The Potential of Nucleotide Analogs as Inhibitors of Retroviruses and Tumors

Roland K. Robins

Abstract: The biologically active form of most purine or pyrimidine analogs is the nucleoside 5'-mono-, di- or triphosphate. The nucleoside form is most often administered because of the ease with which it penetrates cells by facilitated transport. However, many nucleoside derivatives fail to exhibit significant antiviral or antitumor activity because they are not phosphorylated by cellular enzymes to the active nucleotide form. In this review, the potential use of suitable nucleotide analogs as selective inhibitors of ribonucleotide reductase and viral reverse transcriptase is considered. Masked nucleotides such as phosphorimidates or methyl phosphates could be employed to allow transport across cellular membranes. Furthermore, phosphonocarbamyl, phosphonomiformate or sulfamidophosphoramide may mimic nucleotide di- and triphosphates. Tumor cells and virally infected cells are often more permeable to nucleotides and their analogs than normal cells, which could provide a therapeutic advantage. There could be considerable therapeutic potential for nucleotide analogs that can penetrate the tumor cell membranes and that are resistant to enzymatic hydrolysis and are non-incorporable into DNA or RNA.

The Role of Retroviruses in Cellular Transformation

RNA viruses are known to cause malignant neoplasms in a wide range of species from amphibia to primates (1,2). Recently, a unifying theory of malignant transformation involving oncogenic viruses and chemical carcinogens has been proposed (3). Striking evidence for the existence of a unique RNA virus associated with malignant proliferation of human T lymphocytes has emerged from two independent sources (4-6). This virus has been named human T-cell Leukemia Virus (HTLV). Gallo and co-workers (7) have shown that HTLV is acquired by infection; moreover, HTLV proviral DNA is integrated into DNA of peripheral leukemic cells of HTL patients (8, 9). Hinuma and co-workers (9) were able to transmit HTLV into fresh leukocytes from normal humans and to consistently transform these cells by subsequent X-irradiation. Identification of HTLV-like particles in patients with acquired immune deficiency syndrome (AIDS) also implicates this virus in the etiology of AIDS, which is characterized by the development of Kaposi’s sarcoma and various infections (10).

Retrovirus-like particles containing RNA-directed DNA polymerase (reverse transcriptase) have also been isolated from human prostatic cells (11). Gallo and Gelman (12) have reviewed the possibility of a Type-C virus in Hodgkins disease. Moreover, Chandra (13) has found RNA-dependent polymerase activity in human granulocytic sarcoma, human primary melanoma and human osteosarcoma tissue. These studies suggest RNA oncogenic viruses as potential causative agents in certain types of human cancers.

Temin (14) has described some of the possible roles of RNA tumor viruses in the etiology of human cancer: 1. Viruses could act as direct transforming agents, so that genetic information in the virus is responsible for initiating and maintaining tumor cell transformation (such as Rous sarcoma virus in chickens and Friend murine leukemia virus in mice); 2. Viruses could effect genetic changes in an infected host that result in tumorigenesis such as the induction of leukemia in AKR mice, where extended replication of the genetically transmitted Gross virus leads to a mutation or recombination in the viral genome (15). 3. Endogenous virus-related products might cause the induction of tumors by the activation of preexisting “cancer genes”, or by recombination of viral genes and cellular genes to form such “transforming genes”. RNAs related to the genes of certain transforming retroviruses are frequently detectable in human cancer cells (15).

The feature that unites RNA tumor viruses (retroviruses) and distinguishes them from all other animal viruses is the transcription of their single-stranded RNA into double-stranded DNA. Details of this process have been reviewed by Varum (17). RNA tumor viruses (retroviruses) thus are characterized by the presence of reverse transcriptase, an RNA-dependent DNA polymerase (RNA-dependent DNA nucleotidytransferase) that is found in all RNA oncogenic viruses as part of the virion. Mutant RNA viruses lacking reverse transcriptase lost their ability to initiate infection and cell transformation (18-20). The presence of reverse transcriptase in all oncornaviruses strongly suggests its role in the neoplastic transformation by such viruses. The proviral DNA copy of the RNA viral genome is incorporated into the host cell DNA where it carries information for viral replication and for transformation of the normal cell to a neoplastic cell (21). After the incorporation of a DNA-provirus, a virus can replicate by using the DNA and RNA polymerases of the host cell.

Summers and Mason (22) have recently shown that hepatitis B virus resembles retroviruses in that it has a reverse transcription step in the viral life cycle. Since hepatitis B has been linked with an increased incidence of cancer of the liver both in man and animals (23), this suggests that hepatitis B may be carcinogenic in the same way as retroviruses. Chronic hepatitis B infection affects about 200 million people (24) and is associated with the most common fatal cancer of man, primary hepatic carcinoma (25). Some of the unique aspects of the interaction of retroviruses with vertebrate cells have been reviewed by Aaronson (26).
Viral Reverse Transcriptase as a Target for Chemotherapy

Reverse transcriptase from a purified avian myeloblastosis virus (AMV) is a zinc metalloenzyme (27). The purified enzyme also catalyzes a pyrophosphate exchange reaction between deoxyribonucleoside triphosphates. Thus, reverse transcriptase is different from cellular DNA polymerases in that it fails to degrade polydeoxyribonucleotides or hydrolyze deoxynucleoside triphosphates (28). The hydrolytic activity of the enzyme is directed only against the ribo strand of the ribodeoxyribonucleotide complex (29). These properties appear to be general for all classes of the reverse transcriptases present in various RNA tumor viruses (30). The cellular DNA polymerases α, β and γ are distinguished from reverse transcriptase by their inability to copy natural RNA (31).

A transformed murine cell line actively producing a murine sarcoma virus (MSV) and a murine leukemia virus (MLV) was found to contain reverse transcriptase activity indistinguishable from the same enzyme of extracellular virions of MLV (32). Uninfected control cells did not exhibit any reverse transcriptase activity. Thus the potent selective binding of a nucleotide analog substrate to the newly synthesized reverse transcriptase should prevent formation of viable new oncogenic viruses even in the transformed cell. There is a direct correlation between inhibition of reverse transcriptase and the loss of the ability of the retrovirus to cause transformation of cells in culture (33) and to induce leukemia in experimental animals (34).

F. M. Schabel, Jr. (35) was one of the first to suggest strongly antiviral agents as an adjunct to cancer chemotherapy. Following effective chemotherapy of spontaneous leukemia-lymphoma in AKR mice with a very high probability of drug cure of the cellular phase of the disease, all animals ultimately die of the pathologically classical disease, presumably virally induced, often as much as several months after the apparent drug cure. This is an interval well beyond the anticipated maximum time to death from one viable tumor cell that may have survived drug treatment. A chemical compound which would selectively interfere with RNA tumor virus replication of virus-induced cellular transformation should be highly effective in preventing "reinduction" of viral neoplasia in animals and in man (36).

Nucleotide Inhibition of Reverse Transcriptase

The importance of finding specific inhibitors of reverse transcriptase was recognized soon after the discovery (37, 38) of the enzyme. The anthracycline antibiotics, doxorubicin (adriamycin) and daunorubicin are potent inhibitors of the RNA-directed DNA polymerase of Rauscher leukemia virus and avian myeloblastosis virus (17). However, these compounds are also strong inhibitors of cellular DNA polymerases (21). Since the expression of integrated viral genes is responsible for initiation and maintenance of the transformed cell (39, 40), a selective inhibition of retrovirus reverse transcriptase could prevent further virus mediated spread of a tumor (41). A summary of the Russian attempt to find such inhibitors has recently been published (42). Kit (43) and Verma (44) have published similar reviews. Varmus (45) has recently stated that despite enormous synthetic efforts of various investigators, no highly potent specific inhibitor of reverse transcriptase is as yet available.

Holland and co-workers (47) have shown that AKR mice with spontaneously induced lymphoma, despite an apparent cell cure, relapse nonetheless due to probable viral reinduction of the lymphatic leukemia. These investigators showed that ribavirin exhibited 80% inhibition against the Gross (RNA) murine leukemia virus in vitro. Treatment of AKR mice with vincristine plus prednisone followed by ribavirin resulted in a significant delay of the reappearance of viable lymphoma cells and a moderate increase in the life-span of the animals compared with treatment with vincristine plus prednisone alone (46). These authors therefore suggested the application of this combined therapy to certain viral suspected human neoplastic diseases (46). Ribavirin has been reported independently (47) to increase significantly survivors of spontaneous leukemia in AKR/J female mice. Shannon (48) points out that it is quite possible to have selective antiviral agents that work against RNA tumor viruses that also may possess independent significant antitumor activity. Ribavirin shows activity against L-1210 leukemia (49, 50) and adenocarcinoma 755 in mice (50). Ribavirin also has been shown to suppress the development of adenovirus (49) induced tumors in CBA mice (51) and to inhibit Rauscher murine leukemia virus spleenomegalgy in mice (48). Furthermore, ribavirin inhibits cellular transformation in rat kidney cells by a temperature sensitive mutant of Rous sarcoma virus (50) and the replication of the Rous sarcoma virus in chicken embryo fibroblasts (52). Ribavirin, which was first prepared in our laboratory (53) in 1972 as an antiviral agent (54), is readily converted by adenosine kinase (55, 56) to ribavirin-5'-phosphate and then to the corresponding 5'-di- and triphosphates (57) (Fig. 1).

![Fig. 1](image-url)

One of the most promising approaches to the design of a selective inhibitor of reverse transcriptase is the synthesis of specific substrate analogs. Although RNA is utilized as a template, the substrates should be analogous to the natural substrate; a 5'-triphosphate of a 2'-deoxyribonucleoside. AraCPT is highly inhibitory against the RNA-directed DNA polymerase of Rauscher leukemia virus in vitro (58), and it inhibits the DNA polymerase from oncogenic RNA viruses to a greater extent than the DNA polymerase from mammalian cells (59, 60). Similarly, 2',3'-dideoxy-1-β-ribofuranosylthymine (2',3'-dideoxythymidine) inhibits reverse transcriptase from Rous sarcoma virus and Moloney mouse leukemia virus (61, 62). Since phosphorylation of 2',3'-dideoxythymidine has been demonstrated in mammalian cells (63), it is assumed that its 5'-triphosphate (dDTP) is the active form of the drug. dDTP was found to inhibit the viral reverse transcriptase of AMV 100-fold more than the cellular α polymerase (64). Furthermore, DNA polymerase γ from adenovirus is