MINIREVIEW

Treatment of Viral and Neoplastic Diseases with Double-Stranded RNA Derivatives and Other New Agents

JULIAN L. AMBRUS, SR.,*[,1] KAILASH C. CHADHA,† ANWARUL ISLAM,* SELINA AKHTER,* AND JULIAN L. AMBRUS, JR.*

Department of Internal Medicine, State University of New York at Buffalo, Buffalo General Hospital/ Kaleida Health System, Buffalo, New York 14203; and †Department of Molecular and Cellular Biology, Roswell Park Cancer Institute, Buffalo, New York 14263

Many attempts have been made to inhibit viral and neoplastic diseases by targeting the RNA system. The pathophysiologic significance of the microRNA system and the therapeutic potential of its manipulation are discussed. Studies of double-stranded RNA derivatives are reviewed. The therapeutic potential of one of these compounds, polyI:MPC, is emphasized. Studies of other related antiviral and antineoplastic agents are discussed, including 2'-deoxyoligocytidilates and telomerase inhibitors. Exp Biol Med 231:1283–1286, 2006

Key words: AIDS; double-stranded and single-stranded RNA; HIV infection; neoplastic diseases; thiolated polyI:polyC (pI:MPC); 2'-deoxyoligocytidilates; telomerase inhibitors; viral diseases

Introduction

With the rapidly developing drug resistance of some viruses and the difficulty in the timing of vaccine production and activity, there is great concern about viral disorders. Of particular concern are newly emerging viruses, hybrid viruses, and viruses bioengineered for use in bioterrorism. Similar problems exist with certain types of neoplastic disease. For this reason, there is increasing interest in therapeutic manipulation of the RNA system. We will briefly summarize the related studies.

RNA Interference

Gene-silencing techniques (RNA interference) are of particular interest in cancer research. In a series of recent reports (1–5), the roles of interfering RNAs (microRNAs) in cancer development were discussed. It is anticipated that these types of studies may lead to new diagnostic and therapeutic approaches. MicroRNAs are 21- to 25-nucleotide–long regulatory molecules that affect normal growth and development in plants and animals (6). They inhibit the translation of selected mRNAs into proteins. We know little about the normal function of individual microRNAs or about the development and function of abnormal microRNAs in neoplastic cells (3, 7). Johnson *et al.* (8) reported that the let-7 family of microRNAs regulate the expression of the ras oncogene. Michael *et al.* (9) described two microRNAs that are present in reduced quantities in precancerous and cancerous colorectal tissue. O'Donnell *et al.* (1) found that some abnormal microRNAs are regulated by a protein encoded by the well-known c-*myc* oncogene. Preliminary research indicates that microRNAs controlled by c-*myc* may also inhibit the expression of the *E2F* gene family, known inducers of apoptosis that encode another protein involved in cellular proliferation (10). The two genes mutually promote a vicious regulatory cycle leading to increased proliferation of neoplastic cells. Years of research may be required before adequate therapy can be developed based on these initial findings. Research along

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[1] To whom correspondence should be addressed at the Department of Internal Medicine, State University of New York at Buffalo, Buffalo General Hospital/Kaleida Health System, 100 High Street, Buffalo, NY 14203. E-mail: jlambrus@netscape.net

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these lines may be of importance in the therapy of certain viral disorders, as well as neoplastic diseases.

Studies of Double-Stranded RNA Derivatives

Double-stranded RNA derivatives have experimentally been found to have antiviral and antineoplastic effects, thought to be due in part to their capacity to induce interferons (IFNs). Haines *et al.* (11), Torrence and DeClereq (12), DeClereq (13), and Sen *et al.* (14) reported that double-stranded RNA has the capacity to induce IFNs in animal and human cells and to increase resistance to viral infections. A prototype of double-stranded RNA, polyI:polyC, has been reported to inhibit the growth of some tumor cell lines and the growth rate of human tumor xenografts in mice (15, 16). While polyI:polyC is the most potent among the double-stranded RNAs tested, it has proved to be too toxic for therapeutic application (17, 18). Selective thiolation at the 5 position of the cytosine bases in polyI:polyC resulted in partially thiolated double-stranded RNA, polyI:MPC (Fig. 1, Refs. 19, 20). PolyI:MPC was found to be an important inducer of IFN-α, IFN-β, and IFN-γ (20–29). It is of minimal toxicity *in vivo* in mice, rabbits, and guinea pigs and is subject to less degradation by plasma ribonucleases than its parent compound (22). PolyI:MPC has significant antitemplate activity against DNA and RNA polymerases, including reverse transcriptase (which is of great significance in the treatment of human immunodeficiency virus [HIV] infection) (23–27). This compound activates IFN-induced double-stranded RNA–dependent protein kinase (which inhibits protein synthesis) and activates 2′,5′-oligoadenylate synthetase, which in turn activates a latent endoribonuclease and activates adenylate cyclase, thus increasing the concentration of cAMP. PolyI:MPC activates macrophages and augments the natural killer cell activity (20, 27). In primary human lymphocyte cultures, polyI:MPC was found to be a potent inhibitor of HIV replication *in vitro*, including multidrug-resistant HIV lines in preliminary results (23). A recent study indicated that 7.5% thiolation is optimal for this compound from the point of view of IFN-α, IFN-β, and IFN-γ production and antiviral and antiproliferative activity (25). The effect of polyI:MPC is being explored alone and in combination with other anti-HIV agents, including those reported in the retroviral MAIDS model in mice (30). This model was found to be a useful, inexpensive, and quick early screening approach to acquired immunodeficiency syndrome (AIDS) and AIDS-related lymphoma (31). Investigations will proceed to nonhuman primate models and eventually to clinical studies. We anticipate that these agents and derivatives may contribute to the treatment of viral and neoplastic diseases, overcome disease-related IFN resistance (32), and stimulate other factors of the immune system. Figure 1 shows the chemical structure and partial 5-thiolation of cytosine residues of polynucleotides.

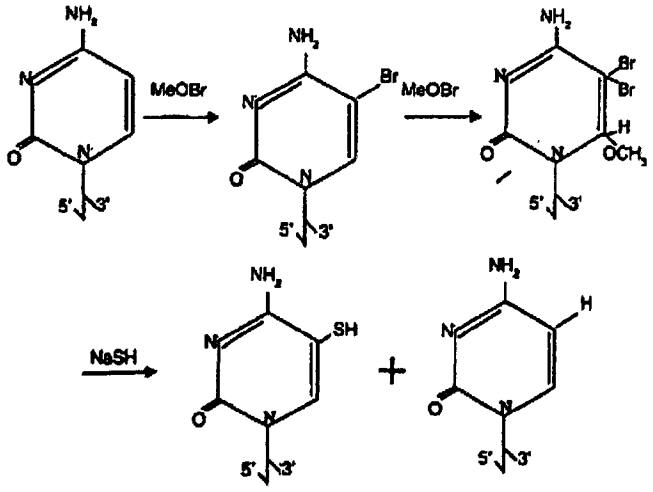

Figure 1. Chemical structure and partial 5-thiolation of cytosine residues of polynucleotides. MeOBr, methyl hypobromide; NaSH, sodium sulfhydride.

2′-Deoxyoligocytidilates

Investigators have produced and evaluated a series of polyI:MPC compounds that are closely and distantly related, as well as others in viral and neoplastic pathology. The most effective included oligonucleotides converted to 4 thiodeoxyuridylic acid $(s^4d\ UMP)_{35}$ (Fig. 2) containing oligomers of the 4 amino group of the cytosine bases, which were converted to the corresponding 4-thio group in 2′-deoxyoligocytidilates. 2′-Deoxyoligocytidilates inhibited a number of established tumor cell lines *in vitro* and was not cytotoxic to human granulocyte-macrophage progenitor cells. It was effective *in vitro* against multidrug-resistant HIV lines (23). 2′-Deoxyoligocytidilates also appeared to inhibit attachment of HIV to target cell receptors and viral fusion to cell membranes. In a series of publications, the chemistry and the antiviral effects (29, 33, 34) of this agent

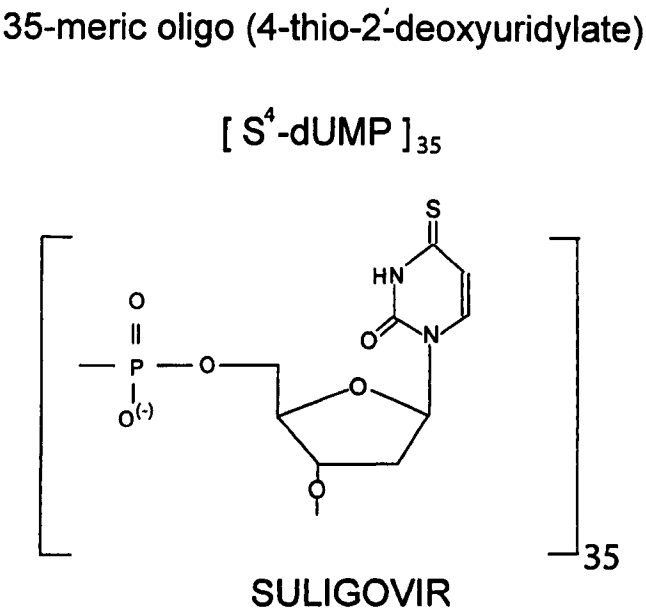

Figure 2. Chemical structure of $(s^4d\ UMP)_{35}$ (Suligovir).

were discussed. Further *in vivo* studies of the possible role of 2′-deoxyoligocytidilates in oncology are under way.

Telomerase Inhibitors

Other synthesized compounds are telomerase inhibitors (35, 36). Telomerase, a ribonucleoprotein complex, includes an RNA template and a catalytic subunit with reverse transcriptase activity and is responsible for the maintenance of normal telomere structure (37). In contrast to healthy adult somatic cells (38–40), most human neoplastic cells exhibit high telomerase activity, with some exceptions, including stem cells of renewable tissues and activated lymphocytes (41). Telomerases thus protect cells against apoptosis (40). Telomerase inhibitors have been described previously (41). Matthes and Lehmann (42) were the first to propose the evaluation of antitelomerase chimeric oligonucleotides, which contain a moiety targeting the primer binding sites and a sequence targeting the RNA as well. One compound that is fairly effective in inhibiting telomerases is a chimeric oligonucleotide composed of an antisense sequence directed against RNA template regions and a base modified moiety. The chemical synthesis has been described (29, 33, 35). Evaluation in several biological model studies is under way, including studies of various therapeutic combinations. In a recent study, retinoid and arsenic combination therapy was effective in patients with active promyelocytic leukemia, including those who were resistant to retinoic acid alone (43). This appears to be due in part to partial telomerase inhibition. Future studies will investigate specific telomerase inhibitors relative to their effect in combination therapy of promyelocytic leukemia and related diseases.

Summary

This brief review demonstrates the potent antiviral activity (such as agents to treat multidrug-resistant HIV) and antineoplastic effects of new therapies that are based on double-stranded RNA derivatives (including polyI:polyC), deoxyoligocytidilate compounds, and telomerase inhibitors (such as those containing methylated deoxyuridine moiety). Further *in vitro* studies are planned to investigate the effects of these agents in animal and human models.

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