Review
Polyanions – a lost chance in the fight against HIV and other virus diseases?

M Lüscher-Mattli

Institute of Biochemistry, University of Berne, Freiestrasse 3, CH 3000 Berne, Switzerland

For correspondence: Tel: +41 31 879 09 18; E-mail: luescher@thenet.ch

Polyanions are known to exhibit potent antiviral activity *in vitro*, and may represent future therapeutic agents. This review summarizes literature reports, pertinent to anionic polymers as antiviral agents. The *in vitro* antiviral effects of numerous polyanionic compounds (sulphated polysaccharides, negatively charged serum albumin and milk proteins, synthetic sulphated polymers, polymerized anionic surfactants and polyphosphates) are described. This class of antiviral agent exhibits several unique properties that are not shared by other presently known antiviral agents: (i) a remarkable broad-spectrum antiviral activity against HIV-1, HIV-2 and a series of other enveloped viruses; (ii) the ability to inhibit syncytium formation between HIV-infected and normal CD4 T lymphocytes, a mechanism that drastically enhances HIV infectivity; and (iii) a low induction of viral drug-resistance. There is increasing evidence that polyanions interfere with the fusion process, a vital step in the viral replication cycle. The inhibition of virus–cell fusion appears to be the source of the antiviral activity of polyanions. *In vivo*, the pharmacological properties of polyanions result in a low bioavailability of the drugs to their viral targets, and hence a poor antiviral activity *in vivo*. It is suggested that polyanions must be used in combination with drug delivery systems in order to become therapeutically useful antiviral agents. Some drug delivery systems are briefly discussed.

Keywords: Antiviral agents, polyanions, dextran sulphate, influenza, fusion

Introduction

It has been known for a long time that polyanions (PA) possess antiviral activity. The interest in this class of antiviral agents has however, been moderate, until the potent anti-HIV activity of dextran sulphate *in vitro* was published (Ueno & Kuno, 1987; Nakashima *et al.*, 1987; Ito *et al.*, 1987; Mitsuya *et al.*, 1988). These reports attained worldwide attention. Two years later, dextran sulphate was tested as an anti-HIV agent in a clinical trial with orally administered dextran sulphate (Abrams *et al.*, 1989). The negative outcome of this trial caused some serious controversy and dextran sulphate was soon 'lost in a sea of confusion' (Anonymous, 1989). Although it was inconclusive, this negative result stopped any further development of polyanions as therapeutic antiviral agents.

In the past decade, a large number of PAs have been investigated *in vitro*. Today it is known that anionic polymers exhibit several important and unique properties: (i) a remarkable broad-spectrum antiviral activity against HIV and a number of other enveloped viruses; (ii) inhibition of syncytium formation between HIV-infected and normal CD4 T cells; and (iii) low induction of viral resistance in cell culture. Furthermore, it has been shown that PAs inhibit the viral fusion process, a vital step in the virus replication cycle during which the viral genome is injected into the host cell cytoplasm.

In vivo, anionic polymers encounter the difficulties common to all highly charged polymeric compounds, such as oligonucleotide or nucleic acid drugs. Novel drug delivery systems, developed to circumvent these difficulties, are now known and are briefly discussed herein.

In view of the fact that the availability of safe and potent antiviral agents is still far from satisfactory, the activity of antiviral polyanions merits further investigation. The aim of this review is to summarize the current knowledge regarding the antiviral properties of anionic polymers and to briefly discuss possibilities for improving their *in vivo* effectiveness.

The data included in this review were obtained in a series of database searches (Medline and internet virology

websites), and were selected according to their pertinence and scientific impact on the different topics discussed.

The mechanism of action of polyanions

It has long been postulated (Bengtson, 1965) that PAs interfere with the attachment of the virus to its target cells. The anionic polymers appear to interact specifically with the virus rather than the (negatively charged) cell membranes, as shown for the Sendai virus and dextran sulphate (Ohki *et al*., 1991).

Mechanism of action with HIV

Numerous investigations on the mechanism of the action of PAs have been conducted for HIV. They were shown to non-specifically inhibit the reverse transcriptase of various retroviruses (Baba *et al*., 1988; Nakashima *et al*., 1987). Since PAs do not penetrate the cell interior, this mechanism of action can be assumed to be of minor practical importance.

The inhibitory effect of PAs on HIV replication appears, however, to be based on another PA property – their ability to interfere with the attachment of the virus to CD4 target cells. Using several experimental techniques – such as radiolabelled HIV particles (Baba *et al*., 1988), a radioimmunoassay (Nakashima *et al*., 1989), flow cytometry (Schols, 1992) and a p24 ELISA assay (Witvrouw *et al*., 1994) – this process was investigated. The results obtained are interpreted as a polyanion-dependent inhibition of binding of the viral gp120–V3 loop to CD4 receptors on the target cell membrane. In a recent study (Kuipers *et al*., 1996), it was reported that negatively charged albumins interact equally with the V3 loop of gp120, and preferentially inhibit virus–cell fusion and syncytium formation. The fusion of HIV-infected cells with uninfected CD4 cells (syncytium formation) was also reported to be strongly suppressed by sulphated polysaccharides (Witvrouv *et al*., 1991; Schols *et al*., 1989, 1992).

HIV is known to fuse with CD4 cells at and below pH 7 in a pH-independent manner (Sinangil *et al*., 1988), comparable to the fusion of the Sendai virus. In addition to this interaction with the V3 loop of the viral binding glycoprotein (gp 120), it has been demontrated that dextran sulphate and anionic HSA bind to the N terminus of gp41, the viral fusion peptide (Gordon *et al.,* 1995)

Mechanism of action with influenza virus

The site(s) of interaction of anionic polymers with the virus envelope glycoproteins has not been investigated with this virus. The binding and fusion of influenza virus with erythrocytes and model membranes was investigated using fluorescence measurements. In binding experiments at 4°C using octodecyl-rhodamine-HCl (R18)-labelled virions it was shown that polyanions selectively inhibit the hydrophobic virus–membrane attachment at approximately pH 5, but have no measurable effect on receptor-mediated attachment at a neutral pH (Herrmann *et al*., 1992; M Lüscher-Mattli, unpublished results). This result was confirmed in a similar binding study (pyrene-PC fluorescence measurements) with influenza virus and negatively charged human serum albumin (Schoen *et al*., 1997).

In a series of fusion experiments (R18 fluorescence-dequeching) it was shown that the PAs, dextran sulphate (DS) and pentosan polysulphate (PPS), strongly inhibit the fusion of influenza virus with model membranes (Lüscher-Mattli & Glück 1990). The anti-fusion effect of DS was confirmed in fusion experiments with erythrocytes (Herrmann *et al*., 1992). Recently reported fusion experiments (PC-pyrene dequeching) with negatively charged human serum albumin (Schoen *et al*., 1997) showed that this PA equally inhibits the fusion of influenza virus with erythrocytes.

Further R18 fusion experiments with different influenza subtypes (H1N1 and H3N2) and with a number of enveloped viruses [Semliki forest virus (SFV), vesicular stomatitis virus (VSV), rabies virus, Sendai virus and mumps virus] demonstrated that DS 8 and 500 kDa and PPS strongly inhibit the fusion activity as well as the *in vitro* replication of influenza virus, SFV, VSV and rabies virus, but do not inhibit the fusion activity of Sendai virus and mumps virus (Lüscher-Mattli *et al*., 1993). The good correlation of anti-fusion and anti-replication effects observed in these experiments, strongly suggests that the antiviral properties of polymeric anions might be based on their anti-fusion activity.

Following different experimental techniques (trypsin digestion and electron microscopy), it has been demonstrated that the conformational change in haemagglutinin (HA), which is a pre-requisite for the exposure of the hydrophobic fusion peptide, is not inhibited by the anionic polymers (Herrmann *et al*., 1992; M Lüscher-Mattli, unpublished results).

From this experimental evidence, the mechanism of the anti-fusion activity of PAs may be tentatively described as follows: PAs selectively inhibit the hydrophobic binding of influenza virus at low pH, which is known to be mediated by the viral fusion peptide (HA2 N-terminus). This virus–cell interaction product is known to represent the key intermediate in the induction of the viral fusion process (Tsurudome *et al*., 1992; Stegmann *et al*., 1991). The low pH-induced conformational change in HA (by which the fusogenic HA2–N-terminus is ejected from its buried position in the HA trimer), is not inhibited by the PAs investigated. The fusion peptide appears to be exposed, but loses its hydrophobic properties in the presence of PAs. The mechanism of this inactivation of the viral fusion peptide is

not known. It can be hypothesized that PAs bind to and inactivate the viral fusion peptide. This binding may involve electrostatic interactions between anionic polymers and positively charged amino acid residues, present in the various viral N-terminus fusion peptides at the respective pH values of the viral fusion process (Hiti *et al.*, 1991).

It has been demonstrated that DS and HSA bind to the viral fusion peptide gp41 in the case of HIV (Gordon *et al.*, 1995).

A more detailed investigation of the mechanism of the anti-fusion effect of PAs would be highly desirable, and may increase our understanding of their structure–activity relationships.

The antiviral activity of polyanions *in vitro*

The majority of the research in the field of antiviral PAs has been carried out by De Clercq and his coworkers, who have made a substantial contribution to the current knowledge of the *in vitro* properties of this class of antiviral agents.

In order to exhibit antiviral activity, the molecular weight of the PAs must be 5–10 kDa and contain a sufficiently high number of negative charges. On first approximation, it can be said that the composition of the polymer chain (polysaccaride, carbon–carbon or polypeptide), as well as the chemical nature of the anionic groups (sulphates, sulphonates, carboxylates and phophates), appear to be of minor importance for the antiviral activity of PAs. In Figure 1 the formulae of some PAs discussed in the following text are given.

It must, however, be emphasized that structural and conformational aspects of the polymer, as well as charge density and/or distribution also play an important role. Different virus strains, cell types and experimental conditions can also influence the outcome of the antiviral tests of PAs (Witvrouw & De Clercq, 1997).

The different polyanionic compounds were tested with a large number of RNA and DNA viruses. Only enveloped virus types were found to respond to the polyanionic inhibitors, whereas non-enveloped viruses (reo-, picorna-, parvo-, papova- and adenoviruses) are not sensitive to PAs.

In most, if not all, of the *in vitro* tests reported below, the polyanionic inhibitors were added before or immediately after inoculation of the cell cultures, i.e. under optimal conditions for drug exposure of the virus.

Sulphated polysaccharides

Polysaccharides are linear or branched polymers of glycosidically linked sugar or aminosugar residues. The synthetic compounds DS and PPS were obtained by sulphation with chlorosulphonic acid.

In a recent review (Witvrouw & De Clercq, 1997), the antiviral activities of synthetic and natural sulphated polysaccharides have been described and discussed. The polymers must contain >2 SO_3^- groups per sugar residue in order to exhibit antiviral activity. In numerous studies, sulphated polysaccharides have been shown to be potent inhibitors of HIV-1 and HIV-2 replication *in vitro*, and to inhibit syncytium (giant cell) formation in cell cultures.

Natural and synthetic polysaccharides were also tested with herpes simplex viruses (HSV-1 and HSV-2), togaviruses (Sindbis virus and SFV), arenaviruses (Junin virus and Tacaribe virus), rhabdoviruses (VSV), orthomyxoviruses (influenza A and B viruses) and paramyxoviruses [parainfluenza 3 virus, measles virus and respiratory syncytial virus (RSV)].

Several of the natural sulphated polysaccharides, such as calcium spirulan (Hayashi *et al.*, 1996), ascidians sulphated polysaccharides (Pavao, 1996) and sulphated bacterial glycosaminoglycans (Baba *et al.*, 1990), do not exhibit discernible anticoagulant activity, a side-effect that represents a major drawback in the therapeutic use of sulphated polysaccharides. Sulphated polysaccharides are known to lead very slowly to virus drug resistance (Witvrouw & De Clercq, 1997).

Synthetic polyanionic polysaccharide derivatives. The following derivatives were synthesized and investigated:

(i) Carboxymethyl dextran benzylamide and carboxymethyl dextran benzylamide sulphonate (Carre *et al.*, 1995; Neyts *et al.*, 1995).

(ii) Carboxylated cyclodextrin derivatives, bearing 18–48 carboxylate groups per polymer (Leydet, 1998).

(iii) Sulphated octadecyl-ribofuranans of molecular weight 3–9 kDa (Choi *et al.*, 1996).

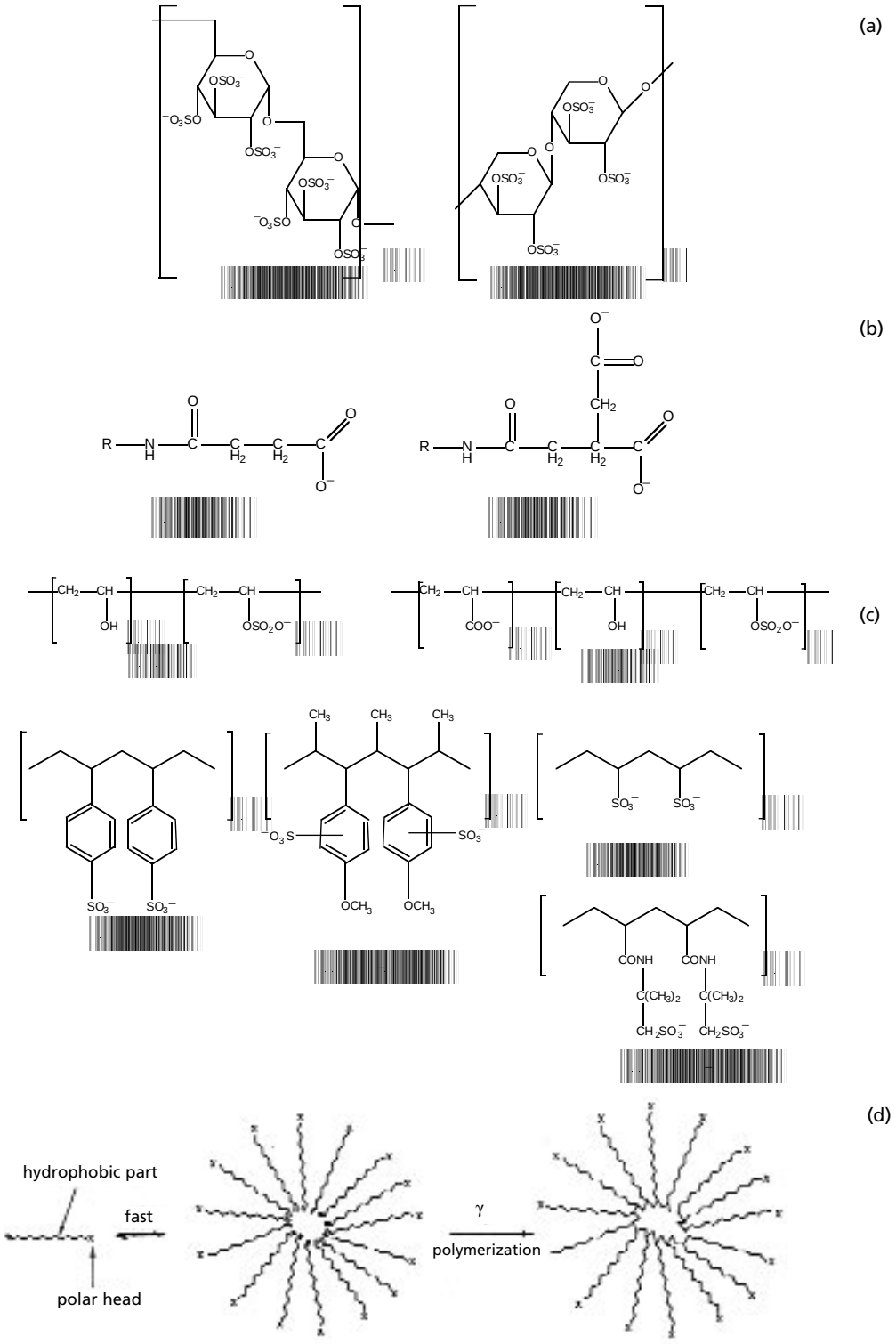
The antiviral activity of these compounds was tested with HIV-1, HIV-2 and other enveloped viruses [human cytomegalovirus (HCMV), HSV and RSV). The concentrations required to inhibit virus replication or cytopathic effects by 50% (IC_{50}) were very low, in the range 0.1 to approximately 3 μg/ml and anticoagulant effects were largely absent (Jansen *et al.*, 1993).

Negatively charged proteins

Human serum albumin (HSA). HSA is a globular protein with a molecular weight of 67500. HSA was modified by the introduction of one or two carboxylic groups by reaction of the protein-lysine groups with succinic and aconitic acid, respectively (Jansen *et al.*, 1991, 1993; Swart & Mejier, 1994). Neither compounds exhibited anticoagulant properties.

The negatively charged HSA derivatives showed very good antiviral activity with HIV-1 and influenza virus A. The inhibitory effects with HIV-2 were less pronounced, and were completely absent with 13 other enveloped RNA

Figure 1. The formulae of some of the polyanions



(a) Sulphated polysaccharides, dextran sulphate and pentosan sulphate. (b) Negatively charged human serum albumin suc- and aco-HSA (R represents the lysine residues of HSA). (c) Synthetic sulphated polymers (Schols *et al.,* 1990; Mohan *et al.,* 1992 with permission). (d) Polymerized anionic surfactants (X represents the polar head groups) (Leydet *et al.,* 1995 with permission).

Table 1. Antiviral activities of the different classes of polyanions

	IC_{50} (μg/ml)									
Compound	HIV-1	HIV-2	VSV	SFV	Sindbis virus	Influenza virus A	Influenza virus B	RSV	Measles virus	Parainfluenza virus
Sulphated polysaccharides										
DS										
MW 40000	1.3[b]	–	0.2[a]	14[a]	2.0[a]	1.0[c]	>200[c]	0.1[c]	>200[c]	>200[c]
MW 110000	3.0[b]	–	0.7[a]	20[a]	2[a]	1.4[c]	>200[c]	0.14[c]	>200	>200
PPS										
MW 3100	1.0[b]	–	4.0[a]	>400[a]	20[a]	20[c]	>200[c]	0.8[c]	>200[c]	>200[c]
Mannan sulphate										
MW 30000	1.6[b]	–	–	–	–	8.0[c]	>200[c]	0.86[c]	>200[c]	>200[c]
Carrageenan κ	6.4[b]	–	–	–	–	–	–	–	–	–
Carrageenan λ	2.2[b]	–	–	–	–	–				
Negatively charged proteins										
Aco HSA	0.023[d]	5.9[d]	>400[d]	>400[d]	>400[d]	0.8[d]	–	–	–	>400[d]
Suc-HSA	0.9[d]	78[d]	–	–	–	–	–	–	–	–
Suc/aco			–	–	–	–	–	–	–	–
α-lactalbumin	0.3–3[e]	500[e]	–	–	–	–	–	–	–	–
β-lactoglobulin	0.3–3[e]	500[e]	–	–	–	–	–	–	–	–
-lactoferrin	0.3–3[e]	500[e]								
Synthetic sulphated polymers										
PVAS	0.3[f]	0.35[f]	0.5[f]	30[f]	2.7[f]	>200[f]	>200[f]	2.3[f]	>200[f]	>400[f]
PAVAS	0.31[f]	0.50[f]	0.7	30[f]	2.7[f]	>200[f]	>200[f]	3.6[f]	>200[f]	>400[f]
PSS	6.0[g]	7.0[g]	–	–	–	20[h]	>100[h]	1.2[h]	–	–
PAS	3[g]	7[g]	–	–	–	4.0[h]	20[h]	1.2[h]	–	–
PVS	3[g]	1[g]	–	–	–	200[h]	>200[h]	20[h]	–	–
PAMPS	1[g]	3[g]	–	–	–	0.8[h]	>200[h]	0.16[h]	–	–
Polymerized anionic surfactants										
$[C{=}C{-}(CH_2)_9{-}X]n$	0.1–55[i]	1.3–88[i]	>400[i]	>400[i]	> 400[i]	4–>20[i]	>200[i]	4–>200[i]	–	>400[i]
Polyphosphates										
Inorganic Polyphosphates	33[k]	–	–	–	–	–	–	–	–	–
Poly (A)–poly (U)	0.5[l]	–	–	–	–	–	–	–	–	–
Low molecular weight polyanions										
Suramin	32[m]									
Aurintricarboxylic acid (ATA)	5[m]									

The abbreviations used in Table 1 are explained in the text.
The antiviral activity of the different compounds is expressed as the inhibitor concentration required to achieve 50% inhibition of virus replication, or inhibition of virus-induced cytopathogenicity (IC_{50}, given in μg/ml). The molecular weights of the polymers listed in Table 1 are >5–10 kDa. The data presented in Table 1 are from references: (a) Witvrouw & De Clercq (1997); (b) Baba *et al.* (1990); (c) Hosoya *et al.* (1991); (d) Jansen *et al.* (1993); (e) Swart *et al.* (1996); (f) Schols *et al.* (1990); (g) Mohan *et al.* (1992); (h) Ikeda *et al.* (1994); (i) Leydet *et al.* (1995, 1996, 1997); (k) Lorenz *et al.* (1997); (l) Krust *et al.* (1993); and (m) De Clercq (1989).

and DNA viruses tested (Jansen *et al.*, 1993).

The mechanism of action of these novel antiviral agents was investigated by *in vitro* antiviral assays with HIV. Negatively charged HSA derivatives appear to block virus–cell fusion and syncytium formation (Kuipers *et al.*, 1996). It is interesting to note that the same compounds

also inhibit fusion of influenza virus (Schoen *et al*., 1997). There is a striking analogy with the anti-fusion effects of dextran sulphate, as reported in the preceding section.

Milk proteins. The milk proteins lactoferrin, alpha-lactalbumin and beta lactoglobulin A and B were also derived by acylation of the amino function of lysine residues using anhydrides of succinic- or *cis*-aconitic acid (Swart *et al*., 1996). The resulting negatively charged proteins all showed strong antiviral activity against HIV-1 and virtually no cytotoxicity at the concentrations used.

Synthetic sulphated polymers

A new class of anionic polymers, in which the polysaccharide or polypeptide backbone of the above-mentioned compounds is replaced by carbon–carbon polymer chains, has also been investigated. The antiviral activity of these compounds increases with increasing molecular weight and increasing negative charge.

Sulphated polyvinylalcohol (PVAS) and its co-polymer with acrylic acid (PAVAS) have been shown to exhibit broad-spectrum antiviral activity against a number of enveloped viruses (HSV, HCMV, VSV, RSV, togavirus, arenavirus and HIV-1 and -2) (Schols *et al*., 1990; Hosoya *et al*., 1991) and to block HIV-induced syncytium formation.

Sulphonic acid polymers such as poly(4-styrene sulphonic acid) (PSS), poly(anetholesulphonic acid) (PAS), poly(vinylsulphonic acid) (PVS) and poly(2-acrylamido-2-methyl-1-propanesulphonic acid) (PAMPS) are reported to suppress HIV-1 and -2 replication and syncytium formation *in vitro* (Mohan *et al*., 1992; Tan *et al*., 1993). The novel sulphonic acid derivatives were also shown to possess antiviral activity against RSV and influenza A virus *in vitro*. *In vivo* PAMPS proved to be effective against influenza A virus infection in mice, only if administered simultaneously with virus infection (Ikeda *et al*., 1994).

Polymerized anionic surfactants

A series of new PAs were synthesized by polymerization of omega unsaturated anionic surfactants whose polar head group was derived from amino acids or dipeptides (Leydet *et al*., 1995, 1996, 1997).

In order to exhibit antiviral activity, a minimal number of anionic groups must be present, and they must be located on the exterior side of the molecule.

The new PAs were tested *in vitro* against HIV-1 and HIV-2, HCMV and HSV (IC_{50} = 5–10 and 20–40 μg/ml, respectively). Influenza virus A virus, RSV and arena viruses (Junin and Tacaribe) were also found to be sensitive to the new PAs. The other enveloped viruses tested (VSV, SFV, Sindbis virus, parainfluenza virus, etc.) did not respond to the new PAs. No cytotoxicity was observed for the host cells at concentrations up to 200 μg/ml.

Polyphosphates

Another type of anionic polymer that exhibits antiviral activity is represented by the following polyphosphates:

(i) Inorganic polyphosphates of chain lengths of 15, 34 and 91 phosphate residues were shown to inhibit HIV replication and syncytium formation (Lorenz *et al*., 1997).

(ii) The double-stranded, synthetic polynucleotides poly(A)-poly(U) (Krust *et al*., 1993) and poly(I)-poly(C) (Montefiori & Mitchell, 1987) were reported to inhibit HIV replication *in vitro*. The authors suggest that the mechanism of action of the polynucleotides is by inhibition of a post-binding step in the virus cell-entry process.

Low molecular weight polyanions

Suramin and aurin tricarboxylic acid (ATA) are polycyclic polysulphate and polycarboxylate compounds, respectively, and were the first polyanionic compounds shown to exhibit anti-HIV activity and inhibition of HIV-1 attachment to cellular CD4 receptors (Mitsuya *et al*., 1984; Balzarini *et al*., 1986).

Recent studies with a novel suramin-analogue (Ono *et al*., 1997) showed that this compound (FP21399) blocks HIV-mediated fusion with CD4 cells and generates only weakly resistant virus variants. *In vivo*, the PA showed significant protection against HIV-1 infection (SCID-hu mice), and was found to be concentrated in lymph nodes.

Table 1 summarizes the results reported for a series of enveloped viruses that have been investigated with the different anionic polymers discussed in this section. An inspection of Table 1 reveals the following: (i) the sulphated polysaccharides have the most pronounced broad-spectrum antiviral activity, being inhibitory to HIV-1, HIV-2, VSV, SFV, Sindbis virus, influenza virus-A and RSV. As shown for heparin, these compounds behave in solution as an extended random coil (Casu, 1985). In contrast, the antiviral activity of the bulky globular HSA is restricted to HIV-1 (and to a lesser extent to HIV-2) and to influenza virus-A. This relationship between the spatial structure of the PAs and their antiviral activity appears interesting, and may be useful in the design of future antiviral PAs. (ii) For HIV-1 the negatively charged proteins and the synthetic sulphated polymers PVAS and PAVAS exhibit the lowest IC_{50} values, and therefore the highest antiviral activity. (iii) With the exception of RSV, the paramyxoviruses and the orthomyxovirus influenza virus-B do not respond to all PAs tested.

Polyanions *in vivo*

Toxic side-effects

Anticoagulant activity. The major undesirable side-effect of anionic polymers is their well-known anticoagulant activ-

ity, which limits the therapeutically administrable doses. This adverse effect can be avoided by using PAs that have no discernible anticoagulant activity, such as negatively charged human serum albumins (Jansen *et al*., 1993), novel sulphated polysaccharides (Baba *et al*., 1990), two natural sulphated polysaccharides Ca-spirulan (Hayashi *et al*., 1996) and an ascidian, dermatan-like polysulphate (Pavào, 1996).

Thrombocytopoenia. Continuous intravenous infusion of dextran sulphate was reported to produce profound, but reversible, thrombocytopoenia (a decrease in platelet count) (Flexner *et al*., 1991).

Pharmacology of polyanions

The pharmacokinetics of dextran sulphate were investigated in humans using an APTT (activated partial thromboplastin time) assay (Lorentsen *et al*., 1989), with ^{3}H-labelled dextran sulphate in rats (Hartmann *et al*., 1990), with a new sensitive bioassay in rabbits (Witvrouw *et al*., 1990) and with fluorescein-labelled dextran in rats (Mehvar & Shepard, 1992).

From these studies, the following pharmacokinetic information was obtained: (i) oral dextran sulphate is poorly absorbed and strongly degraded (MW 200), resulting in a plasma concentration of intact DS of approximately zero. After intravenous administration, the plasma levels were found to be dose-dependently increased, and decreased biphasically with time, the mean half-life being approximately 1.5–2 h. (ii) DS was found to be strongly degraded in plasma. The fraction of high molecular weight DS (8000) decreases by a factor of 10 within 2 h (HPLC studies; Hartmann *et al*., 1990). The antiviral activity of sulphated polysaccharides is known to be dependent on their molecular weight, and their content of negatively charged groups, and the partially degraded (deglycosylation and/or desulphation) metabolites exhibit a strongly reduced or absent antiviral activity. (iii) The antiviral effect of DS was strongly reduced in the presence of human serum. In the presence of increasing concentrations of fresh human serum, the concentration of DS required for complete inhibition of HIV infectivity was significantly increased from 5–10 μg/ml (10–15% serum) to >50 μg/ml (85% serum) (Hartmann *et al*., 1990). As a PA, DS is probably adsorbed non-specifically to cationic serum components. (iv) The volume of distribution of dermatan sulphate is 5.1–7.2 l (Gianese & Lucchelli, 1991). This value is slightly higher than the theoretical total plasma volume. If given intravenously, the high molecular weight PAs appear to be restricted to the systemic circulation. (v) Pentosan polysulphate, and probably DS, are cleared extensively by the reticuloendothelial system, and have been found to be accumulate in kidneys, liver and spleen (Taugner *et al*., 1971).

The pharmacological results reported above refer to sulphated polysaccharides. It is possible that the low molecular weight PAs like suramin and ATA, as well as some of the novel polyanionic compounds, reported in the previous section, will show different pharmacokinetic properties.

Clinical trials with HIV

As pointed out by Drusano (1993) and Fletcher (1996), it is of primary importance to recognize that the bioavailability, or exposure of a given drug to its target, is essential for the therapeutic outcome of clinical tests. The consequences of not distinguishing between administered dose and actual drug exposure are discussed by these authors, and the case of DS is used as an example to demonstrate the pitfalls associated with ignoring pharmacological information.

In the trial reported by Abrams *et al*. (1989), DS was administered orally and in the same year it was shown that DS is poorly absorbed when administered by this route (Lorentsen *et al*., 1989). It is evident that the negative results of this trial are irrelevant. Flexner *et al*. (1991) report a second clinical trial using intravenous DS, given by continuous infusion. Despite high plasma levels of the drug (7.6 μg/ml), no measurable short-term therapeutic effect was observed, but the p24 serum concentrations increased in all patients. An interpretation of this result is difficult. Complex side-effects (lymphocyte activation) or experimental artefacts may be the source of the observed increase in p24.

In view of the pharmacological properties of PAs outlined above, it must be expected that the clinical antiviral efficiency of PAs will be far from satisfactory. The drawbacks of PAs as therapeutic agents (short plasma half-life, toxic side-effects, partial inactivation by plasma components and a poor ability to penetrate infected tissues and cells) hamper their therapeutic efficacy and explain the striking difference beween their *in vitro* and *in vivo* antiviral activity.

Drug delivery systems

Similar pharmacological difficulties are encountered by other chemo-therapeutically important agents, such as polypeptides, proteins, oligonucleotides and nucleic acids, and considerable investigational efforts are presently being made to develop drug delivery systems to improve the therapeutic effectiveness of these drugs. An extensive review of these new technologies may be found in: 'Drug delivery systems: technologies and commercial opportunities' (DR reports 1998). Some of these drug delivery systems may be useful for polyanionic antivirals and are briefly described below.

Lipophilic drug derivatives

Derivatives of anionic polymers, coupled to hydrophobic moieties may have an improved pharmacokinetic profile, compared to their parent compounds. Sulphated octadecyl ribofuranans (Choi *et al*., 1996), liposome-attached aco-HSA (Kamps *et al*., 1996, 1997) and polymerized anionic

surfactants (Leydet *et al*., 1995, 1996, 1997) may represent promising approaches along this line.

Polymeric lipo-polyethylenimines

These novel drug-delivery systems were designed to transport DNA into the cell cytoplasm (Erbacher *et al*., 1999; Bettinger *et al*., 1998). Since the pharmacokinetics of nucleic acids are determined by the physicochemical properties of PAs (Takakura, 1996), it can be assumed that this drug delivery system might also work for polyanionic antiviral agents.

Nanoparticles

Nanoparticles are polymeric particles in the nanometer size range, into which drugs may be incorporated as solid solutions or dispersions. These particles were shown to enhance the delivery of certain drugs across membranes and to improve their bioavailability.

Liposomes

Liposomes are recognized as important and versatile drug-delivery systems and have been investigated for approximately three decades. Pharmaceutical technologies, allowing cost-effective and large-scale production of liposomal drugs, are presently available (Rubas & Schreier, 1991) and it is expected that there will soon be more commercially available liposome-based drugs.

For antiviral drug-delivery, there are numerous studies of liposome-encapsulation in the literature (for reviews see Désormeaux & Bergeron, 1998; Meijer *et al*., 1992; Koff & Fidler, 1985). Liposome-encapsulation is reported to result in prolonged plasma half-life, reduced toxic side-effects and in enhanced antiviral activity of the encapsulated compounds (ddC, foscarnet, zidovudine, etc). To our knowlege, no studies of liposome-encapsulated PAs have been reported.

Investigations into the effect of polyanion-drug carrier systems on virus replication *in vivo* or in suitable *in vitro* model systems are urgently needed as they may improve the therapeutic usefulness of this class of antiviral agents.

Summary

This review summarizes the informations currently available in the literature on anionic polymers as antiviral agents.

In vitro activity of synthetic and natural polyanions

Sulphated and sulphonated polysaccharides, carboxylated proteins (human serum albumin, milk proteins), sulphated and sulphonated synthetic polyvinylic and acrylic acid polymers, synthetic detergent-amino acid polymers and polyphosphates (inorganic and polynucleotide derivatives) have been investigated with respect to their antiviral activity *in vitro*. The main requirements for PAs to exhibit significant antiviral activity (molecular weight >5–10 kDa, high content of negative charge) have been determined. Structural and conformational properties of the polymer, as well as charge density and distribution also play an important role. The different types of PAs were tested for their antiviral activity with a large number of enveloped viruses (Table 1). In cell culture the anionic polymers investigated were found to have several important advantages compared to other antiviral agents: (i) broad-spectrum antiviral activity against a large number of enveloped viruses, including HIV-1 and HIV-2; (ii) inhibition of syncytium formation–fusion between HIV-infected and normal CD4 T cells. This mechanism, which drastically enhances the infectivity of HIV, is not inhibited by other anti-HIV agents, such as proteinase inhibitors or nucleoside analogues; (iii) low induction of viral drug resistance, a major problem in antiviral chemotherapy; and (iv) low cytotoxicity in the *in vitro* assays.

The mechanism of action

There is increasing evidence that PAs inhibit the fusion of the virus with its target membrane. This inhibitory effect can be the result of an inhibition of virus–cell attachment (suggested for HIV), or of a direct interaction of the PAs with the viral fusion peptides (suggested for IV). For dextran sulphate and anionic human serum albumin, binding to the gp41 fusion peptide of HIV, has been demonstrated.

The fusion peptides, being vital to the viral replication process, are highly conserved regions of the virus surface glycoproteins. It is expected that they will not be altered by antigenic shifts and drifts of the virus strains, or by amino acid changes, leading to drug resistance. PAs that are directed towards this target are therefore very good candidates for future antiviral agents.

In this context, it is interesting to note that recent investigations revealed structural parallels among the fusion proteins HA2 of influenza virus and the gp41 of HIV (Joshi *et al*., 1998), the paramyxovirus F protein (Dutch *et al*., 1998) and the murine leukaemia virus fusion protein (Fass & Kim, 1995). Enveloped viruses appear to use very similar 'tools' to enter their target cells.

The antiviral activity of polymeric anions *in vivo*

The activity of PAs *in vivo* is hampered by several drawbacks common to other macromolecular drugs (proteins, polypetides, nucleic acids, oligonucleotides, etc). These are short plasma half-life (approximately 1–2 h), rapid degradation in the gut and in plasma, and a poor ability to penetrate and target infected tissues and cells. It is therefore expected that their therapeutic usefulness *in vivo* will be far from satisfactory, an expectation that was borne out in two clinical trials.

Drug delivery systems

Development of therapeutically effective antiviral PAs requires the use of a drug adjuvant system, and lipophilic drug-derivatives may improve the pharmacokinetics of PAs. In addition, drug delivery systems that are currently being developed (encapsulation in lipo-polyethylenimines or in nanoparticles and liposomes) may improve the therapeutic usefulness of anionic polymers. Liposome-encapsulation has been reported to improve the antiviral activity and to reduce the toxic side-effects of antiviral agents.

It can be hoped that in the near future more attention will be given to improving the *in vivo* effectiveness and reduce the toxicity of antiviral agents, including anionic polymers, which have been shown to be potent and safe broad-spectrum antiviral agents *in vitro*.

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