# Human Papillomavirus Infection

Chapter 1

# Povidone-Iodine as Treatment for Human Papillomavirus

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## 1. Introduction

Verruca vulgaris, also known as common warts, are benign skin growths caused by a viral infection of the skin. Treatment is recommended for patients with extensive, spreading, or symptomatic warts [1]. Many patients feel the condition is socially stigmatizing and thus seek treatment. Treatment methods that are commonly employed include topical agents, intralesional injections, cryotherapy, laser, electrodessication and surgical excision [2,3]. Clinical evidence favoring one therapeutic route over another is limited, which provides rationale as to why there are so many anecdotal treatment options [4]. The current treatment options lack specific anti-viral options, and instead focus on physical destruction, chemical destruction, and localized irritants that are intended to upregulate the immune system and immunomodulatory therapies. This may provide an explanation as to why some common warts are resistant to treatment.

## 2. History of Early Iodophor Preparations

An iodophor is a preparation containing iodine complexed with a solubilizing agent. The result is a water-soluble material that releases free iodine when in solution. Iodophors are prepared by mixing iodine with the solubilizing agent; heat can be used to speed up the reaction. Iodophor preparations are commonly used for skin antisepsis prior to percutaneous procedures and surgery [5]. They are rarely used outside of this purpose; however, when formulated properly, they have the potential to be effective across a broad array of infections spanning numerous fields of medicine due to their potent, safe and effective abilities as anti-

microbials. Of the known iodophor preparations, povidone-iodine (PVP-I) is the formulation used in clinical practice today. It is composed of a polymer, polyvinylpryrrolidone, and iodine. The derivation of this formulation will be discussed below.



## 3. Elemental Iodine

Iodine is the heaviest element commonly used by most life forms. Iodine was discovered by the French chemist Barnard Courtois in 1811 when he was extracting sodium and potassium compounds from seaweed ash. Once these compounds were removed, he added sulfuric acid ($H_2SO_4$) to further process the ash [6]. It takes its name from *iode*, the Greek word for "violet," as suggested by Gay Lussac, a reference to the deep violet gas that can be seen sublimating from the solid element at room temperature and pressure. Situated below bromine in the Group 17 halogen family of the periodic table, iodine derives much of its clinical utility from the combination of its low toxicity, high molecular weight, and reactivity with organic molecules. Like all halogens, it occurs in nature as organoiodine compounds, as components of ionic salts, or as molecular iodine [5]. The unique chemical reactivity has enabled the development of an array of iodine-containing preparations for use in medicinal chemistry.

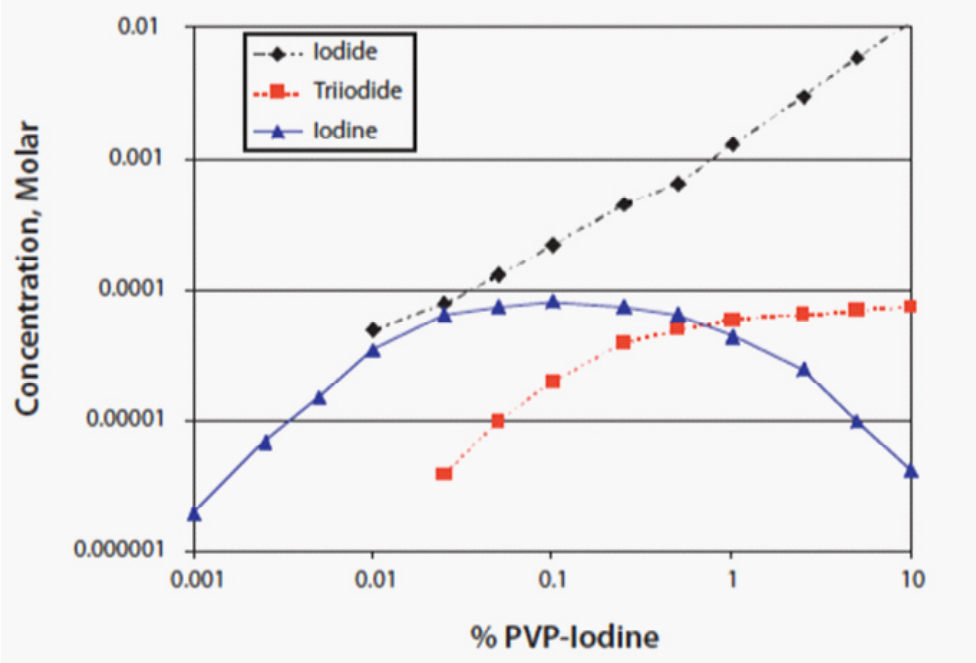
## 4. Early Iodine Preparations

Although its antiseptic effect in medicinal preparations was recognized soon after its discovery, widespread use was limited by poor solubility in water, limited chemical stability, and high local toxicity. Attempts were made to ameliorate all of these shortcomings and these attempts led to the first generation of medicinal iodine compounds, known as Lugol's solution. Lugol's solution, first prepared in 1829 by Lugol, was made to overcome the poor aqueous solubility of iodine by elemental iodine with potassium iodide (KI) [5]. This solution became the universally accepted way of administering iodine by mouth at the time [7]. Iodide and molecular iodine combine to shift the equilibrium distribution of aqueous iodine species to favor increased disproportionation towards easily soluble triiodide ($I_3$-) (**Figure 1**) [8]. Lugol's solution is an effective microbiocide, a starch indicator, a nuclear stain, and a moderator of thyroid activity. It has been used historically for treatment of hyperthyroidism by inhibiting the production of iodinated thyroid hormones [9]. Until recently, Lugol's solution was available as an OTC preparation in most US pharmacies, although its sale is now restricted because it is used in the clandestine production of methamphetamine [10].

While Lugol's solution solved the stability problem inherent in the aqueous delivery of elemental iodine as an antiseptic, the solubility problem was more effectively addressed by the development of iodine "tinctures". These formulations further address solubility by employing up to 70 percent (w/w or w/v) of alcohol (almost always ethanol). In this way, tinctures achieve higher iodine solubility and deliver a higher concentration of elemental iodine per given aqueous unit [10].

It took about a hundred years from the creation of Lugol's solution to the next major improvement in the delivery of medicinal iodine compounds. First publicly disclosed in a US Patent in 1952 Shalanski, complexation of elemental iodine with organic polymers that could subsequently "release" free molecular iodine in aqueous systems quickly became the most common way to solve the three problems associated with iodine: stability, solubility, and toxicity [11].

**Figure 1**: Aqueous Equilibrium Reactions of PVP-I




## 5. Current Iodine Preparations

What Shalanski first described in 1952 became what we now know as povidone-iodine (PVP-I) or "Betadine." This is a complex of triiodide ($I_3$-) and the organic polymer polyvinylpyrrolidone. PVP-I solved the combined problems of low solubility, poor chemical stability, and local toxicity by wrapping active free iodine in a soluble polymer matrix. The carbonyl groups of the pyrrolidone ring complex negatively charged ions and thus allowed higher effective concentrations to be safely delivered to target tissues without local toxicity [11].

Polyvinylpyrrolidone (PVP) is an off-white powder, readily soluble in water and many organic solvents. It resembles the proteins of natural plasma in its capacity for binding water and absorbing other substances and also the ability to render diffusible coarsely dispersed dyes [12]. In attempting to titrate the iodine when in it is complexed with PVP, it was discovered that not all the iodine could be recovered. Repeated extractions with chloroform were unsuccessful in retrieving the missing portion of iodine. Further additions of Lugol's solutions or tinctures to the iodine-containing PVP, however, resulted in no similar loss of iodine. Shelanski and coworkers hypothesized that the lost iodine was bound to the PVP in the form of organically bound iodide that acts to stabilize iodine in the same manner as potassium iodide in Lugol's solution. They prepared an aqueous solution of iodine combined with PVP without the aid of

usual solvents or solubilizing substances. They thusly showed that PVP itself acts as a carrier of iodine in water solutions. The chemical nature of PVP-I has been elucidated as showing a small fraction of iodine being converted directly into inorganic iodide. The reaction occurs at the ends of the PVP molecules, and the amount of inorganic iodide formed is determined by the number of free end groups and thereby by the molecular weight of any given PVP sample. About 30 percent of the iodine is converted into organic iodide, and the remainder of the iodine exists as free elemental iodine [13].

## 6. Povidone-Iodine as an Anti-Microbial

In aqueous solution systems, the reactions between the PVP-I complex and water leads to the generation of seven different forms of iodine at equilibrium. Hydrolysis, dissociation, and disproportionation all contribute to the equilibrium distribution of iodine species. Of the seven iodine forms, a biocidal effect is only attributed to molecular iodine ($I_2$), hypoiodous acid (HOI), and the iodine cation ($H_2O$+I). The PVP portion itself has no biocidal effect, but owing to its affinity for cell membranes is able to deliver the iodine-containing preparation to the target. For PVP-I, free molecular iodine ($I_2$) is almost entirely responsible for the observed microbiocidal activity [14]. Solutions with the same total concentration of iodine but different amounts of free iodine vary greatly in their antiseptic efficacy [15]. Irritation, stinging and burning are potential complications of iodine use at higher concentrations but the PVP-I system eliminates these toxicities by carrying the iodine in a complexed, non-irritating vehicle. Most PVP-I used for medicine is standardized to deliver between 0.5 percent and 1.0 percent free molecular iodine on dissolution. Thus, the common pre-surgical 10 percent Betadine delivers about 1 percent of biocidal, free molecular iodine [10].

Iodine has been recognized as a broad spectrum, resistance-free biocidal agent for many years. The microbiocidal action of povidone-iodine is a result of the non-complexed, freely mobile elemental iodine ($I_2$). Molecular $I_2$ can freely enter cells and works via a variety of pathways to eliminate microorganisms in a non-specific manner. It is likely that free iodine poisons electron transport, inhibits cellular respiration, destabilizes membranes, inhibits protein synthesis and denatures nucleic acids [10]. A synopsis of the electron-microscopic and biochemical observations supports the conclusion that PVP-I interacts with the cell walls of micro-organisms which causes permanent or transient pore formations or generates solid-liquid interfaces at the lipid membrane level, leading to loss of cytosolic material in addition to enzyme denaturation [16]. All of these mechanisms derive from fundamental electron-electrophile reactions whose inhibition, though possible, would require mutations inconsistent with the definition of "living organisms" [17]. This provides explanation of the lack of development of resistance. Povidone-iodine kills microorganisms including bacteria, viruses, yeasts, molds, fungi, and protozoa [18].

## 7. Antiviral Properties of PVP-I

Human papillomavirus (HPV) is a small, non-enveloped deoxyribonucleic acid (DNA) virus that infects skin or mucosal cells. The circular, double-stranded viral genome is approximately 8-kb in length.

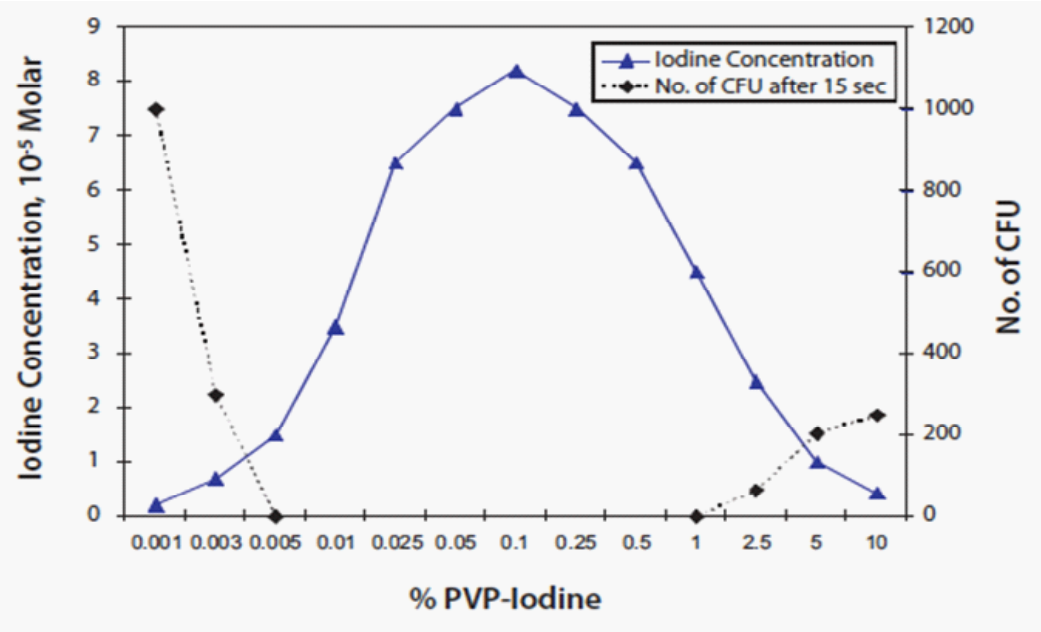
*In vitro* studies have demonstrated the ability of PVP-I to eradicate numerous classifications of viruses. Inactivation of a range of viruses, such as adeno, mumps, rota, polio (types 1 and 3), coxsackie, rhino, herpes simplex, rubella, measles, influenza and human immunodeficiency viruses have been studied. In these experiments, antiseptics such as PVP-I solution, PVP-I gargle, PVP-I cream were used. PVP-I was effective against all the virus species tested. PVP-I drug products inactivated all the viruses within a short period of time and had a wide virucidal spectrum, covering both enveloped and non-enveloped viruses [19]. Low dose PVP-I preparations have also demonstrated considerable efficacy against the highly pathogenic avian influenza. The in vitro antiviral activity of low concentration PVP-I products (2% PVP-I solution, 0.5% PVP-I scrub, 0.25% PVP-I palm, 0.23% PVP-I gargle, 0.23% PVP-I throat spray and 2% PVP-I solution) reduced viral infectious titers to levels below the detection limits by incubation for only 10 seconds with the PVP-I products, indicating that PVP-I products have virucidal activity against avian influenza A viruses [20].

Ebola virus (EBOV) epidemics highlight the need for efficacious virucidal products to help prevent infection and limit the spread of EBOV disease. However, there is limited data on the efficacy of virucidal products against EBOV because the virus has a high biosafety level and is only available in a few laboratories worldwide. Egger et al. studied the in vitro efficacy of four povidone iodine (PVP-I) formulations against EBOV: 4 % PVP-I skin cleanser; 7.5 % PVP-I surgical scrub; 10 % PVP-I solution; and 3.2 % PVP-I and 78 % alcohol solution. Viral titers of EBOV were reduced by >99.99 % to >99.999 % under clean and dirty conditions after application of the test products for 15 seconds [21].

Animal models also point to the antiviral capabilities of PVP-I. Specific in vitro data evaluating efficacy of PVP-I against HPV is lacking, however bovine papillomavirus is very similar to HPV and showed approximately 90% inactivation with exposure to 0.1% PVP-I, and 99.9% inactivation with 0.3%, suggesting PVP-I may reduce the rate of sexual transmission of the human papillomavirus strains associated with cervical cancer [22]. HPV disrupts the normal cell cycle via proteins E6 and E7, whereas bovine papilloma virus (BPV) can disrupt cell cycle protein E5, E6 or E7 depending upon the specific viral serotype. The genomic plan of the BPV-1, BPV-2 and BPV-5 is similar to that of most other papilloma viruses, whereas BPV-3, BPV-4 and BVP-6 lack the E6 gene, which has been replaced by the E5 gene [23]. Overlap amongst between HPV and BPV is further demonstrated by the efforts to development of a therapeutics bovine vaccine against the E6 and E7 proteins [24].

As demonstrated above by Ito et al, it is of particular interest that lower concentrations of PVP-I have been shown in the chemistry literature to be more effectiveantimicrobials [25]. This paradoxical effect is not completely understood but likely stems from the increased free-iodine available in more dilute solutions. It is suggested that as the polymer complex "un-wraps" in more dilute solutions, more free iodine is translocated from inner hydrophilic sites to outer solubilized sites, thus generating more free molecular iodine [26]. Although the exact mechanism remains elusive, the net effect is incontrovertible: 10 percent PVP-I solutions reach a maximum of antimicrobial efficacy *in vitro* as dilutions of 1/100 (**Figure 2**) [8].

**Figure 2**: Correlation of [I2] and %PVP-I With Microbial Reduction After 15s




## 8. Toxicity of PVP-I

There is an abundance of studies focusing on toxicity of iodophor formulations used in ophthalmology, otolaryngology, cardiology and urology, however there are very few studies assessing the toxicity of PVP-I in regards to the skin. The rationale behind the lack of studies is likely the frequency, duration and volume of PVP-I surgeons are exposed to as a pre-operative scrub without any reported systemic absorption or side effects. A single case report exists of a chemical burn on the skin of a 1 year old that was exposed to a surgical sheet immersed in 10% PVP-I solution under occlusion for 4 hours [27]. Other studies have shown the protective effect of povidone-iodine ointment against skin lesions induced by chemical and thermal stimuli [28].

At a molecular level, fibroblast growth has been reported to be progressively retarded with 0.01% and 0.025% PVP-I solutions, and totally inhibited by 0.1% and 1% PVP-I solutions in vitro suggesting caution should be used when PVP-I is placed on an open wound, as it may impede wound healing [29]. The preponderance of evidence, however, disputes this study. A critical review of studies concerning PVP-I and wound healing was undertaken, with emphasis placed on *in vivo* models that replicate, as closely as possible, human wound

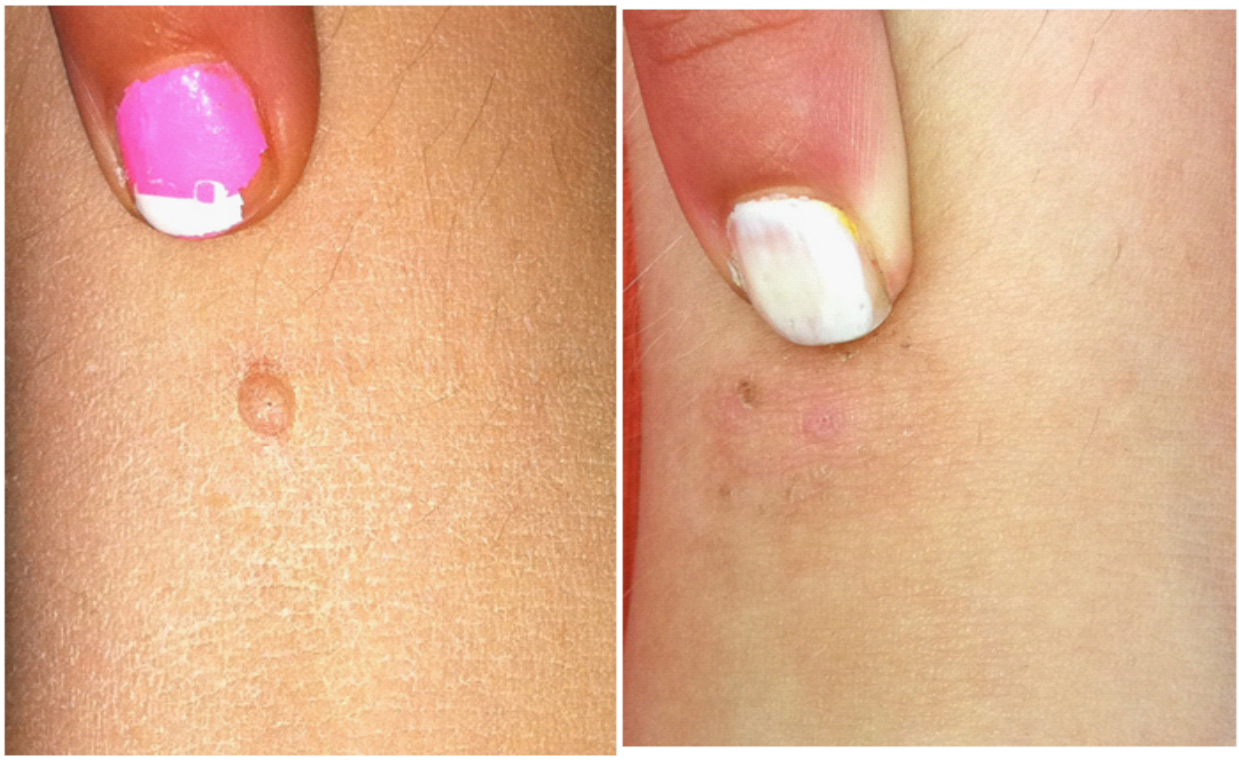
healing. Four forms of PVP-I were evaluated: PVP-I solution, PVP-I skin cleanser/surgical scrub, PVP-I ointment, and PVP-I cream. PVP-I solutions had virtually no deleterious effect on wound healing. In three human studies, no significant difference in healing was observed for 1%, 5% or 10% PVP-I solutions except for a slight delay during the first 24 hours after the application of 5% PVP-I solution in one study; healing was normal by 72 hours. PVP-I solution caused no damage to skin if cleansing of the wound with PVP-I skin cleansers/surgical scrubs was followed by saline irrigation, which is consistent with recommended guidelines. PVP-I ointment showed healing equal to controls and PVP-I cream decreased healing time [30]. There is also data describing PVP-I's effectiveness as potent anti-inflammatory agent, supporting the data reporting accelerated wound healing properties [31].

## 9. Povidone Iodine as Treatment for HPV

PVP-I has been reported as having efficacy in the eradication of HPV. Cervical cancer is caused by sexually acquired infection with certain types of HPV. There are more than 100 types of HPV, of which at least 13 are cancer-causing (also known as high risk type). Two HPV types (16 and 18) cause 70% of cervical cancers and precancerous cervical lesions [32]. Markowska demonstrated PVP-I's efficacy by studying 88 patients, 23-67 years of age (mean of 34.8 years) with abnormal cervical cytology, lesions in the uterine cervix and presence of DNA corresponding to highly oncogenic subtypes. The study included cryotherapy for three minutes, using liquid nitrogen applied via a cervical probe, followed by intravaginal povidone-iodine every day for fourteen nights to a depth guaranteeing the drug reached the uterine cervix. After two months the cryotherapy and local betadine treatment procedures were repeated. Two months later tests were made to check for presence of HPV DNA. Results showed six months after HPV infection detection RT-PCR failed to detect HPV DNA in any of the patients and cytological examination performed three months after the diagnosis was normal in every one of the patients. In seven women, aged 45 to 67, continuing cervical erosion of the vaginal portion led to patients being subjected to conization and histological evaluation of the excised preparation documented the diagnosis of glandular erosion with no traits of koilocytosis [33].

A randomized, double-blind, vehicle-controlled Phase 2 clinical trial for the treatment of common warts assessed twenty-one patients aged 8 years and older for the efficacy, safety and tolerability of twice-daily application of a novel 2% topical povidone–iodine solution in a dimethyl sulfoxide vehicle for 12 weeks duration. Patients were block randomized into two groups consisting of 14 patients in the active arm and 7 patients in the vehicle only arm. All patients were evaluated overall Global Aesthetic Improvement Scale (GAIS) improvement and 77%of subjects in the treatment arm demonstrated significant improvement, whereas only 33% of subjects in the vehicle control arm demonstrated significant improvement. There were no serious safety or tolerability issues reported (**Figure 3**) [34].

**Figure 3**: Improvement in GAIS scale after 4 weeks of treatment.



## 10. Conclusion

Medicinal iodine has evolved from the toxic, insoluble, unstable form first described over a hundred years ago into the universally known iodophor preparation of PVP-I. It affords a well-tolerated, non-toxic, non-irritating broad-spectrum antiviral with capabilities far beyond preoperative surgical preparation utilities. It is poised to be further investigated for HPV in skin and mucosal surfaces.

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