International Journal of Pharmaceutics, 34 (1986) 45–49
Elsevier

IJP 01134

Effect of polyvinylpyrrolidone molecular weight upon the antimicrobial activity of povidone–iodine antiseptics

Ibrahim S.I. Al-Adham and Peter Gilbert

Department of Pharmacy, University of Manchester, Manchester (U.K.)

(Received 2 April 1986)
(Modified version received 18 June 1986)
(Accepted 26 June 1986)

Key words: Polyvinylpyrrolidone molecular weight; Povidone–iodine; Antiseptic; Antimicrobial activity; Iodine antiseptic

Summary

The antimicrobial activity of various povidone–iodine formulations has been assessed against *Escherichia coli*, where the molecular weight of the polyvinylpyrrolidone has been varied between 10 000 and 360 000. Bactericidal activity was directly related to polymer molecular weight and could be attributed to molecular-weight-dependent changes in the proportions of free and available iodine. When bactericidal activities were related to those of diluted Lugol's iodine solutions, then formulation as an iodophor was observed to give an approximate doubling of activity. It is suggested that this molecular-weight-independent potentiation of iodine action could be related to alteration of membrane-conformation, rather than to concentration, by the polymer, of iodine at its target.

Introduction

Iodine has long been employed as a broad spectrum antimicrobial agent. Many of the problems associated with its use can be significantly reduced by formulation as an iodophor. One such iodophor is povidone–iodine (PVP:I). PVP:I is a complex of iodine and polyvinylpyrrolidone, such that it contains not less than 9% and not more than 12% available iodine calculated on a weight basis (USP, 1980). No specification is made as to the molecular weight or molecular weight distribution of the polymer used. These are generally heterodisperse and have molecular weights in the range of 49 000–30 000, but may contain materials of < 10 000 and > 100 000 (Molyneux, 1983).

Correspondence: P. Gilbert, Department of Pharmacy, University of Manchester, Oxford Rd., Manchester M13 9PL, U.K.

PVP:I contains tightly-bound iodine, reacted with the polymer endgroups (Siggia, 1957; Eliassaf, 1966) and loosely-bound iodine complexes as I_3^- in a helical matrix of the polymer (Takikawa et al., 1978a and b; Vratsonas, 1983). This loosely bound iodine is in equilibrium with I_3^-, I_2 and I^- in free solution (Horn and Ditter, 1983). Berkelman et al. (1982) demonstrated that dilution of stock solutions of PVP:I increased activity. Other workers (Anderson et al., 1983; Cantor and Winicov, 1962) related such increases to changes in the loosely-bound and free iodine. Free iodine concentrations increase on dilution of PVP:I due to weakening of the ionic link of the I_3^- and the carrier polymer (Truman, 1971). It has been generally thought that the antimicrobial activity of PVP:I is related to its reservoir effect for free iodine (Cantor and Winicov, 1962), polyvinylpyrrolidone (PVP) per se has, however, been demonstrated to interact non-leth-

ally with cell membranes (Rokem, 1983; Ben-David and Gavendo, 1972). This has led to the suggestion that povidone–iodine formulations potentiate the innate activity of the iodine (Digenis et al., 1983). Such potentiation might well be dependent upon polymer molecular weight, and if present, necessitate regulation of the molecular weight distribution used for PVP:I manufacture. The physicochemical properties of various molecular weight formulations of PVP:I have therefore been evaluated in this study and related to their antimicrobial activity.

Materials and Methods

Organisms and cell suspensions

Escherichia coli ATCC 8739 was used throughout. Cultures were maintained on nutrient agar (Oxoid CM3) at RT in a darkened cupboard, following overnight incubation at 35°C. Cultures were replaced at 2 week intervals. Washed suspensions were prepared from stationary phase (16 h) nutrient broth (Oxoid CM1) cultures (100 ml in 250 ml conical flasks), grown at 35°C in an orbital incubator (150 osc./min). Cultures were harvested by centrifugation at 10000 × *g* for 15 min at 35°C washed twice and finally resuspended to give a cell density of 10^8/ml in sterile normal saline. These suspensions were used immediately.

Chemicals

Povidone–iodine formulations were made from various molecular weight polyvinylpyrrolidones. These were obtained from the Aldrich Chemical Co. (U.K.) as 10000, 24000, 40000 and 360000 molecular weight samples. Cellulose acetate membranes were obtained from Gallenkamp U.K.

Preparation of povidone–iodine formulations

Polyvinylpyrrolidone (10 g), iodine (1 g) and potassium iodide (0.5 g) were accurately weighed and the iodine dissolved in alcohol (1 ml), in a volumetric flask. To this the potassium iodide and a solution containing the polymer (ca. 90 ml) were added and the resultant solution made up to 100 ml with distilled water. The solutions were thoroughly mixed, stoppered and allowed to equilibrate at RT in the dark for 72 h.

Determination of bactericidal activity

Aliquots (1 ml) of washed cell suspension were added to solutions (19 ml) of the antiseptics, diluted where appropriate in sterile distilled water and held in stoppered test-tubes. At various time intervals, aliquots (1 ml) were removed and transferred to tubes containing sodium thiosulphate (0.2% w/v) neutraliser solution. These were thoroughly mixed and left for 5 min. Further serial dilutions were carried out in sterile normal saline. Samples (0.2 ml) were taken from appropriate dilutions and transferred to the surfaces of triplicate, predried, nutrient agar (Oxoid CM3) plates. Viable counts were made after incubation of the plates at 35°C for 16 h and counts expressed as the mean relative to the untreated controls.

Analysis of povidone–iodine formulations

Total available iodine was assayed in each of the formulations at each dilution by potentiometric titration with standardised sodium thiosulphate. Total iodine was calculated from the amount added and the dilutions made. The free iodine species present at each dilution, were assayed spectrometrically at 288 nm and 353 nm before and after addition of excess potassium iodide. This assessed the I_3^- content and reassayed after conversion of the I_2 to I_3^-. Free iodine species were separated prior to assay, from the polymer and loosely-bound species, by dialysis across cellulose acetate membranes. Dialysis was conducted statically at 20°C using donor:recipient volume ratios of 10:1. This minimised the equilibrium changes in the donor solution and achieved equilibrium at 6 h. No polymer was observed to be present in the recipient solution after this time.

Results

The rate of inactivation of *E. coli* cells by Lugol's solutions and the PVP:I formulations was observed to be extremely rapid and completed within 1–2 min of contact; survival was therefore estimated following a fixed contact time of 5 min. Such data, for Lugol's solution, is presented in Fig. 1. At least 25 separate determinations were

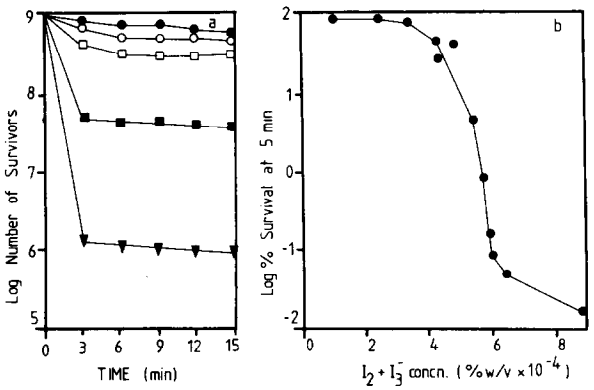

Fig. 1. Bactericidal activity of Lugol's iodine solution against washed suspensions of *Escherichia coli*. Concentration of $I_2 + I_3^-$ (●), 0; (○), 0.0002% w/v; (□), 0.0003% w/v; (■), 0.0004% w/v; (▼), 0.0006% w/v.

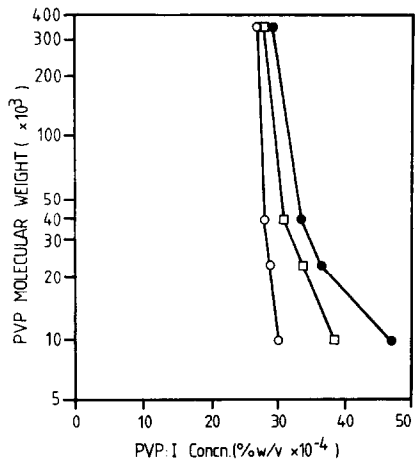

Fig. 3. Bactericidal activity of povidone–iodine formulations as a function of polyvinylpyrrolidone molecular weight. Concentrations of added iodine giving (○), 90%; (□), 99% and (●) 99.9% kill of an *Escherichia coli* washed suspension in 5 min.

made on each PVP:I formulation and related to concentration. These data are illustrated in Fig. 2 for the four formulations. Above certain critical concentrations (ca. 0.002% w/v), activity was observed to be proportional to the molecular weight of the polymer. This is illustrated in Fig. 3, where the concentrations giving 90%, 99% and 99.9% kills are plotted as functions of polymer molecular weight. At these concentrations, the polymers alone were observed to have no lethal effect on the cells (< 1% w/v).

Available iodine (loosely-bound + free) was directly related to PVP:I concentration and polymer molecular weight (Fig. 4). Similarly, the proportion of tightly-bound material, being related to endgroup concentration, decreased with increasing molecular weight of the polymer. Free I_3^- concentrations increased with PVP:I concentration in all cases yet free I_2 maximised at PVP:I con-

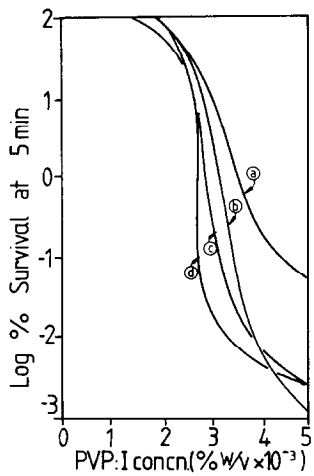

Fig. 2. Collected data to illustrate the antimicrobial activity of various molecular weight povidone–iodine formulations: (a) 10000; (b) 24000; (c) 40000; (d) 360000.

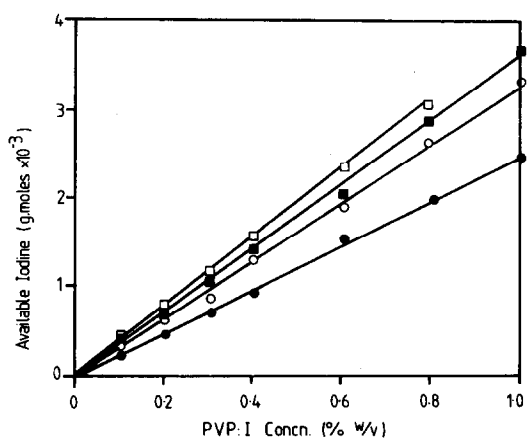

Fig. 4. Interrelation of available iodine and povidone–iodine concentration for various molecular weight formulations: (●), 10000; (○), 24000; (■), 40000; (□), 360000.

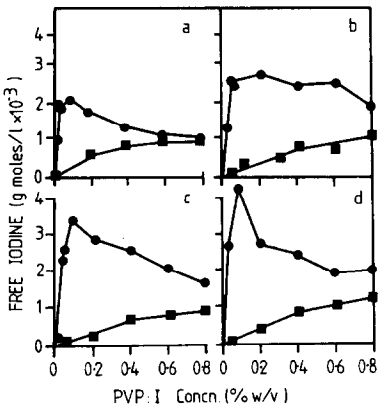

Fig. 5. Interrelation of free iodine (●) and I_3^- (■) concentration for various dilutions of different molecular weight povidone–iodine formulations: (a), 10000; (b), 24000; (c), 40000; (d), 360000.

centrations of ca. 0.1% w/v and subsequently decreased with increasing PVP:I concentration (Fig. 5). Up to these maximal values for free I_2, then all the available iodine was accountable as the free forms, the amount of which was inversely related to polymer molecular weight. Concentrations of free/available iodine ($I_2 + I_3^-$), associated with PVP:I concentrations giving 90, 99 and 99.9% kills are presented in Table 1 and comparison made to Lugol's solutions. These data show that the variation in activity for the PVP:I's could be totally accounted for in terms of available iodine. Activity levels, however, were approximately twice those predicted by the corresponding concentrations of Lugol's solution.

Discussion

Variation in activity for the four formulations (Figs. 2 and 3) could be totally accounted for in terms of available/free iodine vs total iodine (Table 1). This observation is in agreement with the suggestions of Anderson et al. (1983), who related total killing time for dilutions of a commercial PVP:I formulation to their free iodine content. The observed maximal levels of free iodine concentration, with dilution, have been previously reported for commercial PVP:I samples (Truman, 1971; Horn and Ditter, 1983) and related to maximal antimicrobial activity for particular dilutions of the product (Berkelman et al., 1982). The relationships between polymer molecular weight and the fractions of tightly-bound iodine, and maximal free iodine levels reflect the proportion of endgroups present within solutions of the polymers. These will increase with decreasing polymer molecular weight, hence endgroup-iodination will be greatest in solutions of the lowest molecular weight material. Since, in the various Pharmacopoeias, PVP:I is standardised by its available iodine content, assayed titrimetrically, then variation in the molecular weight distribution of the polymer is unlikely to affect activity.

The increased activity of PVP:I over Lugol's solution suggests a potentiation of I_2 action by PVP. This has previously been proposed by various workers (Digenis et al., 1983; Rokem, 1983) to result from interaction of PVP with the cytoplasmic membrane causing a localised release of iodine at its primary target. Since in this study

TABLE 1

AVAILABLE/FREE IODINE CONCENTRATIONS ASSOCIATED WITH SOLUTIONS OF POVIDONE–IODINE AND LUGOL'S SOLUTION WHICH GIVE VARIOUS LEVELS OF KILLING OF AN *ESCHERICHIA COLI* SUSPENSION IN 5 min

	Available/free iodine concentration (gmol/l $\times 10^5$) giving:		
	90% kill	99% kill	99.9% kill
Lugol's solution	2.0	2.2	2.5
PVP:I (10000)	0.75 (1.5) *	0.95 (1.9)	1.1 (2.2)
PVP:I (24000)	0.92 (1.15)	1.1 (1.38)	1.2 (1.5)
PVP:I (40000)	1.0 (1.1)	1.1 (1.21)	1.2 (1.33)
PVP:I (360000)	1.0 (1.0)	1.1 (1.1)	1.2 (1.2)

* Value in brackets indicates total iodine present.

concentrations have been employed for which all the available iodine is present in the free form (Fig. 5) then potentiation is more likely to have resulted from PVP interaction per se at the membrane, increasing the accessibility of membrane proteins towards iodine. In this respect osmotic protection of cells by PVP (Ben-David and Gavendo, 1972) and increased membrane fluidity following PVP treatment of cells (Rokem, 1983) have been reported. In the latter study activity of NADH oxidase, an integral protein of the cytoplasmic membrane, was increased suggesting that PVP had altered the environment of the protein. Since the observed potentiation of iodine action is independent of polymer molecular weight, then, once again, variations in molecular weight and molecular weight distribution of the PVP:I formulations ought not to influence their activity.

References

Anderson, R.L., Berkelman, R.L. and Holland, B.W., Microbiological investigations with iodophors. In Digenis, G.A. Ansell, J. (Eds.), *1st International Symposium on Povidone* University of Kentucky, U.S.A., 1983 pp. 146–157.

Berkelman, R.L., Holland, B.W. and Anderson, R.L., Increased bactericidal activity of dilute preparations of povidone iodine. *J. Clin. Microbiol.*, 15 (1982) 635–639.

Ben-David, A. and Gavendo, S., The protective effect of polyvinyl-pyrrolidone and hydroxyethyl starch on non-cryogenic injury to red blood cells. *Cryobiology* 9 (1972) 192–197.

Cantor, A. and Winicov, M., Germicidal compositions and methods for preparing the same. *US Patent*, (1962) 3,028,300, Cl. 167

Digenis, G.A., Rokem, S., Eckert, D. and Blecher, L., Studies on the association of ^{14}C-povidone-^{131}I with red blood cell and bacterial membranes. In Digenis, G.A. and Ansell, J. (Eds.), *1st International Symposium on Povidone*, University of Kentucky, U.S.A., 1983, pp. 302–311.

Eliassaf, J., The interaction of polyvinyl pyrrolidone with iodine. *Eur. Polymer J.*, 2 (1966) 269–278.

Horn, D. and Ditter, W., Physicochemical fundamentals of the microbicidal action of povidone–iodine. In Digenis, G.A. and Ansell, J. (Eds.), *1st International Symposium on Povidone*, University of Kentucky, U.S.A., 1983, pp. 120–140.

Molyneux, P., The physical chemistry and pharmaceutical applications of polyvinylpyrrolidone. In Digenis, G.A. and Ansell, J. (Eds.), *1st International Symposium on Povidone*, University of Kentucky, U.S.A., 1983, pp. 1–19.

Rokem, S.J., Interaction of polyvinylpyrrolidone with *Acholeplasma laidlawaii*. In Digenis, G.A. and Ansell, J. (Eds.), *1st International Symposium on Povidone*, University of Kentucky, U.S.A., 1983, pp 312–316.

Siggia, S., The chemistry of polyvinylpyrrolidone-iodine. *J. Am. Pharm. Assoc.*, 46 (1957) 201–204.

Takikawa, K., Nakano, M. and Arita, T., Change in apparent permeability of iodine in the presence of polyvinylpyrrolidone. *Chem. Pharm. Bull.*, 26 (1978a) 874–879.

Takikawa, K., Nakano, M. and Arita, T., Spectrometric studies on accelerated hydrolysis of iodine in the presence of polyvinylpyrrolidone. *Chem. Pharm. Bull.*, 26 (1978b) 1370–1374.

Truman, J.R., The halogens. In Hugo W.B. (Ed.), *Inhibition and Destruction of the Microbial Cell*, Academic Press, London, 1971, pp. 137–180.

USP, *United States Pharmacopoeia XX*, Mack Publishing Co., Easton, PA, U.S.A., 1980.

Vratsonas, S.M., On the structure and function of povidone–iodine. In Digenis, G.A. and Ansell, J. (eds.), *1st International Symposium on Povidone*, University of Kentucky, U.S.A., 1983, pp. 289–301.