoic era.

Kerogen is the organic material of the oil shale developed from marine plants as it appears today after several million years of geological processes of sedimentation and metamorphism: A rigid network of high-molecular organic compounds with a comparatively high hydrogen/carbon ratio (Mongenot et al., 1997). By dry distillation under exclusion of air at a temperature below 500 °C, the network structure of kerogen is decomposed and an oil with a high sulfur content is released. After distillative processing, sulfonation and neutralisation of the formed sulphonic acids, Ichthammol appears as a water-soluble ammonium salt of dark sulfonated shale oil.

Originally designated as Ichthammol by ICHTHYOL-GESELLSCHAFT, the name can currently be found in monographs of many pharmacopeias which are acknowledged all over the world (Ph.Eur. "Ichthammolum", USP and BP "Ichthammol"). Other synonyms are Ammonium bituminosulfonate or dark sulfonated shale oil.

In spite of similar outward appearance, sulfonated shale oils should not be mixed up with tars. Natural original materials and manufacturing processes which differ widely lead to raw materials with completely different molecular structure as well as physico-chemical properties. Contrary to tars, the main components of sulfonated shale oils are strongly polar, water-soluble, surface-active and solubilising salts of sulfur-rich compounds (Wernicke, 1953; Koch et al., 1985).

Studies with regard to the acute, sub-acute and chronic toxicity , to the local tolerance as well as to the teratogenic, mutagenic and cancerogenic potential of Ichthammol prove in a high degree of safety for the treated animal and also for the consumer of food which is produced from treated animals whether Ichthammol is administered for a short or a long period of time. There were no indications for teratogenic, mutagenic or cancerogenic effects (Cholcha et. al., 1994). Due to the fact that no sufficient residue data for milk are in hand yet, an application limitation is still existing at present for animals from which milk is produced for human consumption.

Ichthammol has manifold pharmacological actions. Its application for the treatment of inflammatory skin diseases as well as of degenerative and traumatic joint diseases has been established all over the world for a long time. Besides its anti-inflammatory, anti-bacterial and anti-mycotic properties, Ichthammol possesses among other things also anti-pruriginous, hyperemisating and anti-seborrheic actions (Altmeyer, 1998). Considering the list of indications of veterinary medicinal products, this active ingredient is used above all for the treatment of abscesses, furuncles, panaritium, and phlegmons (Ungemach und Kietzmann, 1999). The animals preferably treated with medicinal products containing Ichthammol in Germany are above all cattle and horses. In the USA, Ichthammol seems to be used nearly exclusively for the treatment of horses.

In the meantime, a lot of *in vitro* studies with Ichthammol® from Seefeld are available indicating its mechanism of action.

Anti-inflammatory action *in vitro*

As already known since 1882, the dark sulfonated shale oil is therapeutically effective in the treatment of inflammatory dermatoses. Recently, some of the suitable action mechanisms of Ichthammol, were investigated on a cellular level. In this context, studies on the influence of Ichthammol on the release and biological action of arachidonic acid derivatives (e.g. LTB_4, prostaglandines), respectively, were in the forefront. As it is generally known, these derivatives of arachidonic acid formed through lipoxygenase or cyclooxygenase belong to the extremely effective cellular inflammation mediators. Many classic symptoms of acute inflammations can be explained by the biological effects of the arachidonic acid derivatives.

In vitro studies on the influence of Ichthammol on the formation of arachidonic acid derivatives and their effects on cell migration

Czarnetzki (1986) investigated the calcium ionophore-induced release of chemotactic factors (especially LTB_4) from a mixture of lymphocytes, monocytes, basophilic granulocytes (LMB) as well as from polymorphonuclear neutrophilic granulocytes (PMN).

[1]Ichthammol® is an IR trademark of Ichthyol-Gesellschaft, Hamburg

After an incubation period of 10 minutes of the cells with 0.01–2 mg/ml of Ichthammol, it could be demonstrated that the multicomponent mixture itself does not lead to a release of mediators but on the contrary causes an inhibition of the calcium ionophore-stimulated release of mediators into the cell culture medium. In accompanying tests on the viability of the cells, no cytotoxic effect of Ichthammol could be observed in the indicated concentration range.

Studying the kinetics of the release of chemotactic factors, it could be demonstrated that at a concentration of 0.1 mg/ml of Ichthammol, a contact time of 10 minutes with the leucocytes is necessary to get a significant inhibitory effect during the following ionophore-stimulation. At longer contact times, the effect is more significant. After an incubation period of 15 minutes with 1 mg/ml of Ichthammol, the activity of chemotactic factors as LTB_4 is completely inhibited.

In presence of Ichthammol, the chemotactic migration of leucocytes caused by LTB_4 was also reduced in a concentration-dependent way: 1 mg/ml leads to an inhibitory effect of 90 percent and 2 mg/ml to a complete inhibition action. As Czarnetzki (1986) found out accompanying to this experiment, the inhibition of the migration of leucocytes was observed at a non-cytotoxic concentration. Only at Ichthammol concentrations higher than 2 mg/ml, the number of living cells decreased to 65 percent after an incubation period of 30 minutes.

According to the above-mentioned results, Ichthammol on the one side suppresses the secretion of chemotactic factors from stimulated cells (inhibition of release) and on the other side, it inhibits the biological effects of the chemotactic factors themselves (inhibition of cell migration caused by LTB_4).

However, Ichthammol has no influence on the release of inflammatory mediators of non-stimulated cells (cells in normal condition) but suppresses the biological effects of chemotactic factors which exist in vivo in inflamed tissue.

Diezel et al. (1992) could confirm the above-mentioned results and conclusions. After a 5-minutes preincubation of human polymorphonuclear granulocytes (PMN) with different concentrations of Ichthammol and following addition of ^{14}C-arachidonic acid as well as of a calcium ionophore, it could be noted that Ichthammol inhibits the synthesis of 5-lipoxygenase products (LTB_4, 5-HETE) in PMN depending on its dose (table 1).

The arachidonic acid necessary for the synthesis of LTB4 originates physiologically from the phospholipides of the cell membrane where it is released by phospholipase A_2. Since the incorporation of the exogenous arachidonic acid into the cell membrane of the PMN is not influenced by Ichthammol, the results can be interpreted as a selective inhibition of the 5-lipoxygenase in PMN.

The findings concerning the inhibition of the enzyme lipoxygenase as well as of cyclooxygenase are supported by Schewe et al. (1994). By means of a test model similar to the one of Diezel et al. (1992), the inhibitory power of Ichthammol with regard to the formation of 5-HETE (5-hydroxy-eicosatetraen-acid) from exogenous arachidonic acid after pre-incubation with human PMN was established with a IC_{50} of 0.03 mg/ml. Other animal or plant oxygenases are inhibited by Ichthammol as well. Pure 15-lipoxygenase from reticulocytes of rabbits is inhibited by Ichthammol in a concentration-dependent way. The enzyme was pre-incubated with different concentrations of Ichthammol and the reaction was started through addition of a mixture of potassium linoleate (substrate) and sodium cholate. The IC_{50}-value is of the order of 0.14 mg/ml of Ichthammol.

Also in lipoxygenase-1 of plant origin (from soya-beans), an inhibition of activity by Ichthammol could be observed. This indicates that the substance is able to inhibit the activity of all sorts of lipoxygenase (Schewe et al., 1994). In addition, it could be shown for the first time that on the parallel cyclooxygenase pathway, the activity of the enzyme cyclooxygenase (from vesicular glands of sheep) is also inhibited by Ichthammol (IC_{50}-value = 0.3 mg/ml; Fig. 1). Again it is emphasized by Schewe et al. (1994) that the incorporation of arachidonic acid into the cellular lipids is not restricted in presence of Ichthammol. This suggests that the inhibition of the 5-lipoxygenase does not occur because of an unspecific cell damage.

TABLE 1: *Inhibition of enzyme 5-lipoxygenase (5-LOX) in human polymorphonuclear granulocytes by Ichthammol, measured on the basis of the reduction of the formation of leukotriene B_4 (LTB_4), according to Diezel et al. (1992).*

Polymorphonuclear granulocytes (PMN)[a]	LTB_4 ng/ml[b]	inhibition [%]
+ 1.0 mg/ml Ichthammol	0	100
+ 0.2 mg/ml Ichthammol	6	95
+ 0.05 mg/ml Ichthammol	72	40
+ 0.005 mg/ml Ichthammol	120	0

[a] PMN: 1×10^7 polymorphonuclear granulocytes

[b] ng-marked LTB_4 per 1 ml Hanks solution. Control: Incubation of PMN without Ichthammol

TABLE 2: *Anti-inflammatory action of Ichthammol – summary of in-vitro-studies.*

target parameter	concentration	result	literature
influence on the migration of leucocytes as well as on the formation of inflammation mediators	0.01–2 mg/ml	inhibits the migration of leucocytes and the formation of chemotactic arachidonic acid metabolites (LTB_4)	Czarnetzki, 1986
inhibition of the enzyme 5-LOX	0.005 - 1 mg/ml	concentration-dependent reduction of the formation of LTB_4	Diezel et al., 1992
inhibition of different lipoxygenases and cyclooxygenases	10 - 1000 mg/ml	concentration-dependent inhibition of all tested enzymes	Schewe et al., 1994
release of the enzyme hexosaminidase from PMN by f-MLP	0.8 mg/ml	suppresses completely the action of tripeptide f-MLP	Kownatzki et al., 1986
formation of active oxygen compounds and mobilisation of Ca^{2+} by LTB_4	ca. 5×10^{-3}–0.5 mg/ml	inhibition of the release of O_2^- and H_2O_2 as well as of Ca^{2+} mobilisation	Rabe et al., 1994

Confirmation of the results concerning inhibition of LTB_4-release in animal experiments

The anti-inflammatory action of Ichthammol which became apparent in vitro by the inhibition of LTB_4-

release was checked in an animal trial by treatment of edema induced by application of croton oil to AB/Bln. mouse ears (Diezel et al., 1992; Diezel und Schulz, 1991). This inflammatory edema is primary formed as a result of the action of inflammation mediator LTB_4 (Ruzicka und Printz, 1984). The inflammatory edema were treated with an aqueous solution of 10 % Ichthammol in two different experiments. After 5 hours an inhibition of the inflammatory edema (determined on the basis of the weight gain of the ears) of 27 or 34 percent could be observed compared with a control group.

The results agree with the in vitro findings and lead to the suggestion that Ichthammol suppresses also in vivo the formation of 5-lipoxygenase products.

Further anti-inflammatory actions of Ichthammol

Kownatzki et al. (1984, 1986) were concerned with the effect of the chemotactic factor f-MLP (formyl-methionyl-leucyl-phenylalanin) on human neutrophilic granulocytes (PMN). After having established that the migratory reaction of the cells on the chemotactic factors LTB_4, f-MLP and C5a could be inhibited by sulfonated shale oil (Kownatzki et al., 1984), the authors investigated also the release of the lysosomal enzyme hexosaminidase out of stimulated cells under non-toxic conditions.

It has been found out that Ichthammol in contrast to tripeptide f-MLP is not able to produce a secretion of the enzyme hexosaminidase in presence of cytochalasine B. Again, Ichthammol turned out to be of no influence on the non-stimulated cell. On the other hand, Ichthammol inhibited completely the effect of the tripeptide in case of a simultaneous addition of f-MLP to PMN which were pre-treated with cytochalasine B.

Chemotactic factors as C5a and f-MLP stimulate also the formation of oxygen radicals in neutrophilic granulocytes. Kownatzki et al. (1986) could not observe an influence of Ichthammol on the formation of superoxide anions (O_2^-).

Rabe et al. (1994) investigated also the influence of Ichthammol on some further cell reactions caused by stimulants (LTB_4) in peritoneal macrophages of guinea pigs under non-toxic concentrations in vitro. In their studies, the inhibitory effect of Ichthammol on the formation of reactive oxygen entities as well as on the intracellular mobilisation of Ca^{2+} ions was investigated. The formation of hydrogen peroxide (H_2O_2) caused by LTB_4 was determined by fluorescence measurements. Hydrogen peroxide was produced LTB_4-concentration-dependent. Whereas at 100 nmol/l LTB_4 , about 6.5 nmol H_2O_2 were produced, at 10 µmol/l, about 12 nmol H_2O_2could be found.

Since hydrogen peroxide as well as superoxide anions (O_2^-) cannot be eliminated in case of their immoderate accumulation, as a result peroxidative destruction of membrane lipids, conversion of sulph-hydryl groups (SH groups) of proteins in disulphide bridges as well as damage to DNA is observed. Regarding the elimination of xenobiotics in the course of an inflammatory reaction, however, the highly reactive oxygen compounds are desirably effective as bactericides.

A preincubation of the cells with Ichthammol for 3 minutes resulted in a concentration-dependent inhibition of the formation of hydrogen peroxide. An inhibitory action of Ichthammol could already be confirmed at doses of 1 nmol/l Ichthammol. In case of preincubation

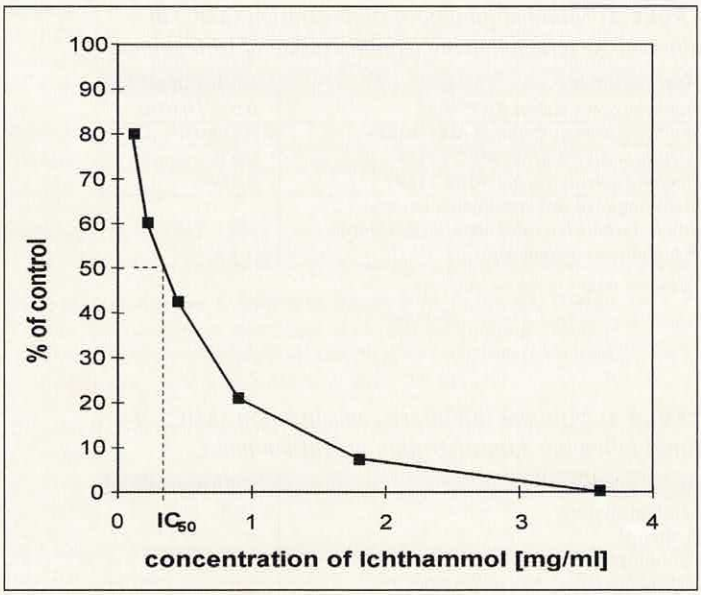

FIGURE 1: *Inhibition of cyclooxygenase from vesicular glands of sheep by Ichthammol. For the duration of 1 minute, the enzyme (450 µg enzyme per sample of 1.91 ml) was pre-incubated with Ichthammol and the reaction was started by addition of 30 mM arachidonic acid. The activity of the control samples was 4.6 ± 0.2 nkat oxygen (according to Schewe et al., 1994).*

with 100 µmol/l Ichthammol (corresponding to about 0.05 mg/ml assuming a molar mass of Ichthammol of about 500 g/mol) and following addition of LTB_4 (100 nmol/l), a maximum inhibition of 32 % was established.

The release of superoxide anions from peritoneal macrophages was started by the addition of 1 nmol/l of the phorbol ester 12-O-tetradecanoylphorbol-13-acetate which is known as tumour promoter and quantified by extinction measurements (reaction with cytochrome C) (Rabe et al., 1994). Between 0.1 nmol/l and 10 µmol/l of Ichthammol, an inhibitory effect of the active ingredient on the release of O_2^- could be observed. Higher concentrations of Ichthammol stimulated the formation of oxygen radicals.

Apart from the release of reactive oxygen entities, the mobilisation of Ca^{2+} ions subsequent to the addition of LTB_4 was investigated in the peritoneal cells by Rabe et al. (1994). The stimulation of the cells with LTB_4 resulted in a time-, temperature- and concentration-dependent increase of the intracellular Ca^{2+} concentration. When the cells came previously into contact with Ichthammol, the addition of LTB_4 resulted in an inhibition of the expected Ca^{2+} signals. The inhibitory effect of Ichthammol was already observed at a concentration of 100 nmol/l; 10 µmol/l caused a complete inhibition.

Summary of the results of *in vitro* studies on the anti-inflammatory action of Ichthammol

Table 2 gives a brief overview of the above-mentioned in vitro studies. As has been proved in all of the studies, Ichthammol was used in non-toxic concentrations. Apart from dark sulfonated shale oil, also pale sulfonated shale oil was partly investigated. It has to be pointed out that the properties of both substances are very similar. In some cases, however, it could be shown that pale sulfonated shale oil is superior to the dark one.

TABLE 3: *Minimal inhibitory concentration (MIC) of different bacteria following administration of Ichthammol.*

Test specimens	Ichthammol® [%]
Staphylococcus aureus ATCC 6538	0.039 / 0.078a
Staphylococcus epidermidis ATCC 12228	0.313
Streptococcus pyogenes ATCC 12344	0.039
Propionibacterium acnes[b] ATCC 11829	0.039
gram-negative test speciments (among others: *Escherichia coli, Enterobacter cloacae, Pseudomonas aeruginosa*)	> 5%[c]

[a] different results in the experiments

[b] incubation under anaerobic conditions

[c] 5 % were accepted as minimum concentration of gram-negativ specimens

TABLE 4: *Minimal inhibitory concentration (MIC) of fungi following administration of Ichthammol.*

test specimen	Ichthammol® [%]
Candida albicans (5 strains)	16.8
Hyphomycetes (*Aspergillus flavus, Aspergillus niger, Scopulariopsis brevicaulis*)	12.9
Dermatophytes (*Trichophyton rubrum, T. mentagrophytes, Microsporum canis, M. gypseum, Epidermophyton floccosum*)	0.02

The *in vitro* studies clearly show that on the cellular level, Ichthammol is able to inhibit the activity of enzymes (lipoxygenase, cyclooxygenase) taking action in the degradation of arachidonic acid and thereby to stop the secretion of inflammation mediators. Besides, Ichthammol has an influence on the biological effect of inflammation mediators (LTB_4): Both an inhibition of cell migration which normally is caused by chemotactic factors could be observed as well as an inhibition of the formation of reactive oxygen compounds and the release of Ca^{2+} ions. The findings of the *in vitro* studies could also be confirmed in animal experiments. The results support the therapeutic response which could be often clinically observed in local therapy of inflammatory skin diseases.

Anti-bacterial action of Ichthammol in vitro

As an example for different indications of Ichthammol, the trichomycosis or furunculosis of horses or cattle can be mentioned which are mostly caused by *staphylococcus aureus*. Concerning inflammations of the skin, only the hyperemisating effect of ointments containing Ichthammol is often placed in the foreground (Rosenberger, 1994). As the following in vitro results on the anti-bacterial action demonstrate, however, Ichthammol is also active in the elimination of several bacteria.

The efficacy of a substance against bacteria is suitably established by determination of the minimal inhibitory concentration (MIC). The MIC-values of Ichthammol with regard to test strains of different bacteria were determined according to DIN 58 940/ICS by Leimbeck and Sonnenschein (1992) (Table 3).

In an earlier study, Pantke (1965) could already show by means of dilution and inhibition zone tests that Ichthammol is significantly effective in an anti-bacterial way also against *Streptococcus haemolyticus* (MIC = 0.20 %) as well as *Streptococcus zymogenes* (MIC = 0.78 %).

It can be noted that with the concentrations of Ichthammol (10 %–50 % in commercially available ointments) that are usually locally or topically applied, a significant anti-bacterial efficacy can be expected.

Anti-mycotic action of Ichthammol

Already at the end of the 19th century, it was reported that a solution of 4 % of dark sulfonated shale oil inhibits the growth of *Trichophyton tonsurans* (Latteux, 1892). Further investigations showed the efficacy of Ichthammol (2.5–20 %) against species of *Trichophyton* and *Microsporum* (Kleine-Nattrop et al., 1950).

In some more recent studies on the anti-mycotic action of Ichthammol, Listemann et al. (1993) applied a test system in which the formation of CO_2 was recorded as a measure for the sensitivity of fungi. The results demonstrate that the output of CO_2 of different strains of *Candida albicans* are absolutely not influenced by Ichthammol in low concentrations. As MIC, a concentration of 16.8 % was determined.

Considering the average CO_2 respiration of *Aspergillus* and *Scopulariopsis* species, it becomes clear that relatively high concentrations (MIC-value: 12.9 %) are necessary to prevent the growth of these fungi; however, at a sub-inhibitory concentration of an average value of 1.25 %, the CO_2 respiration as a measure for growth is already impeded.

Dermatophytes react much more sensitive on the presence of Ichthammol in the medium. Already at a concentration of 0.2 %, growth is no longer possible. The sub-inhibitory concentration is even found at only 0.02 %. In table 4, the results are summarized.

Regarding horses, species of *microsporum* and *trichophyton* are often responsible for the development of dermatomycosis (Wintzer, 1999). As the above-mentioned results clearly show, these dermatophytes could be controlled by a local treatment with Ichthammol. Due to the concentrations in which Ichthammol is offered in commercially available products, an anti-mycotic action can surely be expected.

Conclusions

Dark sulfonated shale oil has been successfully used in dermatotherapy for nearly 120 years now. The recent research results of *in-vitro*-studies on the anti-inflammatory, anti-bacterial and anti-mycotic actions of Ichthammol are the basis of the therapeutic success which can be often observed in practical treatment. Especially the combination of the three mentioned actions offers interesting therapy possibilities since bacterial invasion and/or fungal infection can cause inflammatory skin diseases or inflamed skin is susceptible to immoderate bacterial flora and/or colonisation of fungi. The summary of results demonstrates impressively that the clinical experience of decades made with the active agent Ichthammol could be very well proved using methods of modern pharmacological research.

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