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PERSPECTIVES FOR THE TREATMENT OF GASTROINTESTINAL TRACT VIRUS INFECTIONS

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INTRODUCTION

Gastointestinal infections are one of the leading causes of morbidity and mortality in infants and young animals, both in developing and in developed countries. It has been estimated that between 3-10 billion cases of diarrhea occur annually in humans, resulting either directly or indirectly in approximately 10 million deaths (1,2). The largest number of deaths occur in the age group of 6-12 months, although diarrhea is a major factor in the health of children up to 5 years of age. The causes of acute diarrhea are often multifactorial being determined by nutritional and environmental conditions. For example, significant malnutrition may lead to an increased severity and duration of diarrhea. In addition to the nutritional component, the level of contamination of the environment by specific organisms has a significant effect on both incidence and mortality rate of the resulting diarrhea (3). It is estimated that malnurished children in unhygienic situations have a fatality rate of 6 deaths per 1000 cases of diarrhea presented for treatment (1).

In the present review, we will briefly discuss the specific agents involved, the mechanisms of pathogenesis of enteric virus infections and the potential application of vacines and antiviral agents for the control of enteric viral infections. Attempts will be made to correlate the mechanisms of pathogenesis with the potential use of a specific antiviral agent under specific disease conditions.

In addition to viral causes of acute gastroenteritis, other organisms such as bacteria and parasites can cause severe gastrointestinal infections. The incidence of diarrhea due to bacterial or parasitic gastrointestinal infections demonstrates geographic variability. For example, the incidence of enterotoxigenic E. coli (ETEC) is very high in developing countries as opposed to developed countries (4). In this review we will not discuss agents other than viruses nor other noninfectious causes of diarrhea.

DeClercq, E. (ed.), *CLINICAL USE OF ANTIVIRAL DRUGS*.

CAUSATIVE AGENTS

During the past two decades, improvements in electron microscropic identification of microorganisms and diagnostic tests have greatly increased the efficiency with which viral agents can be identified in fecal samples of animals and humans (5). Although many episodes of nonbacterial diarrhea still remain undiagnosed with respect to the etiologic agent, a wide variety of different viruses have been incriminated as potent causative agents of nonbacterial gastroenteritis. A summary of the various viral agents that have been isolated and associated with acute viral gastroenteritis are listed in Table 1.

The viruses which cause gastrointestinal infections can generally be divided into three groups. In the first group, replication is restricted to the gastrointestinal tract and the viruses induce disease as a direct result of their infection of intestinal cells. The agents in this group generally enter the host directly, via the oral cavity, into the gastrointestinal tract. The disease caused by these agents can be characterized by rapid onset (1-2 days) of acute diarrhea, abdominal cramps, nausea, vomiting and fever. It is this group of viruses that will be addressed predominantly in this review.

The second group of viruses can enter the host via the oral cavity and replicate in the gastrointestinal tract, but do not remain localized, and generally do not cause acute gastrointestinal infections even though they are shed in the feces. These viruses often spread to other target organs such as lymphoid tissues or even to the central nervous system and cause systemic infections (6,7,8). Examples of this group of viruses include hepatitis A, coxsackie virus, polio, etc. The third group of viruses which infect the gastrointestinal tract do so indirectly. Their routes of entry are generally not oral and they reach the gastrointestinal tract via systemic spread, most often via the hematogenous route. Therefore, the gastrointestinal tract serves as a secondary target. In these instances gastrointestinal symptoms may occur but they are not the main clinical features. Examples of such infections are hepatitis B and cytomegalovirus (9,10).

At least nine different virus families can cause infections of the gastrointestinal tract and induce some degree of intestinal damage and diarrhea under appropriate conditions. As is evidenced from Table 1, these viruses fall into a wide variety of different families including RNA

and DNA viruses. Until recently it was felt that the viruses that cause direct infection of the gastrointestinal needed to be acid-stable and

TABLE 1 - SUMMARY OF SOME VIRAL AGENTS CAPABLE OF CAUSING GASTROINTESTINAL INFECTIONS IN HUMANS AND ANIMALS[1]

Family	Biochemical/Biophysical Properties	Human/Animal[2]
Picornaviridae - entero	27-32nm Icosahedral (naked) ss. RNA	+/+
Reoviridae - reo - rota	80nm Icosahedral (naked) ds RNA	+/+
Astroviridae	28nm Icosahedral (naked) ss RNA	+/+
Caliciviridae	30nm Icosahedral (naked) ss RNA	+/+
Coronaviridae	75-150nm Helical (enveloped) ss RNA	+/+
Toroviridae - Breda - Berne	110-140nm Helical (enveloped) ss RNA	?/+
Norwalk	23-34nm Icosahedral (naked) ss RNA	+/-
Adenoviridae	70nm Icosahedral (naked) ss DNA	+/?
Parvoviridae	30nm Icosahedral (naked) ds DNA	?/+

1 All viruses listed here enter via the oral route and cause local infections of the gastrointestinal tract. Virus is shed in feces and acts as a source of environmental contamination.

2 + indicates definite role in acute gastrointestinal infections; ? limited information prevents definite inclusion or exclusion as true gastrointestinal agents; - no involvement known at present.

non-enveloped so as to reach the intestine after an encounter with harsh gastric acids and duodenal bile salts. However, the recent identification of coronaviruses and Breda/Berne viruses which are enveloped clearly indicates that some enveloped viruses do have the capacity to withstand the environmental conditions in route to the gastrointestinal tract where infection will occur (11,12,13,14,15).

The diversity of biochemical and biophysical properties of enteric viruses and different mechanims of viral replication makes the identification of a single antiviral agent to control the majority of gastrointestinal viral infections remote. Thus, it appears that a concerted effort will have to be made to develop a large number of chemotherapeutic agents, each directed towards a specific virus. Furthermore, since enteric viral infections are localized, the incubation period is short and the infection is almost always self limiting, it becomes imperative that antiviral drugs be used in a prophylactic rather than in a therapeutic mode.

Another problem associated with antiviral chemotherapy is the inability to always predict the time when a specific infection will occur. Although epidemiological surveys have aided in elucidating this problem, more studies are needed before accurate predictions can be made. For example, in the case of rotavirus infections, there is generally a peak of activity in winter months in temperate climates (16). However, in tropical countries, the pattern of rotaviral infections may vary depending on the country and various climatic conditions (17). In other instances, such as with Norwalk virus there does not appear to be any seasonal variation in disease incidence (18).

PATHOPHYSIOLOGY OF ENTERIC VIRUS INFECTIONS

In most virus infections of the gastrointestinal tract, regardless of whether the virus has a predelection for the epithelial cells at the tip of the villi or in the crypts, there is severe shortening and occasionally fusion of adjacent villi, resulting in reduced adsorptive surface of the intestine (19,20). Following infection of the epithelial cells at the tips of the villi, the mature adsorptive cells are replaced with immature squamous to cubiodal epithelial cells. Until these cells mature, their absorptive capacity and enzymatic activity is greatly reduced. Since these immature cells also appear to be relatively resistant to virus

infection, the disease is often self-limiting if dehydration is not so significant as to cause hospitalization or death. In virus infections where the crypt cells are not damaged, the rate of recovery is generally rapid. However, in those viral infections where the crypt cells are infected, there is also shortening of villi, but since there are a limited number of new cells available to migrate up the villi, recovery generally takes longer. As more knowledge is gained regarding the virulence and pathogenesis of various gastrointestinal viruses, it is becoming evident that the virulence of the virus will determine the extent of replication within the gastrointestinal tract. Thus, the less virulent viruses may still kill and cause shortening of villi in localized areas, but the viruses are generally restricted to a very small portion of the gastrointestinal tract. Those viruses which are much more virulent appear to have a greater capability of infecting a larger number of cells throughout the gastrointestinal tract, i.e. they are not localized. Thus, an avirulent or mild virus may only infect a certain portion of the jejunum, whereas a more virulent strain of the same virus may infect the jejunum, illeum and even cells of the colon. Since glucose and sodium adsorption are highest in the proximal and middle part of the jejunum, damage here will cause most severe diarrhea. Therefore, the extent of diarrhea will be correlated with the site at which the virus infection occurs. Table 2 illustrates the sites of replication of some of the enteric viruses. Coinfection with virus that replicate in different areas can occur and these mixed infections are often more severe than single infections due to increased intestinal damage. Mixed infections and more common in animals than they are in humans (21).

In most animal species, viral diarrhea is characterized by profuse watery stools containing increased concentrations of sodium, potassium and chloride. The direct destruction of adsorptive epithelial cells and alteration of microvilli leads to diminished glucose, sodium carrier and Na+, K+-ATPase activity. This results in loss of sodium, potassium, chloride, bicarbonate and water. As the virus kills absorptive cells, there is also a loss of enzymes responsible for digestion of disaccharides. These disaccharides, especially lactose, are osmoticaly active and cause an influx of fluid into the gut lumen. The loss of bicarbonate leads to development of acidosis. Acidosis can further create a K^+-H^+ ion exchange across a cell membrane and inhibits cellular

functions required for maintaining normal potassium concentrations with a net loss of potassium from cells. As a result, hypoglycemia occurs, glucose adsorption is impaired which further results in drastic reduction of Na+ adsorption since it is glucose dependent. This series of complex pathophysiological changes, if not promptly corrected, may result in the death of the individual. Fortunately, in many cases, the extent of these pathophysiological changes is not sufficient to require hospitalization.

TABLE 2 - SITE OF REPLICATION OF SOME ENTERITIS VIRUSES

Virus	Horizontal	Longitudinal
Rota	Enterocytes, villus tip	Small intestine
Corona	Enterocytes, top half	Small and large intestine, colon
Breda/Berne	Mid villus, crypts	Small intestine, colon
Astro	?	?
Calici	?	?
Parvo	Crypts, lymphoid	Small and large intestine
Adeno	Enterocytes	Small
Reo	?	?
Entero	?	?

? Insufficient data available for definitive statements to be made.

Effective management of diarrhea requires prompt action to prevent continued loss of fluids and electrolyes. In animals, this is most economically achieved by removal of milk from the diet. This reduces the amount of undigested lactose in the lumen and, therefore, reduces fluid loss and acidosis. Therapy should include administration of balanced electrolyte solutions either orally or by the intravenous route. The use of intravenous fluid replacement and careful monitoring of animals could save a large percentage of severely affected animals; however, the costs are generally too high to recommend this as a standard procedure. In humans, the generalized introduction of oral rehydration therapy has greatly reduced mortality in infants. Antidiarrheal drugs, which reduce gut motility should not be used in viral gastroenteritis.

CONTROL OF GASTROINTESTINAL INFECTIONS

Numerous studies have demonstrated that protection must be directed at preventing the initial infection of the intestinal epithelial cells (22,23). Thus, regardless of whether antiviral agents or vaccines are used to induce protection from gastrointestinal viruses, protection must be directed at the intestinal epithelial cells. As a result, systemic application of drugs or vaccines may not always be efficacious. In this section, we will attempt to address control by vaccination, as well as, the potential for using antiviral drugs to limit viral infection of the intestine.

Vaccination.

The presence of antibody in the lumen of the small intestine, which can neutralize the virus before infection is initiated is at present the most effective means of preventing diarrhea (24,25). Unlike humans, most animals do not transfer antibodies across the placenta. Since animals generally suffer the most severe diarrhea in the first few weeks of life, the most effective method of preventing diarrhea is to hyperimmunize the mothers so that they secrete high levels of antibody in their milk during the neonate's susceptible period. The first convincing evidence that such an approach could work was demonstrated by the practice of feeding Transmissible gastroenteritis virus (TGEV) infected intestines to sows approximately one month prior to farrowing (26). The sows experience a mild subclinical infection and secreted sufficient levels of secretory IgA antibody in their milk to protect their piglets. Since there is a common immune system between the gut associated lymphoid tissue and the mammary gland, such intestinal immunization is very effective in pigs. However, this approach maintains virulent viruses in the environment, so recent work has employed attenuated TGEV given either intranasally or orally. Such vaccines have been only moderately successful since they do not replicate to sufficient levels within the sows to induce high levels of immunity and protection. Similar approaches have been used in other species including cattle to induce high levels of protection in milk (24).

The requirement for local immunity has created an interest in generating active immunity in the neonate or newborn before infection occurs. This is especially attractive in humans where the incidence of most cases of diarrhea does not occur until six months of age. It is also

pertinent in rotavirus infections where it appears that although infection of neonates can occur, a very high percentage of these neonates are asymptomatic shedders (27). As individuals increase in age, the percentage of asymptomatic carriers decreases and the clinical form of the disease is manifested. Although it is not known why children become more susceptible to rotavirus infections as they increase in age between 6 to 24 months of age versus neonates, it has been postulated that there may be age-related changes in the ability of the epithelial cells of the intestine to bind virus. This is in direct contrast to that seen in most other species where the cells appear to be extremely susceptible in the very young, and after a few weeks or months, animals become more resistant to infections (28).

The need for providing local immunity dictates that virus vaccines be administered orally and induce a local immune response. This can be achieved by using 1) live attenuated or naturally avirulent strains, 2) live attenuated heterologous strains, 3) attenuated genetically altered strains, or hyrid viruses, 4) live bacterial vectors capable of colonizing the intestine and contain the appropriate viral antigens, 5) purified viral antigens or synthetic peptides coated for appropriate delivery to the intestine. At present all of these various approaches are being tried with varied success.

One of the greatest impediments to using live virus vaccines for gastrointestinal diseases is that it is very difficult to culture most of these agents *in vitro* to titers that will provide sufficient quantities of antigen for economical vaccine production. Although there is a tremendous amount of interest in developing effective vaccines against gastrointestinal virus infections, the trials are only now beginning and it is too early to confirm their potential efficacy. However, we are optimistic that with the potential tools at our disposal the problems will be overcome and it will be possible to produce a variety of effective vaccines against the majority of important gastrointestinal viral infections.

Anti-viral chemotherapy.

At present a large number of compounds have been identified with potential anti-viral activity in vitro. Although few of these compounds have either been tested or proven to be effective *in vivo* against viruses

which cause gastrointestinal infections, the important advances made in this area over the last decade warrant serious consideration for the potential application of some of these drugs to the control of infections of the gastrointestinal tract. In this section we will discuss the potential sites of activity of a selected number of the drugs and speculate as to their applications in gastrointestinal tract infections. In addition, the limitations of using antiviral drugs in these infections will be addressed. The problems of delivery and maintenance of drugs at the site of infection so as to prevent initiation of infection (prophylaxis) rather than therapy will be emphasized.

In gastrointestinal tract infections as in other viral infections, the targets of antiviral drugs must be at sites that are unique to the virus and not have any physiological effects on the specific cells of the gastrointestinal tract. As is the case in other virus infections the antiviral drugs must be directed at various stages of virus replication cycle such as virus attachment, penetration, uncoating, macromolecular synthesis, viral maturation and assembly. Regardless to which stage of virus replication the antiviral drug is targeted, the major problem with using antiviral drugs in controlling viral induced gastrointestinal infections is the rapidity with which diarrhea occurs following the initial infection. Secondly, in many cases, diarrhea is often self limiting; thereby dictating that antiviral drugs must be used in a prophylactic rather than a therapeutic mode. Thus, prevention of viral activity at the early stages of infection appears to be the most promising but also creates the most problems regarding delivery and maintenance of the antiviral drug within the gastrointestinal tract for considerable periods of time.

Inhibitors of viral attachment are not frequently considered to be the major targets of viral replication, yet in many cases this is the mechanism whereby antibody neutralizes virus. If initial attachment could be prevented this could potentially be one of the best approaches for limiting gastrointestinal infections. This is especially important where onset of disease is extremely rapid. Recently there has been a considerable amount of activity in the area of identification of specific viral receptors on host cells (29). Once these specific receptors are identified it may be possible to either develop compounds or specific synthetic peptides which are capable of interfering with viral cellular

interactions at the receptor level. The advent of anti-idiotypic antibodies to identify receptors for specific viruses will be extremely useful in this regard (30). However one difficulty with developing a specific anti-receptor approach is that there are many different viruses which can cause gastrointestinal infections (Table I). As a result, at present it appears that one would require a specific receptor blocking compound or peptide for each different virus. Whether this would be economical to produce remains to be determined. Nevertheless it appears useful to continue the search for such compounds which may have broad antiviral activity. Some compounds have recently been identified with such activity (31).

Recently, purified bovine lecithin was shown to inhibit in vitro replication of a number of viruses including rotavirus (32). Although there was speculation as to the mechanisms of action, no definitive proof was given. The results do however suggest that inhibition is at the level of viral receptor cell interactions. Since this is a natural product, and is non-toxic, it provides a possible useful approach to antiviral chemotherapy at the early stages of infections.

The step which occurs either simultaneously or immediately after attachment of viruses is viral penetration. Once again, neutralizing antibody plays a major role in preventing viral infectivity and replication at this stage. Thus this stage of virus replication is also attractive as a site for targeting antiviral drugs. Examples of such compounds include protease inhibitors which prevent cleavage of important glycoproteins which are required for viral penetration and virus-cell fusion (33,34). Although at present these examples are mainly limited to respiratory virus infections such as paramyxovirus and respiratory syncytial virus, similar approaches may be possible for some of the viruses which cause gastrointestinal infection. For example, it is well known that proteolytic cleavage of the 84K protein of rotavirus is essential for infectivity (35). Although it is thought that cleavage is not required for viral attachment, it is required for efficient penetration and uncoating (36). Whether additional protease inhibitors present in the intestine of animals and human beings would have deleterious effects on the physiological functions of the gastrointestinal tract remains to be determined.

An extension of the observation that proteolytic cleavage is required to provide active sites for initiation of infectivity, resulted in the synthesis of active oligopeptides with antiviral activity for measles and influenza virus (37). The fact that these oligopeptides are not toxic to cells provides an exciting approach to antiviral therapy in the gastrointestinal tract. However, studies in animals are required to evaluate the usefulness of such an approach to chemotherapy of viral infections of the gastrointestinal tract. Although the oligopeptides are non-toxic, in many cases they may act on the target cell membrane and consequently alter the physiological functions of the cell disrupting their activity and therefore themselves lead to diarrhea. The fact that the epitilial cells of the intestine turn over rapidly dictates that all of the newly susceptible cells continue to be exposed to the oligopeptide to remain refractory to virus infection. Because these oligopeptides are cleaved by the same enzymes that act upon the viruses, high doses are required to prevent virus cleavage. However, the synthesis of non-cleavable analogs of these peptides may reduce the required dose. Whether this will be economically feasible in diseases where it is not possible to predict the exact timing of infections remains to be determined.

Recently aromatic mono- and diamidines with antiviral activity have been identified (38). Althought the mechanism of activity is unknown they appear to act by inhibiting arginine-specific esterproteases and thereby preventing virus envelope-cell membrance fusion and entry (39). Monensin, a carboxylic ionophore, in addition to inhibiting viral glycoprotein processing, also inhibits penetration of viruses into cells (40). The fact that this compound is already used in cattle as a feed additive and an antiparasitic agent suggests that oral administration of it or similar derivatives may also have antiviral activity.

Other compounds that interfere with the uncoating of both envelope and naked viruses have been identified. The most effective compounds would be those that inhibit a broad spectrum of viruses, including DNA and RNA viruses as well as enveloped and naked viruses. One example of a series of compounds with potential as broad spectrum antiviral agents is 4-[6-(2-chloro-4-methoxyphenoxy)hexyl]-3, 5-heptanedione (arildone) (41). To date this compound has proven effective against some viruses which enter via the gastrointestinal tract such as polio and coxsackie virus as

well as systemic viruses including herpes simplex and vesicular stomatitis. These compounds are effective both in vitro and in vivo (42,43). The fact that different derivatives of arildone can be synthesized with variable solubility and viral activity makes them attractive as broad-spectrum drugs. Many of the drugs described in the preceding section need not enter cells to exert their antiviral effect. Thus most of their effects are at the surface of cells therefore there is no need to worry about systemic absorption from the gastrointestinal tract.

The drugs that will be described in the following section inhibit virus infections after entry of the virus into the cell and therefore must actively enter the cell and act at intracellular sites of virus replication. We will not attempt to make an exhaustive list of all the drugs and all the viruses which can be inhibited by these drugs nor will we discuss all the different mechanisms by which each drug acts. In this section we will address classes of antiviral drugs that may have a potential in gastrointestinal infections.

Nucleoside analogs are one of the earliest antiviral drugs identified and such drugs continue to show promise for herpes viruses specifically, but newer analogs are showing activity against a wide variety of virus infections. Thus purine nucleosides such as 2-Amino-2'deoxy-9-β-D-ribofuranosyladenine, (S)-9-(2,3-Dihydroxyprophyl) adenine [(S)-DHPA], and (S)-9-(3-hydroxy-2-phosphonylmethoxyprophyl) adenine [S-HPMPA] have much broader antiviral activity than do the original ara-A derivatives whose activity was mainly restricted to herpes viruses. (44,45,46). Although the exact mechanism of action of these newer analogs is not fully understood the observation that they are active against both RNA viruses and DNA viruses (45,46) indicates their broad spectrum of activity. Specifically these drugs have been shown to be active against a number of adenovirus serotypes which cause infections of the respiratory tract. However, they have not yet been shown to be active against the human enteric adenoviruses nor have they been shown to be active against other enteric viruses such as coronavirus, astroviruses, etc.. If these drugs are found to be effective against these enteric viruses, the fact that they are active when given orally suggests that they should have potential as antiviral agents in vivo (46).

At present most of the pyrimidine nucleosides like the purine nucleosides are active against herpes viruses. However, drugs such as (1R)-(1α , 2β , 3β ,4α)-1(2,3-Dihydroxy-4-hydroxymethyl cyclopentyl) cytosine (carbodine) do have limited activity in vitro against influenza virus infections (47). Unfortunately in vivo activity has not been demonstrated to date. Whether other derivatives can be found that are active in vivo against a wider variety of viruses remains to be determined. The nucleoside ribavirin and its derivatives have been shown to have a broad spectrum antiviral activity (48,49). Once again in the majority of cases these drugs have been used systemically. Whether systemic administration will be affective in transfering antiviral states to the cells of the intestine remains to be determined. Furthermore, whether these drugs will be active if given orally also remains to be determined. The fact that they are active locally when given for respiratory infections indicates that some of these drugs may be effective if given by the oral route. This is further substantiated by the observation that ribavirin was at least partially active in vivo against rotavirus infections (50). Selenazole, (2β-D-Ribofuranosyl selenazole-4 -carboxamide) an analog of ribavirin, appears superior to the parent compound both with respect to its antiviral activity as well as the maintenance of the antiviral state after the drugs is removed (51). Development of these types of drugs with sustained antiviral activity will ensure the antiviral state in the intestine between treatments. However, it must be indicated that drugs would probably still have to be given daily or every second day so that the new cells that are being continuously generated in the intestine continue to exhibit antiviral activity.

So far we have discussed antiviral drugs whose activity was mainly directed at DNA viruses but had some activity against RNA viruses. There are some drugs with in vitro activity against various RNA viruses but are inactive against DNA viruses. One example of such a compound is sodium-5 aminosulfonyl-2, 4-dichlorobenzoate (52). This compound is active against influenza, parainfluenza, respiratory syncytial, vesicular stomatitis, echo and rhinoviruses. Furthermore this drug has been shown to be active when given orally. The fact that this drug is tolerated at very high doses and has a very high therapeutic index indicates its potential for application in young infants and animals. Whether it will be active

against the majority or at least the most serious gastrointestinal infection remains to be determined.

Inhibitors of viral maturation and assembly are also potential sites for directing antiviral chemotherapy. Thus in many cases viral specific processes associated with viral morphogenesis or maturation of specific proteins or polypeptides requires proteolytic cleavage of the precursor proteins to produce mature viral products. If the specific cleavage reactions are mediated by virus-coded proteases it is possible to direct antiviral compounds to these specific targets. Interference with these specific steps of viral replication should generally affect the formation of and release of infectious particles. Examples of such compounds include carbobenzoxyl leucylchloromethyl ketone, which inhibits cleavage of picornavirus precursor proteins without affecting cellular protein synthesis (53). 2-Amino-5 (2'-sulfamoylphenyl)-1,3,4-thiadiazole has been shown to act directly on viral structural proteins and prevent their assembly into virus particles of a variety of different RNA and DNA viruses (54). Although the mechanism of this inhibition remains unknown, studies on the effect of modifying various components of this molecule indicates that alterations in the activity against different viruses are possible. Thus it is possible as we learn more about the mechanisms of action of these specific drugs as well as the assembly and maturation of different virus infections it may be possible to engineer analogs of this compound which have the desired specific antiviral effect. In the case of rotaviruses, calcium is very important for stabilizing the virus and allowing infectious virus particle production. The growth of rotavirus in calcium-free media dramatically reduces virus infectivity (54a). Recent in vitro studies clearly indicate that chelators of calcium can dramatically reduce virus infectivity and replication in vivo as well as in vitro. Unfortunately, calcium chelators in the intestine have profound physiological effects which result in diarrhea (Ijaz, M.K. unpublished results). Thus, this approach does not appear at the present time to be feasible in controlling rotavirus infections.

One of the earliest antiviral mechanisms that the body has available for directly inhibiting virus replication is interferon. This compound was discovered in 1957 by Isaacs and Lindenmann (55) and interest has continued to be generated both in the direct application of interferon as an antiviral agent as well as induction of the bodies' own interferon

system to develop an antiviral state. Since gastrointestinal virus infections are caused by a wide variety of viral agents the broad spectrum antiviral effects of interferon is very attractive for control of virus infections of the gastrointestinal tract. Another advantage of using interferon or interferon inducers as an antiviral compound is that interferons are generally considered to be most effective as prophylactic rather than therapeutic aids in infections. Since it is postulated that antiviral drugs in gastrointestinal infections will be applied prophylactically rather than therapeutically, this makes interferon a very attractive choice. One possible disadvantage of using interferon inducers is the possible induction of hyporesponsiveness after repeated treatment (56). As we learn more about the hyporeactive state and the mechanisms whereby various inducers stimulate interferon production it may be possible to overcome this hyporesponsive state. It is possible also to administer other compounds in combination with interferon inducers to reduce the hyporesponsive state and maybe even in some cases reduce the unwanted side effects of the interferon inducers (57). Polyinosinic: polycytidylic acid, [poly (I·C)], one of the initial synthetic inducers of interferon, was shown to be rapidly degraded by nucleases and generally had poor tolerability in animals. The tolerability was improved by altering the structural composition of poly I·C but maintaining the interferon inducing activity (58). Administration of poly I·C probably will require further modifications to increase the stability and prevent nuclease degradation of the compounds before they are active in vivo. Thus, although interferon inducers have some potential, they have not yet proven to be very effective.

The advent of recombinant DNA technology and availability of different cloned human and animal interferons may provide an effective economical method for future control of enteric infections of viral etiology. Recent studies have clearly indicated that animal interferons are capable of reducing gastrointestinal virus infections (59). However, before interferons can be employed routinely in the control of gastrointestinal infections, methods will have to be implemented to maintain these substances in the intestine for the duration of time that the animals are susceptible to the virus. Another impediment that will need to be overcome is the delivery of interferon to the cells of the gastrointestinal tract. Oral delivery creates problems due to the

tremendous numbers and amounts of proteases that are present in the gastrointestinal tract. Since interferon is rapidly degraded by gastrointestinal proteases, methods of delivery and targeting of interferon will need to be developed to transport the compound to the target site and maintain sufficient levels of interferon in the intestine for a sufficient length of time to develop and maintain the antiviral state. Some of the methods developed for targeting interferon can also be very useful for targeting other antiviral drugs and viruses discussed in the preceding section. The development of enterocoating methods for oral delivery of these compounds will be a tremendous benefit to the oral delivery of specific antiviral drugs.

Combination chemotherapy has proven to be an effective means of dealing with drug toxicitiy and problems of drug resistance in both anti-microbial and cancer chemotherapy (60,61). Recent studies involving combination chemotherapy of viral infections clearly indicate that in some instances the use of two drugs, whose mechanism of action differs, greatly increases their efficacy (62,63). This approach has not been tried in gastrointestinal viral infections to date. In addition to acting synergistically some drugs can be antagonistic (63). Therefore, drug combinations will have to be selected judiciously.

SUMMARY

Presently there are a variety of antiviral drugs which have activity against a wide range of viruses whose mechanisms of replication are different. However few studies have focused on the application of antiviral drugs to specifically controlling gastrointestinal infections. The majority of effort in this area has been directed at controlling herpes virus infections with a considerable amount of success. It is anticipated that control of gastrointestinal infections by antiviral drugs will present a much greater challenge to molecular biologists and pharmacologists due to the variety of viral causative agents and the need to use them prophylactically rather than therapeutically. However, with the recent progress in molecular biology the identification of specific virus cell interactions, and identification of specific viral stages in replication within a cell should clearly provide important information required to develop broad-spectrum and selective antiviral drugs for specific viruses or virus groups. The combined activities of molecular

virologists and pharmacologists to identify the modes of action of a number of the known antiviral compounds and their analogs should greatly enhance our ability to specifically synthesize more effective antiviral chemotherapeutic drugs.

Finally, new methods of drug delivery and/or drug targeting to improve potency and selectivity of antiviral compounds should greatly enhance the therapeutic index of a number of these drugs. In the case of gastrointestinal infections one of the major impediments will be to direct the drug to the epithelial cells and maintain the drug there for a considerable length of time to ensure the antiviral state is present at the time virus infection occurs. Whether this will be possible in cases where gastrointestinal infections can occur over a long period of time remains to be determined. Thus it will be important to be able to predict specific epidemics of gastrointestinal infection using more rapid diagnostic techniques and treat contacts of individuals suffering from these infections so as to greatly reduce the spread of virus infections within the community or hospital environment.

One of the best places for application of antiviral drugs may be in children as they enter hospitals for reasons other than gastrointestinal infections. In these situations it is possible to predict that gastrointestinal nosocomial infections will occur within a few days of hospitalization. Elimination of this added stress would increase the child's rate of recovery. Another application may be in day-care centers where contact with other children shedding gastrointestinal viruses occurs frequently.

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