Volume 252, number 1,2, 1–4 FEB 07404 July 1989

Review Letter

Towards the development of antimicrobial drugs acting by inhibition of pathogen attachment to host cells: a need for polyvalency

Mikhail N. Matrosovich

Institute of Poliomyelitis and Viral Encephalitides, USSR Academy of Medical Sciences, Moscow Region 142782, USSR

Received 15 May 1989

The development of inhibitors of microbial attachment to target cells has been proposed recently as a possible novel approach to antimicrobial chemoprophylaxis and treatment. In this paper an attempt is made to contend that such artificial inhibitors must be polyvalent, i.e. capable of binding to the pathogen or its target by multiple bonds.

Antimicrobial drug; Antiviral drug; Antitumor drug; Microbial attachment; Cooperative binding; Cell adhesion; Sialic acid; Cell adhesion inhibitor

1. INTRODUCTION

The initial step in any viral infection is the attachment of the virus to specific cellular receptors which is mediated by viral attachment proteins (VAPs). This step may determine, in many cases, the outcome of virus-cell encounters, host range and tissue tropism of the virus [1–5]. The possibility has been shown of virus neutralization by blocking the receptor-binding epitopes of VAPs with soluble cellular receptors or their synthetic analogs, for example, cellular membrane lipid fractions for rabies virus [6,7], synthetic sialosides for myxoviruses [8], recombinant soluble forms of CD4 antigen [9,10] or synthetic peptide fragment of CD-4 [11] for human immunodeficiency viruses.

Most natural bacterial infections are also initiated by adhesion of microorganisms to the mucosal surfaces of the host. The attachment proteins of bacteria (the so-called adhesins) interact usually with carbohydrate determinants of host cell glycolipids or glycoproteins [12–14]. In a number of experiments the attachment of microorganisms to target cells in vitro was completely prevented and infected animals were protected by competitive blocking of microbial adhesins with specific sugars, oligosaccharides or glycoproteins [15–19].

It has been postulated that the process of tumor metastasis into certain organs is similar to that of infection of target tissues by bacteria: organotropy of both infectious diseases and metastasis can be mediated by lectins of either the invaded organs or of the invading bacteria or tumor cells [20]. Repeated administration of the hepatic lectin-blocking agents D-galactose or arabinogalactan prevented metastatic dissemination into the liver of mice of experimental sarcoma and lymphoma [21].

These facts support the plausibility of a recently proposed novel approach to the prophylaxis and, possibly, early treatment of microbial infections and tumor metastasis based on the development of drugs which inhibit pathogen attachment to host cells [2,6,12,20–22]. The potential benefits of this approach are blocking of the first stage of infec-

Correspondence address: M.N. Matrosovich, Institute of Poliomyelitis and Viral Encephalitides, USSR Academy of Medical Sciences, Moscow Region 142782, USSR

Published by Elsevier Science Publishers B.V. (Biomedical Division)

tion preceding pathogen multiplication, probable low toxicity of the drug to the host and its wide range of action as many serotypes, types and even unrelated species of viruses and bacteria are known to recognise the same receptors on the target cells [1–5,12,13]. Although in principle two alternative modes of intervention into pathogen-cell interaction are possible, i.e. blocking of (i) either active epitopes of the pathogen's attachment proteins, or (ii) receptors for pathogen on cells, the latter mode [21,22] seems less promising in terms of potential ability to violate the normal physiology of the host cells. In either case, however, in order to develop potent inhibitors of attachment of microorganisms to cells, suitable for medical applications, it is obligatory to consider the multi-bond cooperative character of microbial reception.

2. COOPERATIVE NATURE OF MICROBIAL RECEPTION NECESSITATES THE POLYVALENCY OF INHIBITORS

All viruses, bacteria and cells are polyvalent, i.e. they contain multiple copies of attachment proteins on their surface (up to a few hundred for viruses, and even more for bacteria and cells), and thus can attach to their targets, which are also polyvalent, by multiple bonds. It is calculated that many viruses, when binding to cells, interact with not less than 10 monomeric cellular receptor molecules (the so-called cellular receptor units – CRUs), being organized into polyvalent 'cellular receptor sites' (CRS) [1]. The characteristic feature of such polyvalent cooperative binding is that the high avidity of binding may be mediated by multiple low-affinity individual contacts [1,23,24]. For example, the apparent binding constants of isolated spike glycoproteins of Semliki forest virus, in the form of water-soluble octameric complexes, to cells was shown to be 100–1000-fold lower than that determined for the whole virus containing approx. 260 spikes [25].

Concerning the design of drugs which block microbial attachment to target cells and considering the cooperative nature of this attachment, it was suggested [6,26] that the traditional pharmacological approach – designing of low molecular mass competitive inhibitors of the corresponding active epitope – would not work in this case, as monovalent compounds would not be able to compete effectively with the polyvalent interaction of a microorganism with the cell. Analysis of data in the literature and presented below substantiates this conclusion.

Thus, it is well known that mucoproteins interfere with hemagglutination by myxoviruses as a result of blocking of their attachment proteins with mucoprotein's sialic acid residues, the minimum active determinants, recognised on a host cell surface by many viruses. Orosomucoid, a serum glycoprotein containing *N*-acetylneuraminic acid (NeuAc), does not inhibit hemagglutination, owing to its molecular mass being too low [27]. It was found that incubation of orosomucoid at 80°C caused its polymerization and conversion to a potent inhibitor of hemagglutination by influenza viruses [28]. This shows that an increase in molecular mass, and hence in valency of the inhibitor, augments its activity.

In order to investigate the detailed specificity of the influenza virus hemagglutinin receptor-binding epitope, the ability of some natural and synthetic monovalent sialosides to inhibit adsorption of the virus on human erythrocytes was compared [29]. The inhibition was dose-dependent with a steep slope for the inhibition curve reflecting the highly cooperative nature of the virus-erythrocyte interaction. About 7 mM (4.4 mg/ml) sialyllactose was required for 50% inhibition of binding, and 15 mM for complete inhibition. Although some sialosides were more potent inhibitors than sialyllactose, it seems doubtful that monovalent sialosides can be found which will be sufficiently active for practical use as anti-influenza drugs. The same is true for known monovalent inhibitors of bacterial adhesion, which must be used at very high concentrations (up to 5 mg/ml) to prevent infection completely [17,18]. Recently, high molecular mass sialylglycoprotein inhibitors of influenza virus hemagglutination, low pH-fusion and hemolysis were synthesized starting with the so-called glycoprotein 2 (GP2) from bovine erythrocyte membranes [30]. These artificial sialylglycoproteins contained about 200 NeuAc groups bound per 250 kDa GP2 macromolecule and inhibited hemagglutination by influenza viruses at concentrations of 1.2–4.5 μg/ml (1–4 μM with respect to NeuAc), i.e. they were 1000-times more potent inhibitors than the above-mentioned monovalent sialosides [29].

Similar conclusions about the superiority of polyvalent inhibitors can be drawn from a study [31] in which the nature of the cellular receptors on swine nasal mucosa for *Bordetella bronchiseptica* bacteria, pathogenic to this animal, has been investigated. Among the different mono- and oligosaccharides and glycoproteins tested, only those containing NeuAc inhibited the adhesion of bacteria to target cells in vitro, thus confirming the involvement of sialic acid residues exposed on cellular surfaces. The polyvalent NeuAc-containing inhibitors – bovine submaxillary mucin and gangliosides (the latter form multimeric micelles in solution) – were effective even at 0.075 mg/ml (90–97% inhibition of adhesion), which is 100-times lower than the concentration of monovalent NeuAc and sialyllactose needed to prevent binding by only 55 and 66%, respectively.

The species-specific reaggregation of cells of the marine sponge *Microciona prolifera* cells is mediated by 'sticky' proteoglycan aggregation factor (MAF) having a molecular mass of about 20 MDa and consisting of more than 1300 copies of the monovalent glycopeptide fragment T-10 (10 kDa) [32]. The apparent binding affinity to sponge cells of individual T-10 molecules is comparatively low, i.e. 3×10^6 M which is 13000-times lower than that of native MAF. As a result, the ability of T-10 to inhibit MAF binding to sponge cells is also low: as much as 320 μg unlabeled T-10 was needed for 50% inhibition of adsorption to cells in competitive assays using 0.29 μg ^{125}I-MAF. T-10 can be polymerized by cross-linking with glutaraldehyde; the binding to cells and inhibitory activity of the resulting T-10 polymers increased proportionally with their size. Only 0.51 μg of 15 MDa T-10 polymer (about 1500 copies of T-10) was needed for 50% inhibition of 0.29 μg ^{125}I-MAF adsorption [32].

Just as for viruses, bacteria and other corpuscular organisms polyvalency and cooperativity of attachment to cellular receptors necessitate the polyvalent structure of inhibitors of adsorption for some biopolymers [33,34]. For example, shiga toxin from *Shigella dysenteriae* type 1 specifically binds to cellular glycoproteins or glycolipids having the galabiose disaccharide (Galα1-4Galβ) as a common determinant [34]. The monomeric disaccharide did not inhibit the toxin binding to HeLa cells in monolayers. However, the binding and cytotoxicity of the toxin were inhibited by galabiose covalently linked to BSA. Increasing the number of galabiose residues coupled per BSA molecule from 7 to 25 reduces the concentration of inhibitor required for 50% inhibition from 100 to 1 μg/ml.

All the examples listed show that multimeric polyvalent inhibitors of attachment of viruses, bacteria, cells, toxins and some other biopolymers are several orders of magnitude more active than the analogous monovalent compounds. Furthermore, by polymerising a weak inhibitor (i.e. increasing its valency) a very potent agent can be obtained.

It may be stated therefore that the development of artificial inhibitors of microbial attachment must include as a necessary step the designing of such a form of the inhibitor that will provide multi-site polyvalent binding to the target, i.e. pathogen or its receptor on susceptible cells.

To produce such polyvalent structures, one might utilize some principles of the design of the well-known 'drug delivery systems', for example, coupling the monovalent inhibitory active molecules in multiple copies to soluble biocompatible polymers or microparticulate carriers – liposome, synthetic microcapsule, micelle, polymeric, proteinaceous, carbohydrate or lipid nanoparticle, etc. The contemporary state of the field of drug delivery provides an abundant spectrum of methods and materials that can be used for the production of polyvalent inhibitors – potential drugs for clinical application (reviews [35–37]).

Besides the already mentioned advantage of enhanced activity of drugs solely due to cooperativity of binding to the target, the proposed approach to the design of inhibitors of microbial adhesion may have other possible benefits. Firstly, large polyvalent macromolecular or corpuscular inhibitors would be able to neutralise pathogens not only by competitive inhibition of receptor-binding sites of adhesins, which demands a manyfold excess of inhibitor, but also by steric hindrance of pathogen attachment, endocytosis, fusion with cellular membrane, uncoating, etc. Secondly, by choosing the appropriate carrier or including supplementary functional components in the composition of the 'inhibitor delivery system', one can influence the mode of interaction of inhibitor with its target

and, as a result, bring about an additional increase in its neutralizing potency. Finally, utilization of polyvalent instead of monovalent inhibitors reduces the likelihood of non-neutralizable mutants appearing, since the possible lowering of the avidity of individual contacts as a result of a mutation in the receptor-binding site of the microorganism would be compensated to some extent by the cooperativity of binding.

The correctness of and the prospects for the practical use of this approach to the design of antimicrobial agents could be evaluated in the future by using a model of those pathogens for which the structure of cellular receptor determinants or receptor-binding epitopes of the pathogen are known, for example influenza virus [26], human immunodeficiency virus [11], some bacteria [12–14] and tumors [21].

3. CONCLUSION

The idea of inhibition of a pathogen's attachment to host cells by suitably designed drugs may help to create a novel approach to prophylaxis and treatment of infectious diseases. In this paper, selected data on inhibition of attachment of viruses, bacteria, cells and microbial toxins have been reviewed and discussed, leading to the conclusion that such drugs must be polyvalent, i.e. capable of multi-site cooperative binding to the pathogen or its receptors on susceptible cells.

Acknowledgements: I thank Academician of the USSR AMS Professor M.P. Chumakov for encouragement and helpful discussions and Dr E.V. Koonin for critical reading of the manuscript.

REFERENCES

[1] Lonberg-Holm, K. and Philipson, L. (1981) Receptors and Recognition, ser.B, vol.8, Animal Viruses, Chapman and Hall, London.
[2] Dimmock, N.G. (1982) J. Gen. Virol. 59, 1–22.
[3] Bukrinskaya, A.G. (1982) Adv. Vir. Res. 27, 141–204.
[4] Paulson, J.C. (1985) in: The Receptors (Conn, P.M. ed.) vol.2, pp.131–219, Academic Press, Orlando, FL.
[5] Kohn, A. (1985) Prog. Med. Virol. 31, 109–167.
[6] Karlsson, K.-A., Norrby, E. and Wadell, G. (1986) Patent PCT No.PCT/DK/00007.
[7] Conti, C., Hauttecoeur, B., Morelec, M.J., Bizzini, B., Orsi, N. and Tsiang, H. (1988) Arch. Virol. 98, 73–86.
[8] Zakstelskaya, L.Ya., Molibog, E.V., Yakhno, M.A., Evstigneeva, N.A., Isachenko, V.A., Privalova, I.M. and Khorlin, A.Ya. (1972) Vopr. Virusol. 1972, 223–228.
[9] Smith, D.H., Byrn, R.A., Marsters, S.A., Grevory, T., Groopman, J.E. and Cagnon, D.J. (1987) Science 238, 1704–1707.
[10] Weiss, R.A. (1988) Nature 331, 15.
[11] Jameson, B.A., Rao, P.A., Kong, L.I., Hahn, B.H., Shaw, G.M., Hood, L.E. and Kent, S.B.H. (1988) Science 240, 1335–1339.
[12] Beachey, E.H. (1981) J. Infect. Dis. 143, 325–345.
[13] Sharon, N. (1987) FEBS Lett. 217, 145–157.
[14] Reid, G. and Sobel, J.D. (1987) Rev. Infect. Dis. 9, 470–487.
[15] Aronson, M., Medalia, O., Schori, L., Mirelman, D., Sharon, N. and Ofek, I. (1979) J. Infect. Dis. 139, 329–332.
[16] Fader, R.C. and Davis, C.P. (1980) Infect. Immun. 30, 554–561.
[17] Ko, H.L., Beuth, J., Solter, J., Schroten, H., Uhlenbruck, G. and Pulverer, G. (1978) Infection 15, 237–240.
[18] Beuth, J., Ko, H.L., Schroten, H., Solter, J., Uhlenbruck, G. and Pulverer, G. (1987) Zbl. Bakt. Hyg. A 265, 160–168.
[19] Chadee, K., Petri, W.A., jr, Innes, D.J. and Ravdin, J.I. (1987) J. Clin. Invest. 80, 1245–1254.
[20] Uhlenbruck, G., Beuth, J., Oette, K., Roszkowski, W., Ko, H.L. and Pulverer, G. (1986) Naturwissenschaften 73, 626–627.
[21] Beuth, J., Ko, H.L., Schirrmacher, V., Uhlenbruck, G. and Pulverer, G. (1988) Clin. Exp. Metastasis 6, 115–120.
[22] Colonno, R.J., Tomassini, J.E. and Callahan, P.L. (1987) in: Positive Strand RNA Viruses, pp.93–102, A.R. Liss, New York.
[23] Marsh, M. (1984) Biochem. J. 218, 1–10.
[24] West, C.M. (1986) Mol. Cell. Biochem. 72, 3–20.
[25] Fries, E. and Helenius, A. (1979) Eur. J. Biochem. 97, 213–220.
[26] Weis, W., Brown, J.H., Cusack, S., Paulson, J.C., Skehel, J.J. and Wiley, D.C. (1988) Nature 333, 426–431.
[27] Gottschalk, A. (1960) The Chemistry and Biology of Sialic Acids, Cambridge University Press, London.
[28] Whitehead, P.H., Flewett, T.H., Foster, J.R. and Sammons, H.G. (1965) Nature 208, 915–916.
[29] Pritchett, T.J., Brossmer, R., Rose, U. and Paulson, J.C. (1987) Virology 160, 502–506.
[30] Suzuki, Y., Nagao, Y., Kato, H., Suzuki, T., Matsumoto, M. and Murayama, J.-I. (1987) Biochim. Biophys. Acta 903, 417–424.
[31] Ishikawa, H. and Isayama, Y. (1987) Infect. Immun. 55, 1607–1609.
[32] Misevic, G.N. and Burger, M.M. (1986) J. Biol. Chem. 261, 2853–2859.
[33] Wong, T.C., Townsend, R.R. and Lee, Y.C. (1987) Carbohydr. Res. 170, 27.
[34] Lindberg, A.A., Brown, J.E., Stromberg, N., Westling-Ryd, M.J., Schultz, E. and Karlsson, K.-A. (1987) J. Biol. Chem. 262, 1779–1785.
[35] Plate, N.A. and Vasilyev, A.E. (1986) Physiologically Active Polymers, Khimia, Moscow.
[36] Tomlinson, E. (1987) Adv. Drug Del. Rev. 1, 87–198.
[37] Friend, D.R. and Pangburn, S. (1987) Med. Res. Rev. 7, 53–106.