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Research Article

Impact of the cosmetic mouthwash “Jack Pro Spülung plus” (“rheodol-Spülung plus”) on the oral cavity flora, tested in a monocentric, controlled, randomized, blind, cross-over comparative study

Beeinflussung der Mundhöhlenflora durch Mundspülung mit dem Kosmetikum Jack Pro Spülung Plus, geprüft in einer monozentrischen kontrollierten randomisierten verblindeten Cross-Over-Vergleichsstudie

Jana Göhring[1]
Gerald Müller[1]
Reiner Biffar[2]
Axel Kramer[1]

1 Institute of Hygiene and Environmental Medicine, University Medicine, Greifswald, Germany

2 Center for Dentistry and Oral Medicine, Department of Prosthodontics, Gerostomatology and Biomaterials, University Medicine, Greifswald, Germany

Abstract

Aim: Jack Pro Spülung Plus (also available as “rheodol-Spülung plus”) is recommended to mechanically maintain oral hygiene as part of an overall oral hygiene concept. Because Jack Pro Spülung Plus contains the active agents polihexanide and tosylchloramide sodium in concentrations below microbicidal efficacy, this study tested the hypothesis that the combination of mechanical rinsing and bacteriostatic effect surpasses the effect of mechanical rinsing alone.

Method: The study was performed with 30 volunteers as a monocentric, controlled, randomized, blind, cross-over comparative study. The efficacy of the test product (active agents polihexanide 0.02–0.03% and tosylchloramide sodium 0.004–0.006%) was compared to an aqueous solution of polihexanide (0.02–0.03%) and to Ringer solution as negative control. The efficacy was measured as the reduction of colony forming units (cfu) on buccal mucosa after aerobic and anaerobic cultivation. After determination of pre-values, the volunteers performed mouthrinsing for 30 sec with each of the 3 tested solutions. After 1, 10 and 60 minutes, cfu numbers were determined again. The reduction factor was calculated as the difference between $\log_{10}$ of the measured cfu before and after mouthrinsing with the test solution. The sampling was performed using a template with a smear area of 1 x 1 cm.

Results: Using Ringer solution led to a slight mechanically-induced reduction of cfu in the oral cavity 1 min after rinsing the mouth cavity with the solution. After 10 min and 60 min, no influence on the cfu number could be detected. Using Jack pro Spülung Plus led to a bacteriostatic effect up to 60 min after mouthrinsing; 10 min and 60 min after rinsing the efficacy of Ringer solution was also significantly surpassed. The aqueous solution of polihexanide was less effective than Jack pro Spülung Plus after 10 and 60 min.

Conclusion: Based on these observations, we conclude that Jack pro Spülung Plus is suitable for improvement of oral hygiene if patients have sensitive oral mucosa, e.g., in cases of aggressive cancer therapy or for patients with tracheostoma.

Keywords: polihexanide, tosylchloramide sodium, mouthwash, reduction of cfu

Zusammenfassung

Zielsetzung: Jack Pro Spülung Plus wird zur Unterstützung der mechanischen Mundhygiene empfohlen. Da die Wirkstoffe Polihexanid und Tosylchloramidnatrium in der Jack Pro Spülung Plus unterhalb der mikrobiozid wirksamen Konzentration enthalten sind, sollte in der vorlie-

genden Anwendungsstudie die Hypothese überprüft werden, ob durch Addition von mechanischer Spülwirkung und bakteriostatischer Wirksamkeit der Effekt der mechanischen Spülwirkung übertroffen wird.
Methode: Die Studie wurde als monozentrische kontrollierte randomisierte verblindete Cross-Over-Vergleichsstudie an 30 freiwilligen Probanden durchgeführt. Die Wirksamkeit des Prüfprodukts (Wirkstoffbasis Polihexanid 0,02–0,03% und Tosylchloramidnatrium 0,004–0,006%) wurde mit einer wässrigen Lösung von Polihexanid (0,020–0,030%) und mit Ringerlösung als Negativkontrolle verglichen. Als Maß für die Wirksamkeit diente die Reduktion der Erregerzahl auf der Wangenschleimhaut nach aerober und anarober Kultivierung. Nach Erhebung der Vorwerte wurde eine 30-sekündige Mundspülung mit je einer der drei Prüflösungen durchgeführt. Nach 1, 10 und 60 min wurde erneut die Erregerzahl bestimmt und der Reduktionsfaktor aus der Differenz zwischen dem $\log_{10}$ des Vorwerts und des Nachwerts berechnet. Die Probenahme erfolgte mittels Schablone mit einer Abstrichfläche von 1 x 1 cm.
Ergebnisse: Durch Ringerlösung wurde 1 min nach Mundhöhlenspülung eine geringe mechanische Verringerung der Mundhöhlenflora erreicht. Nach 10 min und 60 min war kein Einfluss mehr feststellbar. Durch Jack pro Spülung Plus wurde zusätzlich zum mechanischen Effekt der Ringerlösung eine bakteriostatische Wirksamkeit erzielt, die bis zu 60 min anhielt. Sowohl nach 10 min als auch nach 60 min wurde die Wirksamkeit von Ringerlösung signifikant übertroffen. Die wässrige Polihexanidlösung war nach 10 min und 60 min tendentiell geringer wirksam als Jack pro Spülung Plus.
Schlussfolgerung: Auf Grund der bakteriostatischen Wirkung ist Jack pro Spülung Plus zur Unterstützung der Mundhygiene vor allem bei empfindlicher Mundschleimhaut, z.B. bei geriatrischen Patienten und während aggressiver Krebschemotherapie, als geeignet anzusehen.

Schlüsselwörter: Polihexanid, Tosylchloramidnatrium, Mundspüllösung, keimzahlvermindernde Wirksamkeit

1 Introduction

Jack Pro Spülung Plus contains polihexanide and tosylchloramide sodium as active agents in concentrations below microbicidally active levels, as used in antiseptic mouth-rinsing solutions [1], [2]. The recommended field of application for Jack pro Spülung Plus is supportive mechanical oral hygiene. This is based on the hypothesis that by combining mechanical rinsing and bacteriostatic activity, the efficacy of mechanical rinsing alone is surpassed.
The purpose of this study was to determine whether Jack pro Spülung Plus is more antimicrobially effective than Ringer solution. Further, the same concentration of an aqueous solution of the active agent polihexanide was compared with Jack pro Spülung Plus to determine whether polihexanide is more effective in Jack pro Spülung Plus than in the aqueous solution.

2 Method

Study design: The study was performed with 30 volunteers as a monocentric, controlled, randomized, blind, cross-over comparative study (Table 1). The test products were encoded with the letters A to C.

Table 1: Use of the test products in the cross-over design

Volunteer	1st week	2nd week	3rd week
1–10	A	B	C
11–20	B	C	A
21–30	C	A	B

The study was confirmed by the ethics committee of the Ernst Moritz Arndt University, Greifswald, Germany (registration number BB 65/12).

Inclusion and exclusion criteria: The selection of volunteers was done without restricting for sex or ethnic origin. The following requirements had to be met:

- age: at least 18 years
- written informed consent
- willingness and ability to meet the requirements of the study protocol.

The following exclusion criteria specific to this study were determined:

- persons with macroscopically visible lesions of the oral mucosa
- persons with known, pre-existing oral diseases
- participation in another clinical trial within the last 30 days
- pregnancy or lactation
- discontinued other mouth-rinsing solutions at least one week before starting the trial.

Only volunteers that met all inclusion and exclusion criteria were accepted.
A detailed explanation about the study procedures was given to all volunteers. Subsequently, they received written information about the procedures. After providing written consent, volunteers were included in the study.

Randomization and decoding: Volunteers were assigned an increasing, consecutive number corresponding to their admission to the trial. They kept the number over the course of the study. Case report forms were numbered corresponding to the volunteers' respective numbers. Thus, unambiguous assignment was possible.

Test products: The following solutions were tested:

- Jack pro Spülung Plus (ELISCHA Medical GmbH, Halberstadt, Germany), active agent polihexanide 0.02–0.03% (g/g), tosylchloramide sodium 0.004–0.006% (g/g), application undiluted in accordance with manufacturer's instructions (test solution A)
- Control: aqueous solution of polihexanide 0.02–0.03% (g/g) made by ELISCHA Medical GmbH, Halberstadt, Germany, application undiluted (test solution B)
- Ringer solution as negative control (test solution C).

Study procedure: The measure of the antimicrobial activity of the tested products is the reduction of cfu on buccal mucosa. After determination of the pre-treatment values the volunteers rinsed their mouths for 30 sec with each of the 3 test products in a cross-over fashion. The buccal swab was taken after 1, 10 and 60 min.
The reduction factor (RF) was calculated using the formula

$$RF = \log_{10} \text{pre-treatment value} - \log_{10} \text{post-treatment value}.$$

Volunteers (10 each) received the one of the 3 tested products on each of the three dates, with a different product at each date (see Table 1). Between the 3 dates, an interval of 7 days was ensured to exclude overlapping effects.
Sampling was done by taking smears from the buccal mucosa with a template (Figure 1) having an area of 1 x 1 cm.
To acquire post-treatment cfu numbers, smears were taken from different locations than the pre-treatment samples (right cranial region 14–17) in the following temoral sequence: right caudal region 44–47, left cranial region 24–27, and the left caudal region 34–37.
Samples were diluted 1:100 and plated on two agar plates (bacteria were detached from the swab in 10 ml trypton/0.89% NaCl solution using a vortexer, 0.1 ml was plated out from this solution). To detect facultative anaerobic bacteria, we used Schädler blood agar. The plates were incubated aerobically at 37 °C or in a microaerophilic atmosphere using the AnaeroGen system (OXOID GmbH; Wesel, Germany).
To inactivate residues of the active agents in the diluted solutions, the samples were neutralized using lipofundin® MCT 20% (B. Braun, Melsungen, Germany) + 0.1% sodium thiosulfate (A2833 AppliChem, Germany).
Volunteers' acceptance of the test products was evaluated in parallel using a questionnaire with the following parameters:

- acceptance: pleasant, tolerable, intolerable, disgusting.
- quality of taste: sweet, sour, salty, bitter, hot, refreshing, neutral.

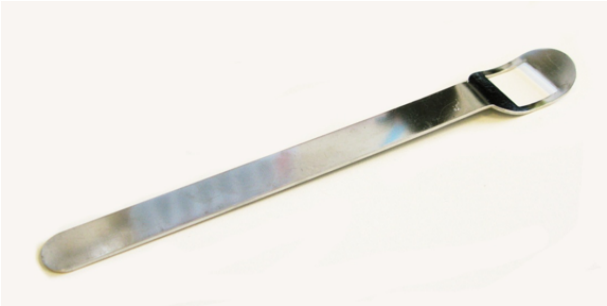

Figure 1: Autoclavable stainless steel template for standardized sampling

Statistical analysis: The statistical analysis was performed with GraphPad Prism 5.0 using one-way ANOVA and Tukey's multiple comparison test.

3 Results and discussion

3. 1 Preliminary tests to determine the methodical approach

Number of colony forming units dependent on dilution of the sample: To determine whether the dilution factor 1:1000 enables a reproducible recovery from the swab, recovery was examined on 5 volunteers. In both aerobic and anaerobic conditions, the results were easily countable (Table 2).
Number of colony forming units dependent on sampling: Because in contrast to other studys [3], [4] – in which sampling was done with a swab without marking a defined area – this study employed a template of 1 cm^2 to standardize the sampling, the number of recovered cfu using the two procedures was compared in 10 subjects. No significant difference in number of cfu CFU was detectable between the two sampling methods. Sampling with the template led to a lower standard deviation, which can

Table 2: Colony forming units dependent on dilution of the sample

Volunteer	Dilution	Colony forming units*							
		plate 1	plate 2	mean**	lg	plate 1	plate 2	mean	lg
		aerob	anaerob			aerob	anaerob		
1	10^{-3}	3	3	3	3.48	19	16	17.5	4.24
	10^{-4}	0	0			1	3		
	10^{-5}	0	0						
2	10^{-3}	78	60	69.5	4.84	112	120	118	5.07
	10^{-4}	4	10			13	11		
	10^{-5}	0	0			4	2		
3	10^{-3}	n.c.***	n.c.	1125	5.93	n.c.	n.c.	687.5	5.84
	10^{-4}	59	71			65	50		
	10^{-5}	4	28			8	8		
4	10^{-3}	n.c.	n.c.	855	5.93	n.c.	n.c.	1535	6.19
	10^{-4}	86	86			146	168		
	10^{-5}	8	9			13	17		
5	10^{-3}	32	33	31.25	4.49	67	61	69.5	4.84
	10^{-4}	4	2			7	8		
	10^{-5}	0	0			2	0		

*Conversion in cfu/ cm^2 with factor 1000

**Mean of countable cfu of all dilution steps

***not countable

Table 3: Number of colony forming units depending on sampling procedure (mean of 10 subjects)

Parameter	Swab without template	Swab with template
	CFU	CFU
Mean (s)	219,395 ± 201,800	174,470 ± 154,957
Logarithm (s)	5.34 ± 5.3	5.24 ± 5.19

be attributed to the defined swab area (Table 3). Therefore, this sampling technique tends to be more favorable.

Determination of the appropriate neutralizing agent: To neutralize Jack pro Spülung Plus (A) and aqueous polihexanide solution (B), three neutralizing agents were tested:

- 3% Tween 80 + 0.3% Lecithin + 0.1% Histidin + 0.1% sodium thiosulfate (TLH-Thio)
- lipofundin
- lipofundin-Thio (Lipofundin + 0.1% sodium thiosulfate).

Approaches were tested with and without addition of the different neutralizing agents to the test solutions A and B (Table 4). The increase in tested microorganisms was inhibited the least using Lipofundin-Thio. Thus, this combination was chosen (Table 4).

Number of colony forming units on 4 different swab areas on buccal mucosa: Sampling 1, 10 and 60 min after mouthrinsing on only one area of the mucosa could lead to a falsification of the results. To exclude this possibility, samples were compared on 4 areas of the mucosa (right and left, each cranial and caudal). No significant differences between the different areas were detected (Table 5). Thus, the method is reproducible.

3.2 Efficacy of the test products

A pre-condition for comparison of the test products was that the pre-treatment values between the test groups were not different after both aerobic and anaerobic cultivation (Table 6).

As expected, Ringer solution (test product C) was the least effective in terms of both aerobic and anaerobic oral cavity flora (Table 6). Under aerobic conditions, no effect was detectable 10 min after rinsing. After 60 min, the number of cfu increased slightly in comparison to the initial value. Under anaerobic conditions, the recolonization of the oral cavity was slightly delayed in comparison to aerobic conditions. This is irrelevant for oral cavity hy-

Table 4: Determination of neutralizing agent (each determined twice)

Component	Trypton/NaCl [ml]	Inactivator [ml]	Aqua [ml]	Test solution [ml]	Test microorganisms* [ml]	Log CFI/plate
NaCl	0.9				0.1	4.9
TLH-Thio		0.9			0.1	4.99
LF		0.9			0.1	5.0
LF-Thio		0.9			0.1	4.88
TLH-Thio/Aqua		0.8	0.1		0.1	4.99
LF/Aqua		0.8	0.1		0.1	4.83
LF-Thio/Aqua		0.8	0.1		0.1	4.9
A-TLH-Thio		0.8		0.1	0.1	4.93
A-LF		0.8		0.1	0.1	4.82
A-LF-Thio		0.8		0.1	0.1	5.03
B-TLH-Thio		0.8		0.1	0.1	4.86
B-LF		0.8		0.1	0.1	4.8
B-LF-Thio		0.8		0.1	0.1	4.91

* 10^4 – 10^5 CFU/ml (Sum parameter of the salivary flora)

Table 5: Number of colony forming units on different swab areas of the buccal mucosa (average from 4 volunteers, pre-treatment values)

Swab area	log CFU	
	average	standard deviation
right cranial region 14–17	4.98	0.52
right caudal region 44–47	5.35	0.62
left cranial region 24–27	4.82	0.76
left caudal region 34–37	5.05	0.47

Table 6: Efficacy of test products 1 min, 10 min and 60 min after mouthrinsing

Test product	Cultivation	Pre-value (log) x, s	Reduction factor (mean, s) after		
			1 min	10 min	60 min
A	Aerobic	5.74 ± 0.52	0.57 ± 0.53	0.8 ± 0.68	0.6 ± 0.74
	Anaerobic	5.89 ± 0.47	0.54 ± 0.46	0.78 ± 0.57	0.65 ± 0.64
B	Aerobic	5.58 ± 0.46	0.89 ± 0.58	0.65 ± 0.53	0.4 ± 0.47
	Anaerobic	5.66 ± 0.45	0.78 ± 0.42	0.61 ± 0.42	0.39 ± 0.45
C	Aerobic	5.75 ± 0.46	0.33 ± 0.36	–0.01 ± 0.45	–0.22 ± 0.44
	Anaerobic	5.79 ± 0.46	0.45 ± 0.31	0.2 ± 0.55	0.07 ± 0.5

giene, because the slight effect 1 min after mouthrinsing is a mechanical effect.

Test product B, an aqueous solution with a polihexanide content identical to that of test product A without additional tosylchloramide sodium, was significantly more effective 1 min after rinsing than test product A after both aerobic and anaerobic culturing (Table 7). Compared to Ringer solutions, both polihexanide-containing test products showed a significantly increased efficacy up to 60 min after rinsing on both aerobic and anaerobic oral-cavity flora (Table 6). Both test product A and test product B showed decreased efficacy within increasing exposure time. Nevertheless, after 60 min, test product A was more efficacious under aerobic and anaerobic conditions compared to Ringer solution immediate after rinsing. The test product A showed tendentially a greater efficacy than

Table 7: Statistical evaluation of average colony forming units (log values) for the 3 test products

Comparison	Cultivation	Pre-value	p after		
			1 min	10 min	60 min
C vs. A	Aerobic	ns*	ns	< 0.01	< 0.01
	Anaerobic	ns	ns	< 0.01	< 0.01
C vs. B	Aerobic	ns	< 0.005	< 0.01	< 0.01
	Anaerobic	ns	0.05	< 0.01	< 0.01
B vs. A	Aerobic	ns	ns	< 0.01	ns
	Anaerobic	ns	< 0.05	ns	ns

* not significant

product B both 10 min and 60 min after mouthrinsing (Table 6 and Table 7).
The reason that test product B is significantly more effective after 1 min compared to test product A, but after 10 and 60 min test product A tends to be more effective than test product B could be the influence of tosylchloramide sodium, because its addition modifies the surface tension of the polihexanide solution and delays attachment on oral mucosa. The residual activity of polihexanide [5] and tosylchloramide sodium [4] is expressed only after longer exposure times. The application of tosylchloramide sodium leads to the formation a chlorine coating on the mucosa by fixed N-Cl bonds, which may explain the residual efficacy [6].

3.3 Acceptance of test products

Test product A was perceived as enjoyable and refreshing by all volunteers. Test product B was validated as follows: 11 x disgusting, 18 x intolerable, 1 x tolerable. The taste of product B was validated as bitter. Because of the addition of a taste-improving agent in test product A, the unpleasant taste of polihexanide disappeared.

3.4 Possible applications for test product A

Based on these results, Jack pro Spülung Plus may be used as an adjunct to mechanical oral hygiene due to its bacteriostatic efficacy. This is important for seniors or geriatric patients, because their motoric and/or mental ability to clean the mouth is often limited. Due to their restricted abilty to perform a structured oral hygiene regime, including both toothbrushing and cleaning dental prostheses, a dramatic increase in plaque index was detected on teeth and dental prostheses among nursing-home residents [7]. This situation underlines the necessity of mouthrinsing with solutions which support their mechanical effect with additional bacteriostatic efficacy. A possible further area indication for Jack pro Spülung Plus is in cancer patients. During aggressive cancer chemotherapy, the oral mucosa is highly sensitive, meaning that teeth cannot be brushed and antiseptics such as chlorhexidine are not tolerated. In fact, due to the cytotoxicity of chlorhexidine [8], the rate of mucositis even increases [9].
In addition, the manufacturer of Jack pro Spülung Plus recommends the product for patients with tracheostoma, because long-term use of chlorhexidine is contraindicated [10]. Test product A would be a useful alternative.

4 Conclusion

The current results show that Jack pro Spülung Plus not only possesses the mechanical effect of a mouthwash (as does Ringer solution), but also a bacteriostatic effect that persists up to 60 min, due to its polihexanide and tosylchloramide sodium content.

Notes

Acknowledgements/Competing interests

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Corresponding author:
Prof. Dr. med. Axel Kramer
Institute of Hygiene and Environmental Medicine, University Medicine Greifswald, Walther-Rathenau-Str. 49 a, 17489 Greifswald, Germany, Phone: +49-(0)3834-515542, Fax: +49-(0)3834-515541
kramer@uni-greifswald.de

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