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INHIBITION OF RNA-DEPENDENT DNA POLYMERASE ACTIVITY OF ONCORNAVIRUSES BY CAFFEINE

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SUMMARY

Caffeine was found to inhibit RNA-dependent DNA polymerase activity of Rauscher leukemia virus when endogenous viral RNA and poly(rA)·$(dT)_{12-18}$ were used as templates. Similar results were also obtained with purified RNA-dependent DNA polymerase (deoxynucleoside triphosphate; DNA nucleotidyl transferase; EC 2.7.7.7) from avian myeloblastosis virus (AMV) utilizing 70S and 35S RNA of AMV, poly(rA)·$(dT)_{12-18}$, globin mRNA and activated calf thymus DNA as templates. The "caffeine effect" was evident only when it was present during the initiation of polymerization reaction. Increasing the template concentration in the reaction mixture partly reversed the effect of caffeine. Of the analogs of caffeine tested, only theophylline inhibited AMV DNA polymerase, whereas aminophylline showed no effect.

INTRODUCTION

Caffeine is known to have pleotropic effects at the cellular level and several studies on the cytotoxic and mutagenic effects of caffeine have been documented (1, 2). Inhibitory effect of caffeine on enzymes involved in DNA synthesis and repair has been speculated (1) and the available experimental data on this subject is conflicting. Wragg et al. (3) first reported the inhibition of DNA polymerase by caffeine in cultured human embryonic lung cells. Most other workers, however, showed that caffeine does not have any effect on polymerization reaction catalysed by DNA polymerase (4, 5, 6). Recent work of Solberg et al. (7) on E. coli polymerase I also supports the latter view. This has prompted us to examine the effect of caffeine on another polymerizing enzyme RDDP (RNA-dependent DNA polymerase) of oncogenic viruses which has both RNA directed and DNA directed activity. Here we report that indeed caffeine inhibits reverse transcriptase of murine and avian oncogenic viruses.

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MATERIALS AND METHODS

Viruses and reverse transcriptase. Avian myeloblastosis virus (AMV), purified AMV DNA polymerase and Rauscher leukemia virus were obtained from the office of Program Resources and Logistics of the Virus Cancer Program, NCI. AMV 70S RNA was extracted according to the procedure of Stephenson et al. (8). 35S RNA was then prepared by suspending 70S RNA in buffer and heating at 90°C for 3 minutes followed by refractionation on sucrose gradient.

Reagents and templates. Labeled [^{3}H]-TTP (64.6 Ci/mmole) was purchased from New England Nuclear. Globin mRNA and calf thymus DNA were obtained from Miles Biochemicals. Oligothymidilic acid primer was obtained from Collaborative Research and poly(rA)·$(dT)_{12-18}$ and unlabeled triphosphates were from P-L Biochemicals. All other chemicals were purchased from Sigma Co.

Assay of RDDP. Reverse transcriptase was assayed by using exogenous templates in the disrupted virions as described by Premkumar Reddy et al. (9). Endogenous RNA directed DNA polymerase of R-MuLV was assayed according to Tomita and Kuwata (10). The procedure for assaying purified AMV DNA polymerase with different templates was the same as given by Houts et al. (11).

RESULTS

Inhibition by caffeine of reverse transcriptase in vitro. Caffeine inhibited RNA-dependent DNA polymerase activity of R-MuLV catalyzed by templates

Table 1

Inhibition of reverse transcriptase by caffeine.

Template used	Concentration of caffeine	Percent inhibition
Exogenous template poly(rA)·$(dT)_{12-18}$[a]	9×10^{-3}M	24
	1.4×10^{-2}M	49
	2.15×10^{-2}M	58
Endogenous RNA directed[b]	9×10^{-3}M	35
	1.4×10^{-2}M	59
	2.15×10^{-2}M	66

[a]Reaction mixture for exogenous poly(rA)·$(dT)_{12-18}$ template was composed of 0.2 M glycine-HCl buffer pH 7.8; 0.075 M NaCl; 10^{-3}M DTT; 10^{-4}M manganese acetate; 0.03 A_{260} units of poly(rA); 0.02 A_{260} units of oligo$(dT)_{12-18}$ and 2 µCi of ^{3}H-dTTP (200 pM) which was added to 50 µl of virus suspension disrupted with 0.05% NP40 solution. Reaction was carried out for 60 min. at 37°C. Suitable concentration of caffeine was achieved by adding 2% caffeine stock solution.

[b]Endogenous assay relied on the virions to supply 70S genomic RNA and tRNA, the natural template and primer. Reaction mixture was composed of Tris-HCl pH 7.8; 5 mM DTT; 30 mM NaCl; 1 mM manganese acetate; 0.2 mM each of dATP, dGTP and dCTP and 2 µCi [^{3}H]dTTP. (Percent inhibition was calculated with respect to control where no inhibitor was added.)

poly(rA)·$(dT)_{12-18}$ and viral endogenous RNA. The concentration dependent inhibition is evident from the data presented in Table 1. Purified AMV DNA polymerase was also inhibited by caffeine (Table 2 and Figure 1). The magnitude of inhibitory effect of caffeine on purified AMV DNA polymerase was dependent on the template used. Denatured calf thymus DNA and 70S AMV RNA templates showed maximum caffeine effect.

Kinetics of inhibition. Time course of different template directed AMV DNA polymerase activity showed that the inhibition by caffeine reached maximum

Table 2

Effect of caffeine on DNA synthesis directed by AMV reverse transcriptase with different templates

Template	Caffeine concentration	Percent inhibition
Poly(rA)·$(dT)_{12-18}$	9×10^{-3}M	25
	1.4×10^{-2}M	39
	2.15×10^{-2}M	34
70S AMV RNA	9×10^{-3}M	48
	1.4×10^{-2}M	64
	2.15×10^{-2}M	66
35S AMV RNA + oligo (dT)	9×10^{-3}M	25
	1.4×10^{-2}M	35
	2.15×10^{-2}M	39
Globin mRNA + oligo (dT)	9×10^{-3}M	30
	1.4×10^{-2}M	30
	2.15×10^{-2}M	40
Calf thymus DNA heat denatured	9×10^{-3}M	44
	1.4×10^{-2}	66
	2.15×10^{-2}	67.5

Purified RDDP was assayed using 50 mM Tris-HCl pH 8.3; 6 mM $MgCl_2$, 40 mM KCl; 6 mM dithiothreitol; 2 µCi ^{3}H-dTTP; 0.2 mM of dATP, dGTP and dCTP and appropriate caffeine concentration was achieved by adding 2% stock solution. Template concentrations used were: poly(rA)·$(dT)_{12-18}$, A_{260} 0.035 units; 70S and 35S AMV RNA, 15 µg/ml; rabbit globin mRNA, 10 µg/ml; activated calf-thymus DNA, .226 mg/ml. Incubation was carried out for 60 minutes.

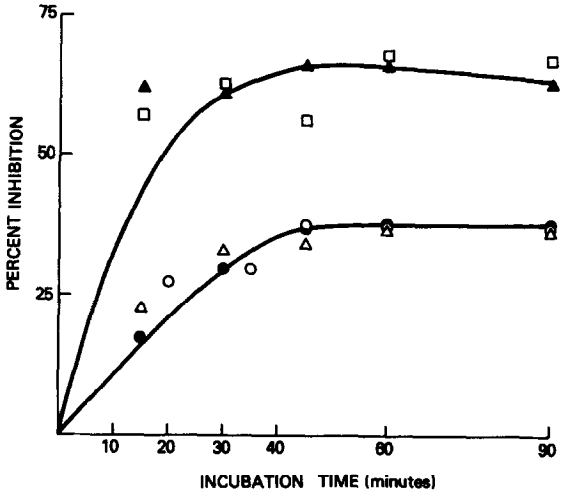

Figure 1. Time course of caffeine inhibition of AMV DNA polymerase with different templates. (●) globin mRNA, (△) poly(rA)·$(dT)_{12-18}$, (○) 35S AMV RNA, (▲) 70S AMV RNA, (□) calf thymus DNA heat denatured. Assay conditions were the same as given in Table 2.

by 30-45 minutes of incubation (Figure 1). The complexity of inhibition was further analyzed by the addition of caffeine at zero time and after initiating the polymerization reaction. The inhibitory effect of caffeine was not seen when it was added after initiating the reaction (Table 3). Also preincubation experiments with various components of the reaction mixture with caffeine showed little difference in inhibition.

To determine the nature of inhibition of RDDP by caffeine, kinetic experiments involving different template concentrations were conducted. The double reciprocal plots of polymerization rate vs. poly(rA)·$(dT)_{12-18}$ concentration showed that inhibition was competitive type (Figure 2).

Effect of caffeine analogs on reverse transcriptase. Analogs of caffeine were also tested for their ability to inhibit AMV DNA polymerase in an effort to determine whether a correlation exists between the structure of the chemical and polymerase inhibitory activity. Theophylline (1,3-dimethyl xanthine) inhibits AMV DNA polymerase and is more effective than caffeine. However, aminophylline (theophylline$_2$-ethylenediamine) completely lacks this effect at the concentration tested.

Table 3

Inhibition of AMV reverse transcriptase by caffeine preincubated with various components of the reaction system and after initiating the reaction.

Reaction conditions	Percent inhibition
No preincubation (complete system containing caffeine)	34
Caffeine preincubated with:	
Assay mixture containing template, no enzyme	35
Assay mixture without template, plus enzyme	34
Assay mixture without template and enzyme	38
Enzyme	38
Template	38
Time of addition of caffeine after initiating the reaction	
5 minutes	0
10 minutes	0
15 minutes	0

After preincubation (15 min. at 37°C), the missing components required for RT reaction were added and the complete system was incubated for 60 min. at 37°C. Percent inhibition was calculated with respect to control where no inhibitor was added.

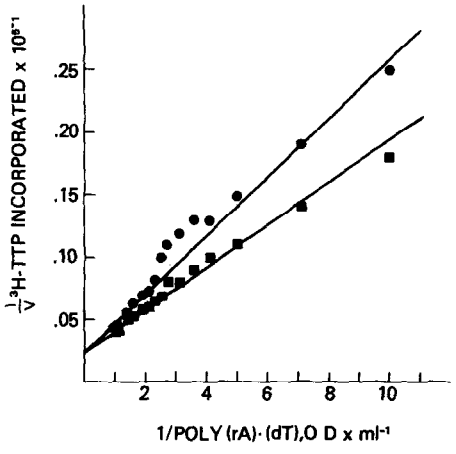

Figure 2. Caffeine inhibition of AMV DNA polymerase. Lineweaver-Burk plot with poly(rA)·$(dT)_{12-18}$ as the variable substrate. (■) control, (●) caffeine.

Table 4

Effect of caffeine analogs (theophylline and aminophylline) on AMV reverse transcriptase.

Concentration of caffeine analog		Percent inhibition
Theophylline	5.5×10^{-3}M	8
	1.1×10^{-2}M	32
	1.6×10^{-2}M	39
Aminophylline	2.4×10^{-3}M	0
	4.7×10^{-3}M	0
	7.1×10^{-3}M	0

Reaction conditions are described in Table 2. Poly(rA)·$(dT)_{12-18}$ was used as template and incubation was carried out for 60 min. at 37°C.

DISCUSSION

RNA containing murine and avian oncogenic viruses have viral DNA polymerase which has both RNA dependent and DNA dependent polymerase activities (12). Results obtained in this study indicate that caffeine inhibits both these activities. Inhibition by caffeine is independent of the purity of the enzyme, as same degree of inhibition was observed with either purified AMV reverse transcriptase or unpurified core enzyme of R-MuLV. Such an inhibitory effect agrees with the finding of Wragg et al. (3) who observed an inhibition of incorporation of ^{3}H-TTP into DNA in cell free extracts of cultured human embryonic lung cells at concentrations greater than 1 mM. However, Grigg (4) and Mouton and Fromageot (5) could not observe such inhibition while using nicked calf thymus DNA with E. coli DNA polymerase.

Despite the vast amount of data on different effects of caffeine, its mechanism of action is not known. Enzyme inhibition, in general, may result from an interaction of the inhibitor with some components of the enzyme system (13). Since RNA-dependent DNA polymerase is a multisubstrate enzyme utilizing template primer, four deoxynucleoside triphosphates and a metal ion activator, interaction of caffeine with any of these reactants could result

in inhibition of enzymatic activity. Preincubation studies with different components of the enzyme system showed similar pattern of inhibition. This seems to suggest that the target of caffeine may be enzyme-template primer complex or initiation complex as a whole.

Kinetic experiments with different concentrations of template-primer suggest that caffeine may inhibit by interacting with the template. Reversal of caffeine effect by high concentrations of template supports such a view. It is of interest to point out that caffeine tends to bind single-stranded and denatured DNA (14, 15). Increasing the Mg^{2+} concentration decreased the inhibition by daunomycin of murine sarcoma virus DNA polymerase (16). Such a study with caffeine did not show any reversing effect (unpublished data).

Of the analogs of caffeine tested, theophylline inhibited RDDP and was more effective than caffeine. Aminophylline, a dimer of theophylline does not exhibit such inhibitory activity. Thus methyl xanthines considered here, which differ only in the number and position of the methyl group on the purine ring, do not conform to the structure-activity relationships observed in some other systems (1).

Based on the results, further studies on the effect of caffeine on DNA polymerases from different sources would be worth attempting. This may help to understand the mechanism of action of caffeine in chemical and physical mutagen-treated systems.

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