VIRUS ASSOCIATED DNA POLYMERIZING ACTIVITIES: THEIR ROLE IN
DESIGNING ANTIVIRAL AND ANTITUMOR AGENTS

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INTRODUCTION

Inhibitors of nucleic acid biosynthesis have an honorable place in the history of chemotherapy. Nucleic acids, or nucleic acid polymerizing enzymes, have served as extremely useful targets in the development of chemotherapeutic agents to combat infectious and malignant diseases. In some cases, such as antiviral agents, this approach has provided virtually the only means to design effective chemotherapeutic compounds.

The strategical role of nucleic acids, or nucleic acid polymerases as targets, involves two distinct features. The investigational drug must have the capacity to recognize distinct bases or base-pair sequences, either by direct interaction between functional groupings on the base-pairs and the drug molecule, or indirectly via recognition of the conformational peculiarity of the nucleic acid molecule. The other alternative is the specific affinity of the drug for one or the other nucleic acid polymerizing enzymes. From the strategical standpoint this is a more specific approach, since a number of enzymes are involved in the polymerization of nucleic acids.

Although the first eukaryotic DNA polymerase, now known as DNA polymerase- α , was discovered in 1958 (1), it was not until 1970 that the existence of multiple forms of DNA polymerases was established in these cells. The search for these multiple species in eukaryotic cells

was, at that time, implicated by the data in prokaryotic cells which documented several types of DNA polymerases present in these cells (2,3,4,5). We now know that several DNA-polymerizing activities are normally present in an eukaryotic cell, and that additional DNA polymerase activities are brought into the cell or induced by RNA tumor viruses (Retroviruses) and by some DNA viruses. These additional or foreign DNA polymerases are coded by the virus genome, and are biochemically as well as serologically different to the constitutive DNA polymerases of eukaryotic cells. Thus, virus-associated DNA polymerases offer an attractive target for designing antiviral and antineoplastic agents.

DNA-POLYMERIZING ACTIVITIES IN NORMAL MAMMALIAN CELLS

The normal mammalian cells contain at least three DNA polymerases, designated as DNA polymerases α , β and γ (6). These polymerases can be distinguished by the differences in their biochemical properties and serological behavior. A number of excellent reviews have appeared in the past covering these details (7,8,9,10,11,12). Based on these reviews, a spectrum of biochemical features distinct for the constitutive eukaryotic DNA polymerases is presented in Table 1.

TABLE 1
Some Important Features of Eukaryotic DNA Polymerases

	POLYMERASE- α	$-\beta$	$-\gamma$
LOCATION	Cytoplasm ^{1†)*}	nucleus	nucleus mitochondria
Mass,kdal	120-220	30-50	100-160
S value	6-8	3-4	7-9
Isoelectric Point	5.6-6	9-9.4	5.4-6.1
Template-Primer preferred	Act.DNA	(dA) _n .(dT) ₁₂	(rA) _n .(dT) ₁₂
pH-optimum	7.2	8.5-9	8.0

* evidence from immunological studies reported by Bollum (11). All other data were accumulated from references 7-12.

While the *in vivo* role of virally induced DNA polymerases is more or less predictable, the physiological functions of normal cellular DNA polymerases are not yet well understood. Studies with mutants have been specially useful in elucidating the functional activity of DNA polymerases (I, II and III) in prokaryotic cells. For lack of polymerase mutants in eukaryotic systems, great reliance is placed on specific inhibitors of various DNA polymerases to characterize their activity and functional role under *in vivo* conditions. For example, the role of DNA polymerase α as the principal replicating enzyme was established by the specific inhibitory action of aphidicolin, a tetracyclic diterpenoid obtained from Cephalosporium - aphidocola. Isolation of an aphidicolin-resistant DNA polymerase α mutant of Drosophila (13) has been very useful in studying *in vivo* role of this polymerase activity. Another experimental approach to study the *in vivo* functions of various cellular DNA polymerases has been developed by Pardee's group. Miller, Castellot and Pardee (14,15) have examined roles of DNA polymerases using lysolecithin-permeabilized baby hamster kidney (BHK) cells. According to their results, this technique yields a cell preparation which can carry out normal, replicative DNA synthesis at *in vivo* rates when supplied with deoxyribonucleoside triphosphates (14,15). They have also investigated the effect of known inhibitors of DNA polymerases on the replication and repair DNA synthesis in permeabilized cells (16). The different sensitivities of replication and repair synthesis to various inhibitors (N-ethylmaleimide, high KCl and aphidicolin etc.) indicated that DNA polymerase α plays a rate-limiting role in replication, whereas DNA polymerase β plays a major role in repair.

Whereas the DNA polymerases α , β and γ are present in all animal cells and tissues, a novel DNA-polymerizing activity located only in the thymus tissue, was discovered by Bollum (17). This enzyme, terminal deoxynucleotidyl-transferase (TdT), catalyzes the polymerization of deoxynucleotides onto the 3'-OH ends of oligo or polydeoxynucleotide initiators in the absence of a template. Thus, TdT, in the strictest sense, is not a DNA polymerase as it does not direct the genetic information of a template DNA. Nevertheless, in a broader sense it does catalyze the incorporation of deoxyribonucleoside monophosphates into DNA and thus we consider it here as one of the DNA synthetic enzymes. Like retroviral reverse transcriptase, terminal transferase merits considerable attention as a useful marker for various types of human leukemia (18,19,20,21,22,23,24). For this reason, terminal transferase, though an enzyme of cellular origin, offers an attractive

target site for developing antineoplastic agents.

VIRUS ASSOCIATED DNA POLYMERIZING ACTIVITIES

The DNA polymerase patterns in virus-infected cells are qualitatively and quantitatively different. To understand this, we will describe the virus-associated DNA polymerase activities in two sections:

1. DNA Virus-associated DNA Polymerases: The animal DNA viruses can be divided into three groups with respect to the nature of DNA polymerase involved in their replication: a) The small transforming viruses (polyoma, SV40) have a limited coding capacity in their genomes and therefore, these viruses cannot specify their own enzymes to support all the replicative and other events involved in their life cycle. However, cells infected by such viruses produce virus coded antigens, such as the T-antigen of SV40, which serve as regulatory signals for the replication of viral DNA (25). It has been known for more than fifteen years that infection of resting cells by polyoma virus leads to an induction of cellular DNA synthesis along with the viral DNA replication, and it has been recognized that these induced functions are accompanied by increases in the activities of cellular enzymes that are involved in DNA synthesis (26,27,28,29). Using a replication complex isolated from adenovirus-2-infected KB cell nuclei, Ito et al. (30) studied the question of the DNA polymerase involved in the replication of viral DNA. The only polymerase that could be extracted and characterized from the nuclear membrane complex from these cells was an activity resembling host DNA polymerase γ . However, recently an active complex of 80-kdal protein-adenoviral DNA with a 140-kdal DNA polymerase could be isolated from cytosol extracts of infected cells. The associated DNA polymerase in the complex resembled DNA polymerase α in its template-primer preference and sensitivity to N-ethylmaleimide, araCTP, and salt, but differed in its insensitivity to aphidicolin. The unusual nature of this strange DNA polymerase needs to be clarified. b) To the second group belong vaccinia and various types of herpes viruses which induce specific DNA polymerases, coded by the virus genome. DNA polymerase activities associated to herpes viruses has been detected in cells infected by HSV-1 (31,32,33), HSV-2 (34,35), equine herpesvirus (36,37), human cytomegalovirus (38,39,40), Marek's disease herpesvirus (41) and Epstein-Barr virus (42). One distinguishable property of the DNA polymerases induced by all the herpes viruses is the characteristic stimulation of viral enzyme by high salt concentration. Therefore, the viral induced

DNA polymerases can be conveniently assayed in crude extracts in the presence of host enzymes by supplementing the assay systems with 100-150 mM ammonium sulfate. Under these conditions host DNA polymerases are almost totally inhibited. The molecular weights of purified herpes-induced DNA polymerases (31-44) are in the range 150-kdal-200-kdal. These enzymes have a pH optimum of 8-8.5 and require Mg-ions and sulfhydryl compounds for activity (32,44). Among the synthetic template-primers, (dG)₁₂.(dC)_n was by far the best primer-template although at very high concentrations this synthetic oligomer-polymer was slightly inhibitory. Weissbach et al. believe (32) that the preferential ability of HSV-induced enzyme to copy (dG)₁₂.(dC)_n may be due to high G+C content (67%) of the DNA of this virus. The herpesvirus-induced DNA polymerases are strongly inhibited by phosphonoacetate & phosphonoformate (45), and acycloguanosine (46,47,48). Aphidicolin, the inhibitor of DNA polymerase α , also inhibits herpes-induced DNA polymerase activity (49). The target for phosphonoacetic acid inhibition of the herpes-induced polymerase was thought to be the inorganic pyrophosphate binding site of the enzyme (51). However, the concurrence of resistance to phosphonoacetate, acycloguanosine, and araA bring this simple mechanism into question. The basis for acycloguanosine action is: i) its conversion to the monophosphate by the virus-coded thymidine kinase only, and ii) its inhibition as a nucleoside triphosphate of the viral DNA polymerase which is ten to thirty times more sensitive than the cellular polymerases (50). c) To the third group belong some small viruses (hepatitis B Dane particle) which carry within the virion a DNA polymerase whose origin is not yet clear. Resistance of Dane particles to solubilization has made the purification and characterization of this DNA polymerase difficult. Analysis of the banding patterns in sucrose density gradients of NP-40-treated plasma concentrates rich in Dane particles showed DNA polymerase activities in the density range covered by the hepatitis B cores (52,53). The only primer-template that has been known to be utilized by this enzyme is the endogenous Dane particle DNA.

In summary, we can conclude three types of DNA-polymerase activities associated to the replication of DNA viruses. The smallest class of DNA viruses do not specify new DNA polymerases in the infected cell. Instead they stimulate the synthesis of cellular DNA polymerases and make use of these to replicate their DNA. On the other hand, the large DNA viruses induce new DNA polymerases coded by their genomes, and therefore these viruses do not depend on the replicative machinery of the host. This

class of viruses offers a selective approach to design antiviral compounds which act via inhibition of the viral induced DNA polymerases. The strategy to combat such viral infections where the replication of viral DNA is solely dependent on cellular enzymes is difficult, and the inhibitors of DNA polymerases may have only a limited application.

2. Retrovirus Associated DNA Polymerases

Perhaps not as fundamental as the discovery of the DNA double helix, but most important to our understanding of the replicative cycle of retroviruses, has been the discovery of an RNA-dependent DNA polymerase (54,55), the reverse transcriptase. Reverse transcriptase is located in the core fraction of the virus as a complex with the viral RNA genome. These core structures can be obtained by treatment of the virus particles with nonionic detergents and fractionated by equilibrium density centrifugation on sucrose gradients, taking advantage of their high density characteristics (approx. 1.25 g/cc). In the ribonucleoprotein complex the reverse transcriptase (RT) catalyzes the transcription of the genomic RNA into a complementary DNA. The viral genome itself is diploid, composed of two single stranded, identical subunits of 35S. Each of these subunits of the genomic RNA has features of eukaryotic messenger RNA, being capped at the 5' end and polyadenylated at the 3' end. Most crucial to the viral genome are five domains (Fig. 1): U5, 76-120 nucleotides at the 5' terminus of the genome; U3, 150-200 nucleotides at the 3' end of the genome; TR (terminal repeats), a direct redundancy of 16-79 nucleotides at both ends of the viral genome; PBS, a sequence of about 18-21 nucleotides that base pairs with the 3' stem region of the tRNA primer; and a short sequence of purines at the 5' boundary of U3 region. These domains account for the genesis of long terminal repeats (LTR), the replication of both ends of a linear template without loss of the genetic content, the initiation and propagation of both strands of viral DNA, and the role of terminal repeats in viral DNA synthesis.

RNA-directed DNA synthesis is unique to the life cycle of retroviruses. No similar reaction is known to occur in normal uninfected cells. Reverse transcriptase is a multifunctional enzyme, with at least four domains that mediate RNA-dependent DNA synthesis, RNase-H activity, DNA-dependent DNA polymerase activity, and a site for nucleic acid binding. All these domain mediated functional activities offer attractive targets for in-

Figure 1: Schematic presentation of molecular events in the provirus integration into host DNA.

hibiting the synthesis of DNA in retroviruses.

The final product of the reactions mediated by

reverse transcriptase is a linear duplex DNA (Fig.1). This linear DNA is longer than the 35S subunit of genomic RNA and contains long terminal repeats (whose composition can be written as 5'-U3 -TR -U5- 3') present at both ends (56,57). The linear duplex viral DNA is synthesized in the cytoplasm, which then migrates to the nucleus where it is converted a closed circular species (Fig. 2). Apparently, two mechanisms are involved in the formation of cir-

Figure 2: Replicative cycle of retroviruses

cular duplexes of viral DNA: (i) circularization may occur by homologous recombination between the redundant ends, leaving a single complete LTR in the circle (58,59), or (ii) by direct ligation of ends of linear duplex DNA (56, 57,60).

Inhibitor studies by Guntaka et al. (61) indicate that the circularization of duplex DNA is a prerequisite for the integration of proviral DNA. The current views on the mechanism and nature of integrated DNA can be summarized as follows (62,63,64,65): (i) The integrated proviral DNA is almost colinear with the linear form of viral DNA, synthesized in the cytoplasm. This means that the integrated proviral DNA has two LTR on terminal ends. (ii) The integration site on viral DNA is specified by direct repeats of short cellular sequences (4-6 nucleotide pairs) (iii) Integration into the host genome can occur in many different regions of cellular DNA, and apparently, there is no homology between the interacting sites on viral and cellular DNA.

The integrated proviral DNA is endowed with specific sequences or domains which mediate signals for the initiation of RNA synthesis, splicing of RNA during the genesis of viral mRNAs, polyadenylation of the mature mRNA, and in case of MMTV (mouse mammary tumor virus), a site exists which signals the induction of viral RNA by steroid hormones (66).

CHEMICAL INHIBITORS OF RETROVIRAL DNA POLYMERASE

Soon after the discovery of an RNA-dependent DNA polymerase (reverse transcriptase) activity in retroviruses (54,55), Gallo and his associates (67) made an exciting observation, that human leukemic cells possess an enzymic activity that biochemically resembles the DNA-polymerase activity associated with retroviruses. This observation infected the idea that retroviruses may be involved in human cancer. Immunological probing of reverse transcriptase has further contributed to our knowledge on the expression of retroviral information in human tumors (68-81). However, the recent discovery of a retrovirus (HTLV= Human T-cell Leukemia or Lymphoma Virus) in T cells (fresh and in culture) from a lymphnode biopsy of a patient with cutaneous T-cell lymphoma is a direct evidence that retroviruses are associated to human cancer, at least to this particular type of human leukemias (82, 83). The search for a specific inhibitor of retroviral reverse transcriptase was, as a matter of fact, induced by these studies which indicated an association of retro-

viruses to human malignancy. In view of the importance of reverse transcription in cancer, and as a biological process, attempts have been made in our laboratory to develop inhibitors of DNA synthesis in RNA tumor viruses.

It may be possible to inhibit proviral DNA formation at several stages (cf. Figures 1 and 2): (a) blocking the penetration of the membrane by the virus; (b) blocking the release of viral components inside the host cell (uncoating); inhibiting the formation of linear duplex DNA by blocking one of the domain-mediated activities of reverse transcriptase; and finally, inhibiting the integration of viral DNA by blocking the circularization of linear duplex DNA. Of all these possibilities, the inhibition of viral DNA polymerase is most attractive since this enzyme is biochemically and immunologically quite different to all the known cellular DNA polymerases, as mentioned in the earlier section.

The *in vitro* assay systems employed for reverse transcriptase determination reveal a variety of substrates, template-primers and interacting compounds which can modulate the catalytic rate of DNA synthesis (84). Some examples of this type of modulation are: the detergent effect on the activity of rifamycins (85), influence of divalent cations (Mg^{++} or Mn^{++}) on the rate of DNA synthesis with different substrates and the role of chelating agents or cation binders in buffers (84,86), and the interaction of thiols with some potential inhibitors or their direct influence on the measured DNA synthesis (87). Thus slight variations in assay conditions may lead to wrong interpretations with respect to the specificity of a particular inhibitor in the viral DNA-polymerase system.

The second problem is the interpretation of the enzymatic data with respect to the antiviral activity of these inhibitors (88). This is particularly the case with such compounds which exert their inhibitory action by complexing with one or more synthetic templates. Such an effect can not be very specific for the viral enzyme. Drugs exhibiting cytotoxic activities to the extent of causing a delayed death, or those which intervene in the replicative cycle of the host cell may give erroneous information about the specific antiviral activity of the compound. Thus antiviral studies *in vitro* should be carried out under conditions and at inhibitor concentration which have little or no effect on the replicative cycle of the cell. Molecular manipulations of parent compounds have proved to be very useful in several instances to develop inhibitors of viral DNA synthesis

which exhibit a low cytotoxicity, and at the same time a high antiviral potential. This is evidenced by our earlier studies on distamycin derivatives (89,90) and tilorone congeners (91,92,93).

Further efforts to develop compounds which inhibit viral DNA polymerases by interacting with templates may lead to the discovery of useful compounds exhibiting a higher therapeutic index, i.e. low cytotoxicity and high antiviral activity. However, this approach will not lead to the development of a specific inhibitor of the viral enzyme, unless one finds a compound which binds specifically to 70S RNA. Though 70S RNA is a novel feature of oncornaviruses, from the chemical and physical standpoint it does not appear to offer any uniqueness to distinguish it from cellular nucleic acids. Thus, the strategical approach of developing an inhibitor of this type is, at the present state of our knowledge, unthinkable.

The second approach, which has proved to be more useful and relatively specific in developing such inhibitors, is to design compounds that bind to the viral enzyme. In searching for this type of inhibitor, the enzymes chosen for comparison are important. Many studies have been done with either avian or mammalian reverse transcriptase, since these two types of enzymes have some different characteristics. A number of studies claim specificity (or selectivity) of a compound by comparing an inhibitory response to a bacterial DNA polymerase with the viral reverse transcriptase. Such a comparison has no relevance to the selective nature of the compound. The most important approach to demonstrate selectivity of a compound is to compare inhibitory effects against various cellular DNA polymerases, such as α , β and γ DNA polymerases.

Our efforts to develop compounds that inhibit viral DNA polymerases by interacting directly to the enzyme led to the discovery of a polycytidylic acid analog, containing 5-mercapto-substituted cytosine bases, a partially thiolated polycytidylic acid (94,95). This compound, abbreviated as MPC (marcapto-polycytidylic acid) was found to inhibit the retroviral DNA polymerase in a very specific manner (94,95,96,97,98,99,100,101). In addition to studies with MPC, we have recently evaluated the effects of several polyadenylic acid analogs on various DNA polymerases. In the following pages we would like to describe the mode of action of polynucleotide analogs on viral reverse transcriptase, and discuss the biological and clinical implications of these studies. In order to document the specificity of action of these analogs, we have compared their

activities against purified cellular DNA polymerases α , β and γ , and terminal deoxynucleotidyl transferase (TdT).

1. Studies with 5-mercapto polycytidylic acid: The inhibition of DNA polymerases from RNA tumor viruses by 5-mercapto polycytidylic acid (MPC) was described earlier (94-101). Partially thiolated polycytidylic acid preparations (MPC I-III), containing 1.7%, 3.5% and 8.6% 5-mercaptocytidylate units inhibited the DNA polymerase activity of Friend leukemia virus (FLV) in the endogenous reaction as well as in the presence of exogenous template-primers, such as (rA)n.(dT)₁₂ and (rC)n.(dG)₁₂. The inhibitory activities were directly related to the percent of thiolation. A maximum inhibition was observed with preparations containing 15-17% of the thiolated cytosine bases. In these experiments, non-thiolated samples of polycytidylic acid showed no inhibition of the reverse transcriptase reaction.

Polyuridylic acid thiolated by the same procedure (94) was also found to inhibit this reaction strongly (102); however, the non-thiolated sample of polyuridylic acid also inhibited the reaction significantly. Besides, the inhibitory effect of thiolated samples of polyuridylic acid was not strictly related to their degree of thiolation, as was observed for the partially thiolated polycytidylic acid. The inhibitory effect of non-thiolated polyuridylic acid is, presumably, due to hydrogen bonding between poly-U and the added template, (rA)n. The inhibition of the endogenous reaction by non-thiolated poly-U is explainable by the fact, that genomic RNA contains large stretches of polyadenylic acid at 3'-terminal. For this reason, it was difficult to designate the role of thiolation on the inhibitory effect of MPU samples.

The mode of action of MPC on the viral DNA synthesis was investigated by product analysis of the DNA polymerase reaction (endogenous) in the absence or in the presence of MPC, as described by Kotler and Becker (103). The reaction mixtures were dissolved with Na-dodecyl sulfate (1%, wt/wt, final concentration), loaded on a hydroxylapatite column (1g, Bio-Rad Lab., Munich), eluted with a Na-Phosphate gradient (0.05-0.4 M), collected into about 40 tubes (total volume approx. 100 ml), and the TCA-insoluble activity collected on GF/C filters (Whatman) and counted in a liquid scintillation counter.

Analysis of the endogenous products of the detergent disrupted virions exhibits three DNA species: single stranded DNA (ss DNA), RNA-DNA hybrids (hy-DNA) and the

Figure 3: Analysis of the DNA species synthesized by FLV-DNA polymerase by elution from hydroxylapatite column. Experimental details are described in the text. The first species to be eluted from the column contained ss-DNA, the second contained hy-DNA and finally, the ds-DNA, eluted in the last peak. The concentration of MPC in the reaction mixture = 20 μ g (95).

double stranded DNA (ds-DNA). As follows from Figure 3, in the presence of MPC (open circles) there is an overall inhibition of ^3H -dTMP incorporation, indicating that the formation of all the 3 species is blocked. This is to be expected since the inhibitor binds to the enzyme. This has been confirmed by the ultracentrifugation studies in which the binding of ^{35}S -labeled MPC to a purified FLV-DNA polymerase was investigated (95).

In view of the fact that all of the oncornaviral DNA polymerases examined so far, do require a primer-template-like double stranded structure for the initiation of DNA synthesis, it is not surprising that single stranded synthetic polynucleotides (unprimed templates) can act as inhibitors of the polymerization reaction. This, presumably, is due to hydrogen bonding of the base sequences between the added polymer and the functional template. Thus, the

specificity of inhibition by such polymers is not limited to the viral enzyme system only. On the other hand, minor modification in the chemical structure of synthetic polynucleotides might be useful to develop inhibitors that interact directly with the enzyme but fail to be transcribed, i.e. they function as "dead template" for the enzyme. The data from our laboratory have shown that the partially thiolated polycytidylic acid is functioning as a "dead template" in the DNA polymerase system of FLV (95). The results of these studies (95) can be summarized as follows: (a) The incorporation of ^3H -dGMP into DNA by the viral enzyme is stimulated to about 9-fold (compared to endogenous value) in the presence of (rC)n.(dG)12. However, under similar conditions a hybrid of (MPC).(dG)12 failed to stimulate the incorporation of ^3H -dGMP into DNA; (b) In the presence of MPC.(dG)12, the increasing concentrations of (rC)n.(dG)12 in the reaction mixture have no effect on the activity of the enzyme; however, at higher enzyme concentrations the stimulatory effect of (rC)n.(dG)12 gradually reappears. These data indicate that the viral enzyme has a higher affinity towards MPC than to its optimal template (rC)n. The presence of zinc in reverse transcriptase molecule makes it attractive to suggest that the mercapto group may undergo an interaction with zinc to form a stable complex with the enzyme.

In order to determine the selectivity of MPC action cellular DNA polymerases (α , β and γ) were purified from normal and FLV-infected mouse spleen. The inhibitory effects of MPC on the cellular polymerases, and on the activity of reverse transcriptase from FLV-infected mouse spleen were compared (101). None of the cellular DNA polymerases, from normal as well as from FLV-infected mouse spleen, was inhibited by MPC. On the other hand, (rA)n.(dT)12 and (rC).(dG)12-catalyzed activities of FLV-reverse transcriptase were highly sensitive to MPC action. Kinetic studies on the FLV-DNA polymerase reaction revealed that MPC inhibition is of a non-competitive nature (Fig. 4).

The studies on the selectivity of MPC action were substantiated using a purified preparation of terminal deoxynucleotidyl transferase (TdT) from calf thymus. The comparative effects of MPC on the (dA)n-catalyzed reaction of TdT, and on the (rC)n.(dG)12-dependent activity of reverse transcriptase is shown in Figure 5. The non-thiolated samples of polycytidylic acid (PC) were, in both cases without any inhibitory effect; on the contrary, at lower concentrations a significant stimulation of the reactions was observed. The thiolated sample (SH= 18.2%) of poly-

cytidylic acid(MPC) inhibited both the enzymic reactions; however the reverse transcriptase reaction was almost 15 times more sensitive to the action of MPC than the TdT-reaction.

Figure 4: Lineweaver.Burk plot of the kinetics of FLV-DNA polymerase reaction in the absence(open circles) or presence of MPC (triangles= 1 μg /reaction mixture; squares= 2 μg).

Of all the enzymatic studies carried out in our laboratory so far, MPC has proved to be relatively specific towards reverse transcriptase.

The protective effect of MPC on leukemogenesis in mice by FLV has been reported earlier (101). In these experiments, the effect of MPC was investigated by pre-incubating the virus containing suspensions with MPC. These studies have been extended using MPC in-vitro and in-vivo.

The animals (mice:Groppel strain) were divided into four groups of five each (donors): 1) Group 1 was injected with a viral suspension (citrate plasma from FLV-infected animals, dose= LD₉₀) preincubated with Tris/HCl buffer, pH 7.6 for 30 min. at 37 °C; 2) Group 2 was injected with

Figure 5: Effect of 5-mercapto polycytidylic acid (SH= 18.2%) on the activities of retroviral reverse transcriptase and on calf thymus terminal deoxynucleotidyl transferase.

TABLE 2

Assay for Leukemogenic Potential of Spleen Extracts from FLV-infected Mice after their in-Vitro/Vivo Treatment with 5-Mercapto Polycytidylic Acid.

TREATMENT OF DONOR MICE	LEUKEMOGENESIS IN RECEPTOR MICE AFTER INFECTION WITH SPLEEN EXTRACT ⁽¹⁾		
	NO. OF POSITIVE TOTAL NO. OF MICE	MEAN SURVIVAL TIME (DAYS)	MEAN SPLEEN WEIGHT(g)
Virus Suspension ⁽²⁾ (0.2 ml) + Tris buffer (37°C, 30 min)	5/5	47	2.41
Virus Suspension + 200 µg of MPC (37°C, 30 min.) + 50 µg MPC, i.p. (day 5 & 9)	2/5	123 ⁽³⁾	1.05 (1.78, 2.10, 0.52, 0.41, 0.44)
Virus Suspension + Tris buffer (37°C, 2hr)	5/5	52.2	1.80
Virus Suspension + 200 µg of MPC (37°C, 2 hr) + 50 µg MPC, i.p. (day 5 & 9)	1/5	110 4 (123) ⁽³⁾ 1 (97)	0.38 (0.74, 0.29, 0.34, 0.22, 0.31)

- (1) Cell-free spleen extracts were prepared from spleens of donor mice on 10th day after virus infection, or other treatments as shown. (2) Citrate plasma (LD₉₀). (3) Experiment terminated on 123rd day.

the viral suspension, as in 1, but preincubated with MPC (200 ug per 0.2 ml suspension) at 37 °C for 30 min. These animals received in addition, on day 5 and day 9 (post infection) 50. µg of MPC, injected intraperitoneally; 3) Group 3 was treated similar to group 1, except that the viral suspensions were preincubated for 2hr; and 4) Group 4 was treated in a similar manner as group 2, except that the viral suspensions were preincubated for 2hr at 37 °C. On the 10th day, animals were sacrificed and spleen extracts were prepared, as described elsewhere (104). The spleen extract from each mouse was then analyzed individually with respect to their leukemogenic potentiality. Each donor spleen specimen was reinjected to a different "receptient" mouse (20 in total), and the leukemogenesis was followed, as shown in Table 2.

All animals in group 1 and 3 developed splenomegally and died between 40-60 days; whereas, in the MPC-treated groups, of the 10 animals only 3 showed signs of splenomegally. In group 2, 2 animals had splenomegally but, in spite of that, all animals survived till the 123rd day, at which time our experiment was terminated. Similarly, in the last group animals survived till the 123rd day; one died on the 97th day. The spleen weights, shown in the last column, also exhibit large differences between the MPC-treated group, and the control group. In another study we have analyzed the effect of MPC on normal mice of the same strain. We failed to observe any effect of MPC on the spleen weights of non-infected mice.

In another study (98, 104) we have analyzed the in-vivo effect of MPC on the leucocytes of mice infected with the active Friend virus. Within 12-24 hrs. after MPC injection (50 µg /mouse) a dramatic fall in the leucocyte count of animals infected with FLV was observed; MPC failed to reduce the leucocyte number in mice not-infected with the virus. It is interesting that, in one of the infected animals MPC failed to suppress the leucocyte number; on the contrary, there was a gradual increase in leucocyte number. This animal died on the 13th day of MPC treatment. Unfortunately, we were not able to analyze the spleen of this animal. It is therefore difficult to interpret the reasons for failure of MPC effect in this animal.

Based on the biochemical selectivity of MPC action and its biological effects, clinical trials of MPC in the treatment of childhood leukemia were initiated. We began these trials on cases with terminal phase of the disease.

TABLE 3

Clinical Data of Patients with ALL and AML submitted to MPC trials.

No.	Init.	Age	Sex	Diag.	Stage	Results
1	D. M.	8	♀	ALL	3 rd rel.	PR, WBC ↓
2	T. I.	6 6/12	♀	ALL	2 nd rel.	?
3	B. M.	6 7/12	♂	ALL	2 nd rel.	?
4	M. M.	8 4/12	♂	ALL	2 nd rel.	CR
5	J. O.	3 6/12	♂	ALL	2 nd rel.	?
6	N. A.	10	♀	ALL	2 nd rel.	CR
7	B. C.	5 8/12	♀	ALL	3 rd rel.	CR
8	N. N.	7 11/12	♂	ALL	3 rd rel.	?
9	M. A.	12	♂	ALL	1 st rel.	?
10	M. I.	8 6/12	♂	ALL	1 st rel.	?
11	K. C.	11 3/12	♀	ALL	4 th rel.	?
12	K. K.	10 9/12	♀	ALL	5 th rel.	PR
13	L. J.	7	♂	ALL	3 rd rel.	WBC ↓
14	F. D.	2 3/12	♂	ALL	init ph.	WBC ↓
15	S. B.	7 11/12	♀	ALL	init ph.	WBC ↓
16	H. B.	12 5/12	♀	ALL	init ph.	WBC ↓
17	S. N.	4	♀	AML	init ph.	WBC ↓
18	W. H.	5 6/12	♂	AML	init ph.	WBC ↓
19	S. K.	4 9/12	♂	ALL	init ph.	WBC ↓
20	F. A.	7 10/12	♀	AML	init pH.	WBC ↓
21	L. F.	3 4/12	♂	ALL	init ph.	WBC ↓
22	F. T.	4 9/12	♂	ALL	init ph.	WBC ↓
23	C. E.	13 4/12	♂	ALL	init ph.	WBC ↓

Clinical data of patients submitted to MPC trials are shown in Table 3. Of the 23 cases treated with MPC, 13 were in the terminal phase of the disease. These patients were resistant to all previous chemotherapeutic regimes which involved drugs such as prednisone, vincristin, daunorubicin, L-asparaginase, Ara-C, 6-mercaptopurine, methotrexate, cyclophosphamide and actinomycin D.

MPC preparation used in our clinical trials contained 15 percent of thiolated cytosine bases. The lyophilized product (MPC) was dissolved in 0.1M Tris/HCl buffer, pH 7.6, and diluted with 0.9% NaCl before use. This solution was sterilized by passing through a membrane filter (Millipore GmbH, Neu Isenburg, Germany). It was kept at 4 °C and used immediately, or within the next five days; solutions older than 5 days were reprecipitated, purified on the column and resterilized. In our clinical trials, MPC (sterile) was given intravenously at a dose 0.5 mg/kg body weight. The injections were given once a week.

Of the 13 terminal cases, complete remission was achieved in 3, and a partial remission achieved in 2 other cases. In the terminal cases, it is not possible to give an empirical evaluation of MPC-effectivity, since these children either lived for a short time only, or no remission could be achieved. The extent to which MPC affected the survival period in these cases can not be evaluated, since the patients were in a very heterogeneous state (1 to 5 relapses). Fever, occasionally accompanied by shivering was frequently observed under MPC treatment in the first hour after injection. However, these symptoms never lasted more than the first hour, and no other side effects could be observed.

On the basis of our experience with MPC on terminal cases, we were motivated to give MPC a clinical trial in the beginning of leukemia treatment. A monotherapy with MPC, as devised for terminal cases is, however, not possible. We therefore decided to introduce MPC therapy in the beginning of treatment of cases which at the time of diagnosis had leucocytosis. The initial treatment, a single injection of MPC, was then followed up by the chemotherapy protocol, devised by Rhiem (106). Irrespective of ALL or AML, 24 hrs after MPC injection, there was a dramatic reduction of leukemic cells in all cases (Table 3).

The selective cytolysis of leukemic cells by MPC indicates that this compound binds to some "novel" component present in these cells, which may be the viral related reverse transcriptase. The reason for this selectivity may be the concentration range, which does not affect the normal cellular polymerases, as indicated by the enzymatic studies. Once the compound is bound to this "target" the cytotoxicity could then be a secondary event, probably by release of lysosomal enzymes.

2. Studies with Polyadenylic Acid Analogs: The fact that polyadenylic acid (Poly A) is easily transcribed by

a complementary primer strand (Fig. 7). However, the K_m of the reverse transcriptase reaction catalyzed by $(dAfl)_n$ is higher than the reaction catalyzed by $(rA)_n \cdot (dT)_{12}$.

Figure 7: Lineweaver-Burk Plot of the Kinetics of Reverse transcriptase reaction, catalyzed by $(dAfl)_n$ and $(rA)_n \cdot (dT)_{12}$.

In contrast, the 2-substituted derivatives, poly(2-methylthio-adenylic acid) and poly(2-ethylthio-adenylic acid), are not able to discriminate between the reactions catalyzed by different template-primers. For example, both the derivatives inhibit $(rA)_n \cdot (dT)_{12}$ - and $(rC)_n \cdot (dG)_{12}$ catalyzed reverse transcriptase reaction to the same extent; though the methylthio derivative is a better inhibitor than the ethylthio analog. The DNA polymerase- α was less sensitive to these inhibitors; whereas, the bacterial DNA polymerase (Polymerase I) was completely resistant to the action of all the derivatives used in this study.

CONCLUSION AND FUTURE PROSPECTS

The concept of antiviral chemotherapy emerges from the basic knowledge about the biochemical differences between the metabolic reactions operating in a normal cell, and in a virus-infected cell. The knowledge about the association of several RNA- and DNA viruses to human cancer has been very rewarding in the exploitation of such differences. We know of several DNA polymerizing activities, not present in the normal eukaryotic cell, are present in virus-infected cells. These virus-specific DNA polymerases are either induced by viruses, or in some

cases, associated as a structural entity of the virus. In both cases, these DNA polymerases are products of the viral genome. Recent advances in the biochemical methodology have contributed to the purification of these "novel" DNA polymerases from viruses, or virus-infected cells. The purified virus-specific DNA polymerases are biochemically and immunologically different to the constitutive cellular DNA polymerases α , β and γ . Thus, virus-specific DNA polymerases offer a unique molecular target for designing potential antiviral compounds.

In spite of a great variety of inhibitors which affect virus-induced DNA synthesis, an absolutely specific inhibitor of such viral polymerases is not yet available. The specificity is relative and often varies with the system studied. Thus, an inhibitor may have a K_i value of less than 10^{-5} M for the viral DNA-polymerase system, but a K_i of 10^{-3} M for the cellular DNA-polymerase system. As a result, it makes sense to evaluate the biological activity of such inhibitors under carefully controlled conditions. The question is: Is it possible to fractionate the antiviral and anticellular activities of such compounds in a particular dose range? We know from our own studies that the cytotoxic and the antiviral activities of a compound can be influenced differently by structural alterations of the compound.

The future of the development of new DNA-polymerase inhibitors is dependent on two parameters: firstly, a good knowledge about the mode of action of the compound under investigation. This is important to localize the structural constraints of the molecule involved in exercising a particular function. On the basis of our present-day knowledge about the molecular processes involved in the replication of such viruses, it is possible to specify the possible target(s) for such inhibitors. This will facilitate the modification of the molecular structure in order to potentiate functional activity of the compound against that particular target reaction. Secondly, to develop new systems and procedures to identify certain binding interactions which occur during the in-vivo polymerization of DNA. The lysolecithin-permeabilized baby hamster kidney cell system, developed by Miller et al. (14,15) could contribute to study the interaction of such inhibitors with their molecular targets under semi-in vivo conditions.

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REFERENCES

1. BOLLUM, F.J.: J. Am. Chem. Soc. 80, 1766, 1958.
2. KORNBERG, T. and GEFTER, M.L.: Biochem. Biophys. Res. Commun. 40, 1348, 1970.
3. MOSES, R.E. and RICHARDSON, C.C.: Proc. Natl. Acad. Sci. U.S. 67, 674, 1970.
4. KNIPPERS, R.: Nature 228, 1050, 1970.
5. KORNBERG, T. and GEFTER, M.L.: Proc. Natl. Acad. Sci. U.S. 68, 761, 1971.
6. WEISSBACH, A., BALTIMORE, D., BOLLUM, F.J., GALLO, R.C. and KORN, D.: Science 190, 401, 1975.
7. SARANGADHARAN, M.G., ROBERT GUROFF, M. and GALLO, R.C.: Biochim. Biophys. Acta 516, 419, 1978.
8. LOEB, L.A.: In, The Enzymes (Edt. P.D. Boyer), vol 10, 173, 1974. Academic Press, New York.
9. BOLLUM, F.J.: In, Progress in Nucleic Acid Research & Molecular Biology 10, 109, 1975. Academic Press, New York.
10. WEISSBACH, A.: Cell 5, 101, 1975.
11. BOLLUM, F.J.: In, Antiviral Mechanisms in the Control of Neoplasia (Edt. P. Chandra) pp.587-601, 1979. Plenum Press, New York.
12. FALASCHI, A. and SPADARI, S.: In, DNA-Synthesis (NATO Symposium) pp 487, 1978. Plenum Press, New York.
13. SUGINO, A. and NAKAYAMA, K.: Proc. Natl. Acad. Sci. U.S. 77, 7049, 1980.
14. MILLER, M.R., CASTELLOTT, J.J. and PARDEE, A.B.: Biochemistry 17, 1073, 1978.
15. MILLER, M.R., CASTELLOTT, J.J. and PARDEE, A.B.: Exptl. Cell Res. 120, 421, 1979.
16. CASTELLOTT, J.J., MILLER, M.R., LEHTOMAKI, D.M. and PARDEE, A.B.: J. Biol. Chem. 254, 6904, 1979.
17. BOLLUM, F.J.: J. Biol. Chem. 237, 1945, 1962.
18. McCaffrey, R., Smoler, D., and Baltimore, D.: Proc. Natl. Acad. Sci. U.S. 70, 521, 1973.
19. Coleman, M.S., Hutton, J.J., Simone, P.D. and BOLLUM, F.J.: Proc. Natl. Acad. Sci. U.S. 71, 4404, 1974.
20. SARIN, P.S. and GALLO, R.C.: J. Biol. Chem. 249, 8051, 1974.

21. SRIVASTAVA, B.I.S.: *Cancer Res.* 34, 1015, 1974.
22. BERTAZZONI, U. and BOLLUM, F.J.: *Terminal Transferase in Immunobiology and Leukemia 1982*. Plenum Press, New York.
23. BERTAZZONI, U., SCOVASSI, A.I., TORSELLO, S., BRUSAMOLINO, E., ISERNIA, P., BERNASCONI, C., GIENLLI, E. and SACCHI, N.: In, *Biochemical and Biological Markers of Neoplastic Transformation* (Edt. P. Chandra), pp 217-232, 1983. Plenum Press, New York.
24. SARIN, P.S., VIRMANI, M., PANTAZIS, P. and GALLO, R.C.: In, *Biochemical and Biological Markers of Neoplastic Transformation* (Edt. P. Chandra), pp 193-216, 1983. Plenum Press New York.
25. TEGTMEYER, P. and ANDERSEN, B.: *Virology* 115, 67, 1981.
26. DULBECCO, R., AHRTWELL, L.H. and VOGT, M.: *Proc. Natl. Acad. Sci. U.S.A.* 53, 403, 1965.
27. WEIL, R., MICHEL, M.R. and RUSCHMANN, G.K.: *Proc. Natl. Acad. Sci. U.S.A.* 53, 1468, 1965.
28. KIT, S., DUBBS, D.R. and FREARSON, P.M.: *Cancer Res.* 26, 262, 1966.
29. MARTIN, R.G.: *Adv. Cancer Res.* 34, 1, 1981.
30. ITO, K., ARENS, M. and GREEN, M.: *J. Virology* 15, 1507, 1975.
31. KEIR, H.M., SUBAK-SHARPE, H., SHEDDEN, W.I.H., WATSON, D.H. and WILDY, P.: *Virology* 30, 154, 1966.
32. WEISSBACH, A., HONG, S.C.L., AUCKER, J. and MÜLLER, R.: *J. Biol. Chem.* 248, 6270, 1973.
33. ARON, G.M., PURIFOY, D.J.M. and SCHAFFER, P.A.: *J. Virology* 16, 498, 1975.
34. HAY, J., MOSS, H. and HALLIBURTON, I.W.: *Biochem. J.* 124, 64p, 1971.
35. PURIFOY, D.J.M. and BENYESH-MELNICK, M.: *Virology* 68, 374, 1975.
36. KEMP, M.C., COHEN, J.C., O'CALLAGHAN, D.J. and RANDALL, C.C.: *Virology* 68, 467, 1975.
37. ALLEN, G.P., O'CALLAGHAN, D.J. and RANDALL, C.C.: *Virology* 76, 395, 1977.
38. MILLER, R.L. and RAPP, F.: *J. Virology* 20, 564, 1976.
39. HUANG, E.S.: *J. Virology* 16, 298, 1975.
40. HIRAI, K. and WATANABE, Y.: *Biochim. Biophys. Acta* 447, 328, 1976.

41. BOEZI, J.A., LEE, L.F., BLAKSLEY, R.W., KOENIG, M. and TOWLE, H.C.: J. Virol. 14, 1209, 1974.
42. MILLER, R.L., GLASER, R. and RAPP, F.: Virology 76, 494, 1977.
43. KNOPF, K.: Europ. J. Biochem. 98, 231, 1979.
44. POWELL, K.L. and PURIFOY, D.J.M.: J. Virol. 24, 618, 1977.
45. ERIKSSON, B., LARSSON, A., HELGSTRAND, E., JOHANSSON, N.G. and ÖBERG, B.: Biochim. Biophys. Acta 607, 53, 1980.
46. FURMAN, P.A., St. CLAIR, M.H., FYFE, J.A., RIDEOT, J.L., KELLER, P.M. and ELION, G.B.: J. Virol. 32, 72, 1979.
47. MILLER, W.H. and MILLER, R.L.: J. Biol. Chem. 255, 7204, 1980.
48. DARBY, G., FIELD, H.J. and SALISBURY, S.A.: Nature 289, 81, 1981.
49. PEDRALI-NOY, G. and SPADARI, S.: J. Virol. 36, 457, 1980.
50. DERSE, D., CHENG, Y.C., FURMAN, P.A., St. CLAIR, M.H. and ELION, G.B.: J. Biol. Chem. 256, 11447, 1981.
51. LEINBACH, S.S., RENO, J.M., LEE, L.F., ISBELL, A.F. and BOEZI, J.A.: Biochemistry 15, 426, 1976.
52. KAPLAN, P.M., GREENMAN, R.L., GERIN, J.L., PURCELL, R.H. and ROBINSON, W.S.: J. Virol. 12, 995, 1973.
53. ROBINSON, W.S. and GREENMAN, R.L.: J. Virol. 13, 1231, 1974.
54. TEMIN, H.M. and MIZUTANI, S.: Nature 226, 1211, 1970.
55. BALTIMORE, D.: Nature 226, 1209, 1970.
56. SHANK, P.R., COHEN, J.C., VARMUS, H.E., YAMAMOTO, K.R., and RINGOLD, G.M.: Proc. Natl. Acad. Sci. U.S. 75, 2112, 1978.
57. HSU, T.W., SABRAN, J.L., MARK, G.E., GUNTAKA, E.V. and TAYLOR, J.W.: J. Virol. 28, 810, 1978.
58. DHAR, R., McCLEMENTS, W.L., ENQUIST, L.W. and VANDE WOUDE, G.F.: Proc. Natl. Acad. Sci. U.S. 77, 3971, 1980.
59. SWANSTROM, R., DeLORBE, W., BISHOP, J.M. and VARMUS, H.E.: Proc. Natl. Acad. Sci. U.S. 78, 124, 1981.
60. YOSHIMURA, F.K. and WEINBERG, R.A.: Cell 16, 323, 1979.
61. GUNTAKA, R.V., MAHY, B.W.J., BISHOP, J.M. and VARMUS, H.E.: Nature 253, 507, 1975.
62. TEMIN, H.M.: Cell 21, 599, 1980.
63. MAJORS, J.E. and VARMUS, H.E.: Nature 289, 253, 1981.

64. SHIMOTOHNO, K. and TEMIN, H.M.: Proc.Natl.Acad.Sci.U.S. 77, 7357, 1980.
65. HUGHES, S.H., MUTSCHLER, A., BISHOP, J.M. and VARMUS, H.E. Proc.Natl.Acad.Sci.U.S. 78, 4299, 1981.
66. RINGOLD, G.M.: Biochim. Biophys. Acta 560, 487, 1979.
67. GALLO, R.C., YANG, S.S. and TING, R.C.: Nature 228, 927, 1970.
68. GALLO, R.C., GALLAGHAR, P.E., MILLER, N.R., MONDAL, H., SAXINGER, W.C., MAYER, R.J., SMITH, R.G. and GILLESPIE, D.H.: Cold Spring Harb.Symp.Quant.Biol. 39, 933, 1975.
69. CHANDRA, P.: In, Proceedings of the XIIth International Cancer Congress, Buenos Aires, Vol. 1, pp.29, 1978. Pergamon Press, Oxford.
70. CHANDRA, P.: Advances in Ophthalmology 38, 234, 1979. Karger-Verlag, Basel.
71. CHANDRA, P.: In, Proceedings of the Federation of European Biochemical Societies, Dubrovnik (Edt. P. Mildner) Vol. 61, pp. 215, 1979. Pergamon Press.
72. CHANDRA, P. and STEEL, L.K.: Biochem. J. 167, 513, 1977.
73. CHANDRA, P., BALIKCIOGLU, S. and MILDNER, B.: Cancer Lett. 5, 299, 1978.
74. CHANDRA, P., STEEL, L.K. and CAVDAR, A.O.: In, Modern Trends in Human Leukemia (Edts. R. Neth et al.) Vol. III, pp.497, 1979. Springer-Verlag, Berlin-Heidelberg-New York.
75. CHANDRA, P., STEEL, L.K., LAUBE, H. and KORNHUBER, B.: In, Antiviral Mechanisms in the Control of Neoplasia (Edt. P. Chandra) pp177, 1979. Plenum Press, New York.
76. CHANDRA, P., STEEL, L.K., LAUBE, H., BALIKCIOGLU, S., EBENER, U. and WELTE, K.: In, Proceedings of the 8th International Symposium on the Biological Characterization of Human Tumors, Athens. Vol. 5, pp 359, 1980. Excerpta Medica, Amsterdam.
77. WELTE, K., EBENER, U. and CHANDRA, P.: Cancer Lett. 7, 189, 1979.
78. CHANDRA, P., STEEL, L.K., LAUBE, H., BALIKCIOGLU, S., MILDNER, B., EBENER, U., WELTE, K. and VOGEL, A.: In, Cold Spring Harb. Conference on Cell Proliferation (Viruses in naturally occuring cancer) vol. 7, 775, 1980.

79. CHANDRA, P., BALIKCIOGLU, S. and MILDNER, S.: Cell & Mol. Biol. 27, 239, 1981.
80. CHANDRA, P.: Editorial Review, Survey of Immunologic Res. (in press)
81. OHNO, T. and SPIEGELMAN, S.: Proc. Natl. Acad. Sci. U.S. 74, 2144, 1977.
82. POIEZ, B.J., RUSCETTI, F.W., GAZDAR, A.F., BUNN, P.A., MINNA, J.D. and GALLO, R.C.: Proc. Natl. Acad. Sci. U.S. 77, 7415, 1980.
83. GALLO, R.C., ROBERT-GUROFF, M., KALYANARAMAN, V.S., CECCHERINI, N.L., RUSCETTI, F.W., BRODER, S., SARANGADHARAN, ITO, Y., M. MAEDA, M. WAINBERG and M.S. REITZ: In, Biochemical and Biological Markers of Neoplastic Transformation (Edt. P. Chandra) pp. 503, 1983. Plenum Press, New York.
84. SARIN, P.S. and GALLO, R.C.: International Reviews in Science, Vol. 6 (Edt. K. Butron) pp 219, 1974. Butterworths, London.
85. THOMPSON, F.M., LIBERTINI, L.J., JOSS, U.R. and CALVIN, M.: Science 178, 505, 1972.
86. TEMIN, H.M. and BALTIMORE, D.: Adv. Virus Res. 17, 129, 1972.
87. LEVINSON, W., FARAS, A., WOODSON, B., JACKSON, J. and BISHOP, J.M.: Proc. Natl. Acad. Sci. U.S. 70, 164, 1973.
88. CHANDRA, P., EBENER, U., STEEL, L.K., LAUBE, H., GERICKE, D., MILDNER, B., BARDOS, T.J., HO, Y.K. and GÖTZ, A.: Ann. N.Y. Acad. Sci.: Third Conference on Antiviral Substances 284, 444, 1977.
89. CHANDRA, P., ZUNINO, F., GÖTZ, A., WACKER, A., GERICKE, D., DI MARCO, A., CASSAZA, A.M. and GIULIANI, F.: FEBS-Lett. 21, 154, 1972.
90. CHANDRA, P., DI MARCO, A., ZUNINO, F., CASAZZA, A.M., GERICKE, D., GIULIANI, F., SORANZO, C., THORBECK, R., GÖTZ, A., ARCAMONE, F. and GHIONE, M.: Naturwissenschaften 59, 448, 1972.
91. CHANDRA, P., ZUNINO, F. and GÖTZ, A.: FEBS-Lett. 22, 161, 1972.
92. CHANDRA, P.: In, Medicinal Chemistry : Topics in current Chemistry Vol. 52, 99, 1974. Springer-Verlag.
93. CHANDRA, P., WILL, G., GERICKE, D. and GÖTZ, A.: Biochem. Pharmacol. 23, 3259, 1974.

94. CHANDRA, P. and BARDOS, T.J.: Res. Commun. Chem. Path. and Pharmacol. 4, 615, 1972.
95. CHANDRA, P., EBENER, U. and GÖTZ, A.: FEBS-Lett. 53, 10, 1975.
96. CHANDRA, P., EBNER, U., STEEL, L.K., LAUBE, H., GERICKE, D., MILDNER, B., BARDOS, T.J., HO, Y.K. and GÖTZ, A.: Ann. N.Y. Acad. Sci. 284, 444, 1977.
97. CHANDRA, P., STEEL, L.K., EBENER, U., WOLTERS DORF, M., LAUBE, H., KORNHUBER, B., MILDNER, B. and GÖTZ, A.: Pharmacol. & Ther. A 1, 231, 1977.
98. CHANDRA, P., STEEL, L.K., EBENER, U., WOLTERS DORF, M., LAUBE, H., KORNHUBER, B., MILDNER, B. and GÖTZ, A.: In, Inhibitors of DNA and RNA polymerases Edts.: P.S. Sarin and R.C. Gallo), pp47, Pergamon Press, Oxford, 1980.
99. CHANDRA, P.: In, Topics in current chemistry 52, 99, 1974. Springer-Verlag, Heidelberg-New York.
100. CHANDRA, P., EBENER, U., BARDOS, T.J., GERICKE, D., KORNHUBER, B. and GÖTZ, A.: In, Modulation of host immune resistance in the prevention or treatment of cancer (Edt. M.A. Chirigos), Fogarty International Center Proceedings (USA) Vol. 28, 169, 1977.
101. CHANDRA, P.: In, Antimetabolites in Biochemistry, Biology and Medicine (Edts. J. Skoda and P. Langen) pp.249, 1979. Pergamon Press, Oxford.
102. EBENER, U.: Doctoral Thesis (Frankfurt University), 1977.
103. KOTLER, M. and BECKER, Y.: FEBS-Lett. 22, 222, 1972.
104. CHANDRA, P., KORNHUBER, B., GERICKE, D., GÖTZ, A. and EBENER, U.: Z. Krebsforschg. u. Klin. Onkol. 83, 239, 1975.
105. KORNHUBER, B. and CHANDRA, P.: In, Antiviral Mechanisms in the control of Neoplasia (Edt. P. Chandra) pp. 577, 1979. Plenum Press, New York.
106. RHIEM, H., GARDNER, H., JESSENBERGER, K. and TARIVERDIAN, G.: Proc. Amer. Assoc. Cancer Res. 15, 58, 1974.
107. CHANDRA, P., DEMIRHAN, I. and EBENER, U.: In, Terminal Transferase in Immunobiology and Leukemia (Edts. U. Bertazzoni and F.J. Bollum) pp 87, 1982. Plenum Press, New York.
108. CHANDRA, P., DEMIRHAN, I., EBENER, U., KORNHUBER, B. and GERICKE, D.: In, Developments in Molecular Virology (Edt. Y. Becker) Vol. 3, (in press). Martinus Nijhoff/Dr. W. Junk Publishers, Holland.

109. CHANDRA,P., DEMIRHAN,I. and DE CLERCQ,E.: Cancer Lett. 12, 181, 1981.
110. DE CLERCQ,E., FUKUI,T.,KAKIUCHI,N.,IKEHARA,M.,HATTORI,M. and PFLEIDERER,W.: Cancer Lett.7,27,1979.
111. IKEHARA,M.,FUKUI,T. and KAKIUCHI,N.: Nucleic Acid Res. 5, 1877, 1978.