Oral Citrus Seed Extract in Atopic Eczema: *In Vitro* and *In Vivo* Studies on Intestinal Microflor

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Abstract

Serial dilutions of a citrus seed extract (ParaMycrocidin) were highly effective at concentrations ranging from 0.5 to 2 p.c. against a large number of Gram positive, Gram negative and yeast strains in vitro. The oral administration of the same agent in atopic patients with intestinal dysbiosis (3 x 150 mg daily) resulted in a significant inhibition of Candida Sp., Geotrichum sp. and hemolytic E. Coli growth. Staph, aureus, aerobic spore formers and lacto-bacilli were only slightly inhibited and no activity could be noticed against Klebsiella sp. and Bifidobacteria.

Introduction

Recurrent fungal and bacterial infections have a high frequency of association with atopic eczema (AE). A prospective study in our clinic demonstrated a high colonization density of skin lesions, nasal, pharyngeal and vaginal mucosa with *Staph, aureus,* streptococci and with *Candida, Aspergillus* or *Penicillium sp..*[1]

Quantitative investigations of duodenal aspirates and fecal microflora in the same AE group revealed significantly increased counts of hemolytic coliforms and staphylococci, *Candida/Geotrichum sp.* and pathogenic *Clostridia,* generally associated with dramatically reduced counts of lactic acid producing bacteria.[1] The treatment of these fungal and bacterial infections is often not easy and requires potent antibiotics and antimycotics.

In an alternative approach, we have tested recently the antimicrobial effects of a botanical citrus seed extract (ParaMycrocidin).

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We report here our experience with this natural antimicrobial product against different bacterial and yeast strains in vitro and in AE-patients with intestinal dysbiosis.

Patients and Methods

Twenty-five clinically proved AE-pa-tients[2] (age range 16-41 years) gave their consent to take part in this study. All patients avoided any steroid or antihista-minic treatment for at least 10 days before admission and none of them had asthmatic symptoms. The patients showed severe AE with widespread deep excoriation and weeping or bleeding lesions over the face, limbs and trunk. Intermittent diarrhea, constipation, flatulence, intestinal rushes, bloating and abdominal discomfort (particularly after carbohydrate rich meals) were registered in 14 cases.

ParaMycrocidin Supplementation

A group of 10 patients received orally a 0.5% ParaMycrocidin solution (2 drops in 200 mL water) twice daily for one month. It was difficult to convince the patients to swallow higher concentrations of the liquid antimicrobial agent because of the bitter taste of the product. In order to get higher concentrations of the agent in the intestine we put another group of 15 patients on ParaMycrocidin capsule (50 mg extract/capsule) in a dosage of 3 x 3 daily for one month.

Microbiological Investigations

Sensitivity tests were performed in 794 bacterial and 93 fungal strains against 30 antibiotics, 18 antimycotics and different concentrations of liquid ParaMycrocidin. The antimicrobial activity of the seed extract dilutions

was expressed as perceptual inhibition of the microbial growth. Anaerobically yielded fecal samples were taken before and 4 weeks after ParaMycrocidin and serial dilutions were plated for quantitative investigations of large bowel microflora.

All samples were cultured on appropriate grow-media for Gram-positive, Gram-negative, anaerobic and yeast strains. Grow media, incubation time, isolation, identification and quantitative estimation of micro-organisms were described elsewhere.[3] [4] Intestinal microflora results are expressed in colony forming units/g wet stool.

Results and Discussions

A. By testing liquid ParaMycrocidin against different pathogenic bacterial and yeast strains 'in vitro' we noticed an obvious antimicrobial activity at concentrations ranging between 0.1 and 2 p.c.(Table 1). A 0.5% solution already prevents Gram-positive *(Streptococcus sp., Staph, aureus, enterococci)* as well as Gram-negative *{Enterobacter* and *E. Coli)* bacterial growth. The same concentration was highly effective against different yeasts and molds *(Candida, Geotrichum, Aspergillus* and *PeniciIlium sp.).* Only increased concentrations (1-2 p.c.) eliminated *Proteus* and *Klebsiella sp.* but no effects were noticed upon *Pseudomonas sp.*

The antimicrobial activity of the botanical extract at concentrations exceeding 0.1 p.c. was similar to that of 30 potent antibiotica and 18 antimycotica parallely tested (results will be reported elsewhere).

B. The oral treatment of atopic patients with 2 drops of liquid ParaMycrocidin in 200 mL of water (0.05%), twice daily resulted in no significant changes of fecal microflora. Only 2 of 10 patients reported an improvement of their intestinal symptoms (flatulence, meteorism, diarrhea) after 4 weeks therapy. We believe the low concentration of the liquid extract is respon sible for the low antimicrobial activity.
We therefore suggest to increase the daily dosage to 3 x 4 drops in 200 mL water in order to achieve effective results (see in vitro investigations). On the other hand, it is difficult to convince the patients to swallow higher concentrations of liquid ParaMycrocidin when they are already complaining about the obvious bitter taste of the product.

By using ParaMycrocidin capsules in a dosage of 3 x 3 (3 x 150 mg) daily we noticed a significant activity against several pathogenic intestinal strains in our patients (Table 2).

The extract was mostly effective against *Candida, Geotrichum sp.* and hemolytic *E. Coli.* The growth of *Lactobacillus sp., Staph, aureus* and aerobic spore formers was slightly inhibited at this concentration. No effect could be seen upon *Bifidobacteria* and *Klebsiella sp.*

The antimicrobial activity of the capsules in the dosage used in this study correlated with the in vitro activity of the extract. It is conceivable that a higher dosage of oral ParaMycrocidin leads to similar results as in vivo.

No side-effects were registered during the whole study. Clinically, a definite improvement of constipation, flatulence, abdominal discomfort and night rest was noticed after 4 weeks of ParaMycrocidin intake (capsules) in all 15 patients.

Investigations concerning the intestinal flora of AE-patients after a higher dosage of oral ParaMycrocidin are in progress.

References

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Table 1

In vitro effectiveness of ParaMicrocidin against 194 bacterial and 93 fungal strains

	% Growth Inhibition									
Para Microcidin Concentration %	**Staph. aureus n = 249**	**Streptococcus sp. n = 86**	**Enterococcus sp. n = 232**	**Enterobacter sp. n = 77**	**E. Coli sp. n = 86**	**Klebsiella sp. n = 22**	**Pseudomonas sp. n = 24**	**Proteus sp. n = 18**	**Yeast strains n = 71**	**Mold strains n = 22**
2	100	100	100	100	88	33	0	100	100	100
1	100	100	100	100	88	0	0	100	100	100
0.5	100	91	97	100	88	0	0	0	100	100
0.1	94	38	54	0	0	0	0	0	4	0
0.07	92	68	58	0	0	0	0	0	0	0
0.05	98	18	39	0	7	0	0	0	0	0
0.01	35	0	4	0	0	0	0	0	0	0
0.005	0	0	0	0	0	0	0	0	0	0
0.001	0	0	0	0	0	0	0	0	0	0

Table 2

Fecal Microflora of 15 AE-patients before and after ParaMicrocidin supplementation

	Lacto-bacilli	**Bifido bacteria**	**Haemolytic coliforms**	**Staph. aureus**	**Aerobic spore formers**	**Klebsiella**	**Candida/ Geotrichum**
Normal range c.f.u./g. wet stool	$>10^6$	$>10^8$	$<10^4$	$<10^4$	$<10^4$	$<10^4$	$<10^s$
Before therapy (n = 15)	$5x\ 10^4$	2.55×10^8	$5x10^5$	$2x\ 10^4$	$5x10^4$	$5x\ 10^s$	$4xl0^4$
After 4 weeks ParaMicrocidin (n = 15)	1.5×10^4	1.5×10^8	$8\ x10^s$	$6\ x10^s$	$8\ x10^s$	$5\ x10^s$	2.5×10^2

c.f.u. = colony forming units