

# Recent Advances In Chemotherapy of Mucocutaneous Viral Diseases

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The involvement of the skin and/or mucosa may be either a part of a systemic viral disease or the only manifestation of a viral disease. The former is classified as generalized viral disease involving the skin and mucosa and the latter localized viral disease. Since the mucosa serves as the site of entry for the majority of human viral diseases, the mucosal involvement described in this paper will be limited only to a small area of the body accessible by routine physical examination.

Because of the easy accessibility, viral disease involving the skin and mucosa are often the first ones to be chosen for chemotherapeutic studies of various antiviral agents. As a matter of fact, with a few exceptions, our successful attempts of antiviral chemotherapy have been largely limited to these areas. Thus, a review of chemotherapy of the viral disease involving the skin and mucosa practically covers our present knowledge in antiviral chemotherapy in general.

## *Generalized Viral Diseases Involving the Skin and Mucosa*

Generalized viral diseases involving the skin and mucosa represent a large section of viral disease of childhood (Table 1). Clinically, we may classify them into three groups. The first group consists of clinically defined diseases in which the causal virus has been identified. The clinical features alone are often characteristically sufficient to allow a correct diagnosis. This includes: measles, chickenpox, smallpox, disseminated zoster, hand-foot-and-mouth disease (coxsackie A<sub>16</sub>), herpangina, and hemorrhagic fevers.

The second group comprises exanthematous diseases due to various viruses, which present clinically either in the form of certain syndromes or ill-defined pictures. For example, rubelliform eruptions can be caused, besides rubella virus, by a number of enterovirus or adenovirus. The other syndromes include Boston exanthem (echo<sub>16</sub> virus), febrile eruption with aseptic meningitis (many coxsackie and echoviruses), exanthema subitum-like (echo<sub>16</sub>), urticaria (coxsackie A<sub>9</sub>, B<sub>4</sub>, and echovirus<sub>4,9</sub>), erythema multiforme (coxsackie B<sub>4</sub> and echovirus<sub>6,11</sub>) and infectious mononucleosis (EB virus, 10-15% of cases associated with skin rash). The clinically ill-defined eruptions due to various viruses include a number of descriptions involving eruptive fevers in association with infections by adenoviruses (type 1, 2, 3, 4, 7), parainfluenza (type 3), reovirus (types 1, 2), respiratory syncytial virus and arbovirus. The diagnosis is possible only by either viral isolation and/or demonstration of antibody response.

The third group includes diseases with characteristic clinical features but a specific virus has not been identified. Examples are roseola infantum, "fourth" and "fifth" diseases.

## *Localized Mucocutaneous Viral Diseases*

Localized viral diseases involving the skin and mucosa are listed in Table 2. Recurrent herpes may involve any part of the skin, but the mucocutaneous junctions are the most frequent sites of involvement. Genital herpes, either primary or recurrent variety, is seen more and more frequently in the clinics, especially among the young women. Genital herpes in women is a

symptomatic disease, often causing pain, dysuria, and dyspareunia. *Herpes zoster* represents endogenous reactivation of varicella virus. The sensory involvement is a distinguishing feature from varicella lesions. Smallpox virus may cause conjunctivitis by accidental inoculation in individuals with immunity to smallpox. Vaccinia is an artificial disease, the virus being antigenically more closely related to variola virus than to cowpox virus. Cowpox was described by Jenner who discovered its immunizing properties against smallpox. Milker's nodules, unlike cowpox and vaccinia, result in no immunity to smallpox. Depending on the source of infection, Milker's nodules occur in the farmers who milk the cows, while contagious papular dermatitis often results from contact with diseased sheep. Both diseases present the same clinical features and are caused by paravaccinia viruses.

**Table 1. Generalized Viral Diseases Involving the Skin and Mucosa**

Exanthems	
Macular or Maculopapular	Measles, rubella, roseola Dengue, EB virus, respiratory syncytial virus (rare) Coxsackie A <sub>2,4,5,9,23</sub> ; B <sub>1,2,4,5</sub> Echovirus <sub>4,6,9,14,16,18</sub> Adenovirus <sub>1,2,3,4</sub> Parainfluenza virus
Vesicular	Chickenpox, disseminated zoster, smallpox Generalized herpes simplex Coxsackie A <sub>2,4,5,9,16</sub> ; B <sub>3,5</sub> Echovirus <sub>4,5,9,11,12</sub> Reovirus (rare)
Urticaria	Coxsackie A <sub>9</sub> ; B <sub>5</sub> Echovirus <sub>4,9</sub>
Petechiae	Arbovirus: hemorrhagic fever Coxsackie A <sub>9</sub> ; B <sub>3</sub> Echo <sub>4,9</sub>
Erythema multiforme	Coxsackie B <sub>4</sub>
Enanthems (with or without exanthems)	Echovirus <sub>6,11</sub>
Erythema	Respiratory viruses
Vesicular	Herpangina Coxsackie A <sub>1,2,3,4, 5,6,8,10,12</sub> Rarely: B <sub>2,3,4,5</sub> Echo <sub>9,16,17</sub> Primary herpes: type <sub>1,2</sub>
Nodular	Nodular pharyngitis, Coxsackie A <sub>10</sub>
Miscellaneous lesions	Coxsackie A <sub>5,9,16,13</sub> B <sub>2,3,5</sub> Echo <sub>6,9,16</sub>

**Table 2. Localized Viral Diseases Involving the Skin and Mucosa**

Recurrent herpes simplex	Herpesvirus
Herpes zoster	Herpesvirus
Smallpox (reinfection)	Poxvirus
Vaccinia	Poxvirus
Cowpox	Poxvirus
Milker's nodule or pseudo-cowpox	Poxvirus
Orf or contagious pustular dermatitis	Poxvirus
Molluscum contagiosa	Poxvirus
Warts	Papovavirus

Molluscum contagiosa and warts are small, benign skin tumors caused by a poxvirus and a papova virus respectively. They are often multiple and show spontaneous regression. In both cases, the viruses are not detectable in the basal layer, which is the initial site of hyperplasia.

## Principles of Antiviral Chemotherapy

Compared to antibacterial chemotherapy, our approach to antiviral therapy is limited. Thus far, we have not yet discovered a nontoxic antiviral drug for the treatment of systemic viral diseases, although a few are available for prophylaxis. Our approach to antiviral therapy has been in three directions, based on a close virus-cell relation: inhibition of viral replication, inactivation of viral infectivity and increase of cell resistance (Table 3).

**Table 3. Approaches to Antiviral Therapy**

Inhibition of Viral Replication		
Adsorption		immunoglobulins*
Penetration		immunoglobulins*, Amantadine*
Synthesis of Viral Component		
RNA		HBB, Guanidine
DNA		IUDR (IDU)*, Cytosine Arabinoside (Ara-C)*, Adenine Arabinoside (Ara-A)
RNA-dependent polymerase	DNA	Dimethyl Rifampin, others
Assembly		Methisazone*, Rifampin
Release		Novobiocin, Isoquinolines
Inactivation of Viral Infectivity		
Photo-oxidation*		Isoquinolines
Increase of Cell Resistance		Interferon and its inducers
		Isoprinosine

\*Useful in human viral diseases, either for prophylaxis or for treatment

### 1. Inhibition of Viral Replication

Inhibition of viral replication can be achieved by blocking viral replication at various stages of its replication. Specific immunoglobulin prevents viral adsorption when its concentration is high and blocks viral penetration when the antibody concentration is low. Amantadine works also by preventing viral penetration of influenza virus.

Inhibition of viral but not host RNA synthesis can be achieved by two chemicals, HBB (2-(*o*-hydroxy-benzyl 1)-benzimidazole) and guanidine. These compounds inhibit many picorna viruses *in vitro* by restricting the formation of a functional virus-induced synthetase. *In vivo*, however, they do not show chemotherapeutic effect probably because of the rapid development of drug resistant viral mutants.

Analogues that interfere with viral DNA synthesis include a group of halogenated derivatives of deoxyuridine. FUDR (5-fluorodeoxyuridine), which inhibits thymidyl synthetase, BUDR, IUDR (5-bromo- and 5-iododeoxyuridine), which not only interfere with enzymes synthesizing DNA precursors but also are incorporated into DNA instead of thymidine, thus forming a faulty nucleic acid and non-functional proteins. These compounds inhibit replication of DNA viruses, including papova, adeno, herpes and pox viruses, cytosine arabinoside (Ara-C) and adenine arabinoside (Ara-A) inhibit DNA viruses by interfering with the reduction of ribotide to deoxyribotide, thus impeding DNA synthesis. Viruses resistant to IUDR are sensitive to these compounds.

RNA tumor viruses possess a unique enzyme called RNA dependent DNA polymerase. This is important because the enzyme (reverse transcriptase) is capable of inducing DNA synthesis, which could lead to tumor formation. Specific inhibitor of this unique enzyme will not only be useful in its possible role in neoplasm formation, but may also lead to the discovery of chemotherapy of RNA. These compounds, however, may also inhibit other enzymes involving nucleic acid synthesis or in direct combination with DNA. Their toxicity to cells is therefore difficult to avoid.

Assembly of viral components may be interfered with by inhibiting late protein synthesis. Methisazone and rifampin belong to this category. Methisazone inhibits the replication of vaccinia, variola, virus induced leukemias or breast cancers. Several compounds have been found to possess this activity: derivatives of demethylrifampicin,<sup>1</sup> acridine orange, congo red, heparin, ethidium bromide, etc.,<sup>2</sup> adenovirus as well as several RNA viruses including picorna virus, arbovirus and myxoviruses.<sup>3</sup> Rifampin, on the other hand, only inhibits the replication of vaccinia and adenovirus.<sup>4</sup> Cells infested by vaccinia virus and treated with rifampin contain almost all components of the viral particle. After removal of the antibiotic, these components are rapidly assembled, and complete viral particles are formed readily.<sup>5</sup>

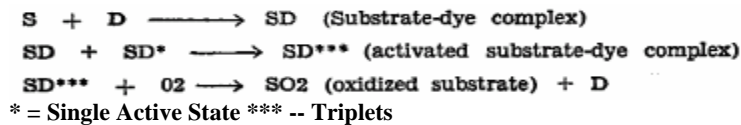
Finally, release of mature virus particles may be interfered with by isoquinolines and novobiocin.<sup>6,7</sup> Isoquinolines inhibit neuraminidase activity of myxovirus, thus preventing the release of the mature virus. It also inactivates myxovirus. Novobiocin treatment of herpes infected cells reduces the extracellular virus yield, while intracellular virus titer is not affected.

Electron microscopic examination reveals a marked reduction in the extracellular virus, and some reduction of the intra-cytoplasmic virus, but full of intra-nuclear virus, suggesting some blockage of viral release.

## 2. Photodynamic Inactivation of Viral Infectivity

Selective toxicity to virus can be made possible by way of photo-dynamic oxidation. By using certain tricyclic dyes, such as neutral red, proflavin or toluidine blue or acridine orange, a complex is formed between a substrate and the dye. On exposure to light the complex is activated and readily oxidized (Figure 1).

**Figure 1. Photodynamic Inactivation: Photo-oxidation**



Viruses vary their sensitivity to photo-oxidation, depending on their affinity to form complexes with the dye.<sup>8-11</sup> The most sensitive ones are those, which combine readily with the dye and form dye-virus complexes. On exposure to light, inactivation of infectivity takes place in a matter of minutes, even after the dye has been removed. Herpesvirus, measles, influenza, papovavirus and poxvirus belong to this category (Table 4). On the other end of the spectrum, the least sensitive viruses are those that do not form dye-virus complexes under ordinary circumstances. Photosensitivity results only when the virus has been purified, which is an artificial situation. Since these reactions require the presence of oxygen, the term photo-oxidation is also used.

**Table 4. Photoinactivation of Viruses**

Sensitive	Herpesvirus
	Measles
	Influenza
	Papovavirus
	Poxvirus
Moderate Sensitive	Adenovirus
	Reovirus
Insensitive	Enterovirus
	Rhinovirus

Photo-oxidation of virus has been demonstrated not only in the test tube, but also in animals. A recent report showed that this phenomenon could be applied for the treatment of herpes keratitis in rabbits.<sup>12</sup> Proflavine (0.1% solution) was instilled into herpes virus infected rabbit eyes. This was followed by light exposure. Twenty-two out of 24 corneas were free from the disease after a single course of treatment that was applied two days after virus infection. Without light exposure, the dye alone did not produce any significant cure rate.

### **3. Enhancement of Cellular Resistance**

Increase of cellular resistance to viral invasion can be brought about by two mechanisms: interferon and its inducers, isoprinosine. Much has been published about interferons and interferon-inducers. Great expectation has been generated again and again about their use as therapeutic agents. While interferon mechanism remains an important biological phenomenon, its therapeutic application has also been a great disappointment.

Interferon(s) exerts broad-spectrum antiviral activity by inducing an endogenous protein, which prevents the translation of viral messenger RNA. As a result, viral proteins are not synthesized and nucleic acid cannot be replicated. Because interferon mechanism is a part of cellular function, the antiviral activity is mediated through strengthened cell resistance against viral invasion.

Interferon-inducers are found in a variety of substances and living organisms. Almost all viruses are inhibited by interferon. Some rickettsia, protozoa, chlamydia and bacteria<sup>13</sup> are also sensitive to the action of interferon. Fungi, on the other hand, are stimulated by interferon inducers.<sup>14</sup> Such a general phenomenon can hardly be applicable as a specific therapy of virus diseases.

A derivative of inosine, called isoprinosine, has only mild or no antiviral activity in tissue cultures. In animals, depending on the methods used for studying the antiviral activity, the results vary from no effect to good therapeutic effects.<sup>15-17</sup> The drug is virtually non-toxic, being catabolized to uric acid. It increases the rate of synthesis of M-RNA and stabilizes polysomes of the cells, thus strengthening the cells to resist viral disruption of the cellular metabolism.<sup>15</sup> In this regard, isoprinosine is not exactly "antiviral" in strict sense. By enhancing host resistance, one would expect that the drug might be useful in more than one situation.

#### *Practical Approaches to Antiviral Therapy*

Several approaches have been proved to be of value in the prophylaxis and treatment of mucocutaneous viral diseases. Most measures are known to be effective, while some are still in the experimental stage. The following is a summary of such measures:

#### **1. Prophylaxis of Viral Diseases**

Prophylaxis of generalized mucocutaneous viral diseases has been most successive by active immunization with live virus vaccines. Even subclinical infection may not be prevented following vaccination; the cutaneous manifestations are almost completely suppressed.

Passive immunization with immune globulins had been the most important means for prophylaxis before the development of viral vaccines. Once an effective vaccine is developed, the use of immune globulin is rarely needed for general prophylactic use.

Chickenpox in individuals with immunological defects may become a life-threatening situation and should be prevented by the administration of zoster immune globulin. The administration of zoster immune globulin (2 ml within 3 days of exposure), reduces the risk of developing chickenpox by over 90%.

Since smallpox vaccination is no longer recommended as a routine procedure in most western countries, the possibility of smallpox outbreaks will be unlikely eliminated in the near future. Methisazone prophylaxis is effective within hours while vaccination may take days to develop significant protection. Therefore, for those with close contact with unsuspected case of smallpox for over a week and with no history of vaccination within the past 10 years, the use of methisazone in addition to prompt vaccination should be considered.

## **2. Iodouridine in the Treatment of Infections by Herpes Simplex and Herpes Zoster Viruses**

5-iodo-2-deoxyuridine (IDU or IUDR) has been used in the treatment of infections by herpes viruses including herpes simplex and herpes zoster. The drug, however, inhibits DNA synthesis of virus as well as the host cells to about equal degree. Toxicity is, therefore, expected especially to the rapidly growing cells in the liver, bone marrow, intestinal mucosa etc. Because of the toxicity, the use of IDU by systemic route is restricted to only life-threatening situations such as herpes encephalitis or generalized herpes.

Local application of IDU has been successfully used in the treatment of herpes keratitis. Its value in treating herpes infection elsewhere, however, is questionable. A recent double blind study indicates clearly the therapeutic effect of 5% IDU in dimethyl sulfoxide (DMSO) intermittently applied, and of 40% IDU in DMSO continuously applied for 4 days, to the lesions in patients with zoster of recent onset. The duration of pain is markedly shortened and healing accelerated after 40% IDU application. DMSO alone, on the other hand, is not only ineffective, but also deleterious to the patient.<sup>18</sup>

## **3. Cytosine Arabinoside in the Treatment of Herpes Virus Infections**

Ara-C (cytosine arabinoside, 1-B-D-arabinofuransylcytosine) has been used as an alternative drug for IDU in the treatment of herpes keratitis, herpes encephalitis, disseminated zoster and generalized cytomegalovirus disease (18-21). It appears that Ara-C is more effective and somewhat better drug than that of IDU. First, mutant herpes virus resistant to IDU is easily obtained, while resistant virus to Ara-C has not or rarely been observed. Secondly, viruses of herpes group are more sensitive to Ara-C than to IDU. Lastly, IDU is mutagenic because it incorporates into DNA, resulting in frequent base pairing mistakes.

Pyrimidine analogues are given by continuous intravenous drip, because they are rapidly metabolized into inactive forms. Only a very small amount is excreted in the urine in unaltered form.

## **4. Treatment of *Herpes labialis* and *Herpes proenitalis* by Photodynamic Inactivation of the Virus.**

A remarkable result in the treatment of recurrent herpes by means of photo-oxidation or photodynamic inactivation of herpes virus has been reported. Proflavine solution 0.1% or 16% neutral red ointment is applied locally to the lesion. This is followed by exposing the treated area to iridescent or fluorescent lights. Clinical improvement is seen in 90% of treated cases within 2-3 days. Recurrences are reduced in at least half of the cases. The proflavine was used as a local antiseptic in the treatment of wound infection. The local application of the drug results in no systemic reaction. Its use in the treatment of superficial herpes infection is, therefore, the simplest and cheapest, and yet most effective method at the present time.

## 5. Miscellaneous Therapeutic Devices

The use of interferon locally to prevent vaccinia of the skin and to treat vaccinia keratitis has been reported with some success. The use of interferon inducers locally to prevent rhinovirus infection of the respiratory tract has also been shown to alleviate some clinical manifestations of the disease.<sup>19</sup>

Rifampin, when given orally, exerted no effect on the development of vaccinia skin lesion. Local application of 15% ointment or cream, however, resulted in a reduction of vaccination take by 50%.<sup>20</sup>

Methisazone, though ineffective in the treatment of smallpox, may be beneficial to patients with eczema vaccinata or vaccinia necrosa.

## CONCLUDING REMARKS

While we have available ever-increasing numbers of antibacterial agents, we are still looking for the first safe and effective antiviral drug to treat systemic viral diseases. It is true that both amantadine and methisazone have been shown to possess antiviral activity against smallpox and A<sub>2</sub> influenza in man, their clinical application has been limited to the prophylaxis of these diseases. A flurry of recent interests has been focused on the use of IUDR and Ara-C in the treatment of diseases caused by herpesvirus, including herpes simplex, varicella, zoster and cytomegalic inclusion disease. It is important to realize, however, that both agents are also poisons when given systemically, and should be reserved only for life threatening situations. It is also important to remember that none of these agents have been critically evaluated by double blind study in the treatment of generalized viral diseases.

Local application of IDU and proflavine in the treatment of superficial herpes infection on the other hand, has been fairly successful. The use of photodynamic inactivation as a therapeutic means is both exciting and intriguing. At the moment, its use is limited to the management of superficial herpes infections. Since a number of viruses are also sensitive to photo-dynamic inactivation, the possibility of its clinical application to treat other diseases will be undoubtedly tested.

## REFERENCES

1. Gurgo C, Ray RK, Thiry L, et al. Effects of rifampicin and actinomycin D on DNA polymerase. *Nature New Biol* 1971; 229:111-114.
2. Muller WEG, Zahn RK. Inhibitors acting on nucleic acid synthesis in oncogenic RNA virus. *Nature New Biol* 1971; 232:143-146.
3. Bauer DJ, Apostolov K, Selway JWT. Activity of methisazone against RNA viruses. *Ann NY Acad Sci* 1970; 173:314.
4. Subak-Sharpe JH, Timbury MC, Williams JF. Rifampicin inhibits the growth of some mammalian viruses. *Nature* 1969; 222:341-345.
5. Katz E, Grimley P, Moss B. Reversal of antiviral effects of rifampicin. *Nature* 1970; 227:1050.
6. Coombes JD, Bramer KW, Herb St-Laier RH, et al. Laboratory studies on two synthetic dihydroisoquinolines. *Ann NY Acad Sci* 1970; 173:462-476.
7. Chang TW, Weinstein L. Antiviral activity of novobiocin. *Antimicrob Agents Chemother* 1970; 165-168.
8. Wallis C, Melnick JL. Irreversible photosensitization of viruses. *Virology* 1963; 23:520-527.
9. Hiatt CW, Kaufman E, Helprin JJ, et al. Inactivation of viruses by photodynamic action of toluidine blue. *J Immunol* 1960; 84:480-484.
10. Wallis C, Melnick J. Photodynamic inactivation of enteroviruses. *J Bacteriol* 1965; 69:41-46.
11. Wallis CS, Trulock S, Melnick JL. Inherent photosensitivity of herpes virus and other enveloped viruses. *J Gen Virol* 1969; 5:53-61.
12. Moore C, Wallis C, Melnick JL, et al. Photodynamic treatment of herpes keratitis. *Infect Immun* 1972; 5:169-171.
13. Gobar LL, Friedman-Kien AE, Vilcek J. Suppression of intracellular growth of *Shigella flexneri* in cell cultures by interferon preparations and poly inosinic-polycytidylic acid. *Infect Immun* 1972; 5:370-376.
14. Worthington M, Hasenclever HF. Effect of an interferon stimulator, polyinosinic-polycytidylic acid on experimental fungus infections. *Infect Immun* 1972; 5:199-202.
15. Gordon P, Brown ER. The antiviral activity of isoprinosine. *Canad J Microbiol*. In press.
16. Glasgow LA, Galasso GJ. Isoprinosine, lack of antiviral activity in experimental model. *J Infect Dis* 1972; 126:162-169.
17. Chang TW, Weinstein L. In vitro and in vivo antiviral activity of isoprinosine. *Am J Med Sci*. In press.

18. Juel-Jensen BE, Maceallum FD, Ma-Kenzie AMR, et al. Treatment of zoster with idoxyuridine in dimethyl sulfaoxide: results of two double-blind controlled trials. *Br Med J* 1970; 4:776-780.
19. Hill DA, Baron S, Perkins JL, et al. Evaluation of an interferon inducer in viral respiratory disease. *JAMA* 1972; 216:199-202.
20. Moshkowitz A, Godblum N, Heller E. Studies on the antiviral effect of rifampicin in volunteers. *Nature* 1970; 228:1184-1190.