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MICROBIOLOGICAL EVALUATION OF A NOVEL IODOPHOR

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ABSTRACT

A new iodophor was designed so that iodine is complexed with the cationic polysaccharide chitosan.The study showed that chitosan-iodine complex (CIC) is readily dispersible in water and gradually releases most of the free iodine. CIC proved to be more effective antimicrobial agent than the widely used povidone-iodine (betadine).

INTRODUCTION

Iodine has a rapid and broad antimicrobiol activity against bacteria; including mycobacteria, fungi, viruses, protozoa, yeasts, and some spores [1]. Its activity is less affected by organic matter than that of other halogens, but is decreased at alkaline pH[2].

Iodine is used as a skin disinfectant in hospitals, and also a domestic antiseptic. Among the disadvantages which limit the use of iodine solutions are their staining of skin and fabrics, and the possible occurrence of skin reactions or irritation [3].

Iodine may be complexed by various nonionic and cationic surface-active agents, the resulting preparations being known as iodophors, since they act as iodine carriers. Active iodine is slowly liberated as the complexes are dispersed on dilution [4].

Iodophors are more stable and less reactive than ordinary iodine solutions. This diminished reactivity results in lower oral toxicity, a reduced corrosive action on metals, a decrease in skin irritation and staining, and a reduction in the volatility of iodine[5].

The addition of a surfactant to a germicide solution may be an important factor in bringing the solution into contact with the organism [6].

In the present work chitosan was utilized as iodine carrier and the antimicrobial activity of the new iodophor (chitosan-iodine complex) was evaluated in comparison with the commonly used povidone-iodine (Betadine). Chitosan is a polyaminosaccharide derived from chitin. It is nontoxic biological polymer having favorable characteristics such as immune adjuvant activity, biodegradability and biological compatibility[7].

MATERIALS AND METHODS

Materials:

Iodine was purchased from Andenex-Chemie Engelhard & Partner GmbH, Raboisen-Hamburg. Chitosan is the product of Sigma Chemical Co. St.Louis, MO, USA. Acetic acid and Tween 80 were obtained from Prolabo Co, France. Betadine (10% w/v povidone-iodine) was the product of the NILE Co.for pharmaceutical products, Cairo, Egypt, Batch No. J4L003, under License by Mundipharma AG, Basal, Switzerland. Nutrient broth and agar and Sabouraud's dextrose broth were the product of Oxoid, England.

Microorganisms:

The following standard strains were used in this investigation: *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 10536, *Pseudomonas aeruginosa* ATCC 9027, *Klebsiella pneumoniae* ATCC 10031 and *Candida albicans* ATCC 1023.

Preparation of chitosan-iodine complex (CIC):

CIC was prepared by mixing stock alcoholic iodine solution with 1% chitosan solution in 1% acetic acid, to yield a final iodine concentration of 0.1% to 0.4%.

Antimicrobial spectrum of chitosan-iodine complex by agar diffusion[8]:

Seeding the medium: Agar diffusion technique has been used successfully for determining microbial susceptibility to the different antimicrobial agents. To pre-seed the medium; nutrient agar for bacterial strains and Sabouraud's dextrose agar for yeasts;were melted and cooled to 48°C. A suspension of tested organism was added to give a concentration of about 10^5 cells/ml.

Mix well and pour into a 9-cm Petri dish on a flat surface. Dry the plates at room temperature for 1 hour. Agar depth of 4 mm is recommended. For yeast, a 24-hour old culture on Sabouraud's dextrose agar was washed from the surface with a small amount of sterile water. The suspension was vigorously pipetted or vortexed to break up large clumps of cells. Yeast suspension should contain 10^5 yeasts/ml.

Test: Holes were made in the medium with a sterile cork borer (10 mm in diameter) and the agar plugs were left out carefully so that the surrounding medium was not disturbed. Holes were completely filled with the tested antimicrobial agents and the plates were incubated for at least 4 hours at 37°C for bacteria and yeast strains.

The size of the zone of inhibition was used as a measure of the degree of susceptibility of the tested strains. The larger the zone of inhibition, the more susceptible the tested organism.

Evaluation of activity of chitosan -iodine by Kelsey-Sykes procedure[9,10].

One ml of the bacterial suspension prepared in broth (for clean) or in 5% (w/v) yeast suspension (for dirty), as required, was added to 3 ml of each disinfectant dilution with gentle shaking. After 8 minutes a sample of the mixture was removed and one drop was transferred to each of five tubes of the liquid recovery medium contains 3% (w/v) tween 80. Two minutes later, i.e., 10 minutes after the first inoculation, the disinfectant mixture was inoculated with a further 1 ml of the bacterial suspension, and 8 minutes later subculture as before. A further 2 minutes later, i.e, 20 minutes after the first inoculation, repeat the process again. The initial test was carried out at 20 to 22°C, and all subcultures were incubated at about 32°C for 48 hours. The number of tubes showing growth in the liquid medium was recorded. No growth of the tested organism in two or more of the five subculture tubes after the second incremental addition signified that the disinfectant was satisfactory for use at the initial concentration used in the test. In other words, a satisfactory concentration was identified where no growth was obtained in 40% or more of the total number of tubes after the second increment.

The test should be repeated several times on different occasions and the mean response calculated for each disinfectant dilution employed.

The 24-hour culture growth on agar slope should be washed from the surface with a small amount of broth, or yeast suspension, as appropriate, and diluted in the same liquid to give a concentration of 10^9 organism/ml.

For tests simulating clean conditions, the organisms were suspended in broth; whereas for those simulating dirty conditions, the organisms were suspended in yeast suspension.

All dilutions of disinfectant were freshly prepared in standard hard water (300 ppm hardness).

RESULTS

The antimicrobial activities of the chitosan-iodine complex (CIC) by agar diffusion method in comparison with that produced by Betadine and iodine solution are summarized in table (1).

The data revealed that CIC has a wide spectrum activity and is more efficient as antimicrobial agent than; the widely employed iodophor; Betadine.

On the other hand, CIC appears to be distinctly less potent than the free iodine preparation. Moreover, chitosan exerted some inhibitory action against the microorganisms under investigation (table 1) Kelsey-Sykes test:

Since CIC is non-phenolic compound, it has been useful to evaluate its activity in comparison with that produced by iodine solution and Betadine by one of the official procedure for evaluation of non-phenolic compounds, the Kelsey-Sykes procedure. The obtained results are summarized in table (2). The table revealed that, CIC is more efficient as antimicrobial agent than Betadine.

CIC passed the test at lower iodine concentration against the tested microorganisms than Betadine. However, the antiseptic activity was slightly reduced (table 2) by carrying out the test under dirty condition. Evidently, free iodine preparation was highly active against the organisms investigated than the iodopher preparations (table 2).

DISCUSSION

Iodine tincture and solutions are used externally as disinfectants and antiseptics for their broad microbicidal

Zagazig J. Pharm. Sci., December 1995.
Vol. 4, No. 2, pp. 79-83

Table (1): Antimicrobial activities of CIC, Betadine, iodine solution, and chitosan by agar diffusion method.

Microorganisms	Diameter of Inhibition Zone (mm)			
	CIC**	Betadine	Iodine solution	Chitosan
Staph. aureus ATCC 6538	22 mm	19 mm	24 mm	15 mm
Candida albicans ATCC 10231	15 mm	14 mm	17 mm	12 mm
E.coli ATCC 10536	18 mm	15 mm	20 mm	13 mm
Ps. aureginosa ATCC 9027	20 mm	16 mm	22 mm	14 mm
Kl. pneumoniae ATCC 10031	16 mm	15 mm	19 mm	13 mm

**CIC = Chitosan -Iodine Complex

Table (2): Antimicrobial Evaluation of CIC, Betadine and iodine solution, by Kelsey-Sykes procedure.

Strain No.	CIC				Betadine				Iodine solution			
	Clean		Dirty		Clean		Dirty		Clean		Dirty	
	*Conc	result	Conc	Result	Conc.	Result	Conc.	Result	Conc.	Result	Conc.	Result
Staph. aureus ATCC 6538	0.03%	Pass	0.04%	pass	0.04%	Pass	0.05%	Pass	0.02%	Pass	0.03%	Pass
Candida albicans ATCC 10231	0.05%	Pass	0.06%	Pass	0.06%	Pass	0.07%	Pass	0.04%	Pass	0.05%	Pass
E.coli ATCC 10536	0.04%	Pass	0.05%	Pass	0.05%	Pass	0.06%	Pass	0.03%	Pass	0.04%	Pass
Ps. aureginosa ATCC 9027	0.03%	Pass	0.04%	Pass	0.03%	Pass	0.05%	Pass	0.02%	Pass	0.03%	Pass
Kl. pneumoniae ATCC 10031	0.04%	Pass	0.05%	Pass	0.05%	Pass	0.06%	Pass	0.03%	Pass	0.04%	Pass

* Conc.=Concentration of iodine content

spectra against bacteria, fungi, viruses, protozoa, and yeasts.

Iodine is believed to destroy microorganisms by inactivation of protein through iodination and oxidation [3].

For the sake of lower toxicity and irritation, iodine has been dispersed in various nonionic and cationic surfactants. These complexes are known as iodophors. In this study, a novel iodophor which is chitosan-iodine complex (CIC) has been tested for its antimicrobial spectrum and activity by agar diffusion method and Kelsey-Sykes test. CIC has been tested in comparison with the commonly used iodophor, Betadine, as well as the free iodine solution.

All preparations had equal iodine content, the only difference is the formulation of iodine content. In CIC, iodine has been complexed with cationic biopolymer chitosan in 1% acetic acid. In Betadine preparation, iodine has been complexed with povidone (polyvinyl pyrrolidone). Aqueous free iodine has been prepared in 1% acetic acid solution. The antimicrobial activity of iodophors, by using agar diffusion (table 1) and Kelsey and Sykes (table 2) methods revealed that, they were less reactive than ordinary iodine solution. CIC had a more inhibitory action upon the tested organisms than that exerted by the povidone-iodine complex (Betadine).

In addition, chitosan biopolymer exerted some inhibitory action against the different organisms (table1).

Briefly then, CIC has an almost all advantages for practical use. The complex possesses quick microbicidal action on a wide array of microorganisms, and is miscible with water. In the use dilution, CIC does not stain skin and neutral fabrics permanently.

Away from the previously mentioned advantages of CIC which are usually shared by most iodophors including Betadine, the biopolymer chitosan, has extra benefits which gave CIC the priority in practical use. Chitosan is an attractive candidate for a wound-healing treatment because it forms a tough, water-absorbent, biocompatible film[11] and this film can also be formed directly on the burn by application of an aqueous solution of chitosan acetate. Surprisingly and unexpectedly, chitosan solutions in acidic water provide a cool and pleasant soothing effect when applied to open wounds [12]. Another advantage of chitosan in the treatment of wounds is its oxygen permeability. Thus, prevent oxygen-deprivation of tissues which occurs in the eye, for example, when low permeability drops are used [13].

Chitosan is slowly degraded by lysozyme which is transported to the wound area by the inflammatory cells (polymorphonuclear leucocytes), means that chitosan film need not to be periodically removed from the wound at all [14].

Chitosan solutions demonstrated growth inhibitory actions against the organisms often encountered in burns which are *Pseudomonas aeruginosa* *Staphylococcus aureus* and *Streptococcus pyogenes* as well as the intestinal bacterium *E.coli* and Candida yeast which is usually present on human skin[12].

Chitosan is a hemostatic agent[15] and induces tissue repair[16]. The inductive and stimulatory activity of chitosan on connective tissue-rebuilding is clearly demonstrated, and it is suggested that chitosan could be considered a primer on which a normal tissue architecture is organized. The good healing produced by chitosan provides evidence for satisfactory biocompatibility[17]. Toxicity tests of the chitin in sutures, including mutagenicity, acute toxicity, pyrogenicity, hemolysis and skin reaction were negative in all respects[18].

In conclusion, the hemostatic action, burn and wound healing properties, as well as the bacteriostatic and fungistatic characteristics of chitosan solution provide us the initial idea of profitable commercial utilization of this polyaminosaccharide in our novel iodophor.

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التقييم الميكروبيولوجي لمستحضر أيودوفور جديد

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تم تصميم هذا الأيودوفور الجديد بحيث توجد مادة الأيودين في صورة تراكب بواسطة مادة الكيتوزان. يعتبر مستحضر الأيودين - كيتوزان سهل الانتشار في الماء، مع خاصية الانطلاق التدريجي لجزيئات الأيودين الحره. أثبتت الدراسة أن مستحضر الأيودين - كيتزان أكثر فاعلية كمادة مضادة للميكروبات من مستحضر المحلول المطهر أيودين - بوفيدون (بيتادين) الأكثر شيوعا.