SPECIFIC INHIBITION BY RIFAMPICIN OF TRANSCRIPTION IN HUMAN LYMPHOCYTES STIMULATED BY PHYTOHEMAGGLUTININ

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INTRODUCTION

Rifampicin, a semisynthetic antibiotic, is known to inhibit the bacterial DNA-dependent RNA polymerase (1), the growth of some animal viruses (2), and the focus formation induced by oncogenic viruses (3, 4). While certain derivatives of rifampicin can inhibit the RNA-dependent DNA polymerase activity of oncogenic viruses, and a similar activity found in normal and virus-transformed cells (5–7), rifampicin itself, when tested in vitro, has no effect on the mammalian DNA-dependent RNA polymerases (1). Nevertheless, toxicity of the drug for some cell lines has been reported (8). Recently, rifampicin has been shown to suppress the humoral and cellular immune response in vivo and in vitro (9) and to inhibit thymidine incorporation by human lymphocytes after stimulation by phytohemagglutinin (PHA) and tuberculin (10).

One of the earliest known events in lymphocyte transformation by PHA is the stimulation of RNA synthesis (11, 12) due to the increase in the activity of RNA polymerases (13). Among the three RNA polymerases examined, RNA polymerase I is stimulated within 1 hr and RNA polymerase II after 1 hr of PHA treatment (13). In this communication, I wish to present results correlating a block in lymphocyte transformation with synthetic events that follow soon after PHA stimulation.

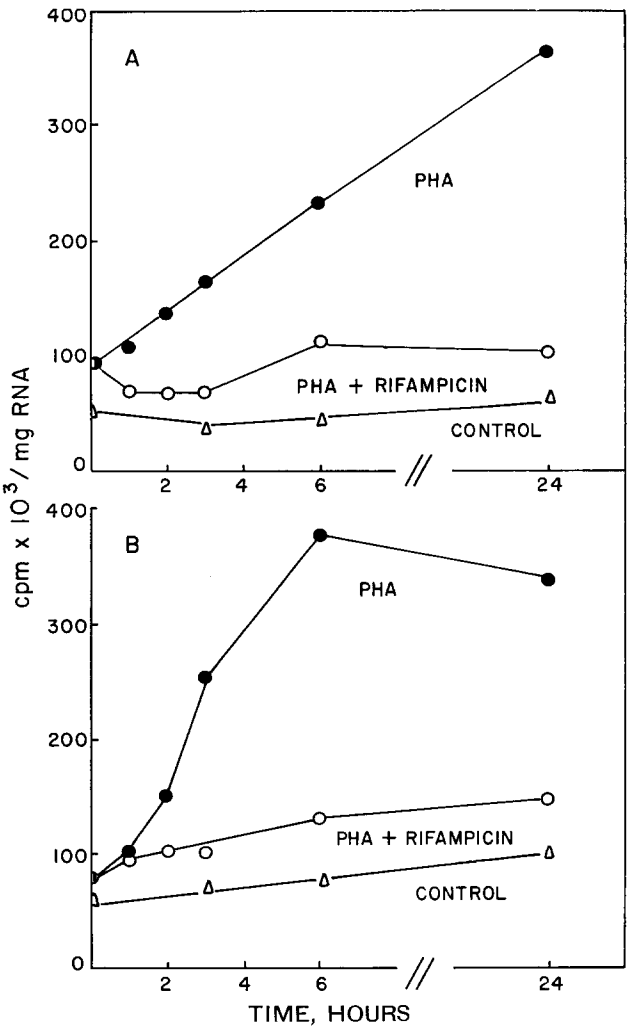

FIGURE 1 Effect of rifampicin on uridine incorporation into RNA by PHA-stimulated lymphocytes. Samples containing 5×10^6 cells/ml were pretreated with PHA for 1 hr before 100 μg/ml of rifampicin were added (0 time in the abscissa). At the times indicated, the cells were incubated with 4 μCi/ml of uridine-^{3}H (sp act 20 Ci/mmole) for 5 and 30 min and then processed for RNA determination and counting. Fig. 1 A represents the incorporation of uridine-^{3}H into RNA after 5 min, and Fig. 1 B the incorporation after 30 min. ●—●, PHA-treated lymphocytes; ○—○, PHA-treated lymphocytes plus rifampicin; and △—△, unstimulated lymphocytes.

MATERIALS AND METHODS

Detailed descriptions of the nutrient media, preparation of human lymphocyte cultures from blood, and other experimental methods have been published (12). Purified PHA (Burroughs Welcome & Co., Inc., Tuckahoe, N. Y.) was added at the concentrations recommended by Milthrop and Forsdyke (14). Isolation of nuclear fractions and assays for the three respective RNA polymerases were detailed previously (13), as were the DNA determinations using Burton's method (15), RNA estimation by alkaline hydrolysis (16), and protein by the procedure of Lowry et al. (17).

RESULTS

Inhibition of PHA-Induced Transformation and DNA Synthesis by Rifampicin

To establish the effective concentration of rifampicin required to block transformation and

DNA synthesis, antibiotic concentrations of 10–200 μg/ml were applied to cell cultures containing 5×10^6 cells/ml. The proportion of transformed cells in the cultures was ascertained 5 days after addition of PHA, and the rate of DNA synthesis, measured as the incorporation of thymidine, after 72 hr. Addition of rifampicin at 100 μg/ml resulted in the presence of only 4% of transformed cells as compared with 70% in untreated companion cultures. Concentrations of 100 and 200 μg/ml of drug inhibited DNA synthesis by 70–90%. The standard dose chosen for all subsequent experiments was 100 μg/ml.

Effect of Rifampicin on RNA and Protein Synthesis

Rates of RNA synthesis were measured by uptake of uridine-^{3}H into RNA. According to Cooper (18), the RNA of lymphocytes, labeled during a 5 min pulse, sediments in a sucrose density gradient as polydisperse material of heterogeneous molecular weight. By comparison, the RNA labeled during a 30 min pulse sediments in the position of ribosomes or ribosomal precursors. Therefore, two intervals of incorporation were employed, of either 5 or 30 min duration. Where appropriate, rifampicin was added to the cells that had been pretreated for 1 hr with PHA. At the times indicated in Fig. 1, uridine-^{3}H was added to the cultures, and the incorporation of label into an RNA product was determined. The results with 5-min pulses are illustrated in Fig. 1 A, and with 30-min pulses in Fig. 1 B, from which it is apparent that within 1 hr after addition of rifampicin the drug inhibited synthesis of the rapidly labeled RNA fraction. The drug also inhibited synthesis of RNA formed during the 30-min pulses within 2 hr after its application. By comparison, 0.15 μg/ml of actinomycin D failed to suppress RNA synthesis related to the 5 min uridine-^{3}H pulse, but did inhibit by 75% the synthesis of RNA formed during the 30-min pulses.

The influence of rifampicin on the synthesis of protein, ascertained by incorporation of leucine-^{3}H into macromolecular form during 30-min intervals, was also monitored at various times after exposure of lymphocytes to PHA. The results, summarized in Fig. 2, showed that the drug had either minimal or no effect on protein synthesis during the first 3 hr after induction. Exposure to rifampicin for longer periods resulted in a decline, until the level

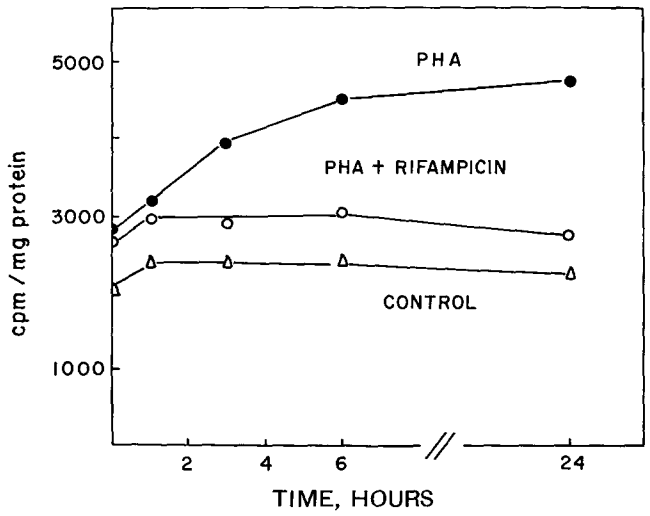

FIGURE 2 Effect of rifampicin on amino acid incorporation into proteins by PHA-stimulated lymphocytes. Samples containing 5×10^6 cells/ml were pretreated with PHA for 1 hr before 100 μg/ml of rifampicin were added (0 time in the abscissa). At the times indicated, the cells were transferred to a leucine-less medium containing 2% fetal calf serum and 1 μCi/ml of L-leucine-4,5-^{3}H (sp act 36 Ci/mmole) and incubated for 30 min. The cells were washed with phosphate-buffered saline, precipitated with 0.5 N perchloric acid (PCA) in the cold, washed with 0.5 N PCA, 95% alcohol, and alcohol ether (3:1). The precipitates were resuspended in 0.1 N NaOH for protein determination and scintillation counting.

of synthesis was suppressed to that observed with unstimulated cells.

Effect of Rifampicin on the Nuclear DNA-Dependent RNA Polymerases

The activity of the DNA-dependent RNA polymerases was assayed in nuclear fractions. Nuclei were isolated from cells that had been exposed for varying time intervals to PHA plus rifampicin. The data, summarized in Table I,

TABLE I

RNA Polymerase Activities in Isolated Nuclei from PHA-Treated Cells Incubated with or without Rifampicin and Control Cells (nmoles UMP-^{3}H/mg DNA per 10 min)

Time after addition of rifampicin	Conditions of incubation	PHA-treated cells	PHA-treated cells rifampicin	Control cells
hr				
1	Mg^{++}	2.10	2.12	1.60
	Mn^{++}	4.20	2.20	2.00
3	Mg^{++}	4.00	3.20	1.60
	Mn^{++}	5.40	3.30	2.30
6	Mg^{++}	6.50	3.80	1.90
	Mn^{++}	6.80	2.00	1.80
24	Mg^{++}	6.20	3.90	2.20
	Mn^{++}	6.00	2.00	2.50

Experimental conditions similar to those for Figs. 1 and 2. At times indicated, 5×10^6/ml cells were washed with phosphate-buffered saline, and then resuspended in 1 ml of 0.01 M Tris-HCl buffer (Schwarz/Mann, Orangeburg, N. Y.), pH 7.8, with 1 mM $MgCl_2$ (or $MnCl_2$) and 10 mM KCl. Swelling of the cells was allowed to proceed for 10 min at 4°C. Triton × 100 was then added at a final concentration of 0.5%, and the cells were disrupted by 10 strokes of a Dounce homogenizer and centrifuged at 800 *g* for 3 min. The pellet was washed with the same solution plus 0.1% Na deoxycholate. The nuclear fraction was then resuspended in 0.25 ml of a mixture containing 0.3 M sucrose in 0.01 M Tris-HCl buffer, pH 8.0, 4 mM $MgCl_2$ (or 1.8 mM $MnCl_2$), 0.06 M NaCl, 30 mM 2-β-mercaptoethanol, 0.1 μmole of adenosine triphosphate (ATP), cytidine triphosphate (CTP), guanosine triphosphate (GTP), and 0.03 μmole of ^{3}H-labeled uridine triphosphate (UTP) (sp act 20 μCi/μmole). The reaction was allowed to proceed for 10 min at 37°C and then stopped by adding 5 ml of 10% trichloroacetic acid (TCA) with 0.05 M sodium pyrophosphate. The precipitates were collected in Millipore filters, washed twice with 10% TCA, and the radioactivity was determined.

showed that in the absence of rifampicin there occurred the expected increase of RNA polymerase activity due to PHA induction (13). Addition of the antibiotic inhibited the Mn^{++}-dependent enzyme activity (polymerase II) by 40% after only 1 hr and by 74% after 24 hr of treatment. The Mg^{++}-dependent activity (polymerase I) was reduced less, and the effect of rifampicin did not become evident during the initial 3 hr of treatment.

DISCUSSION

The data presented indicate that rifampicin inhibits PHA-induced lymphocyte transformation and interferes with RNA synthesis. Synthesis by PHA-treated lymphocytes of rapidly labeled RNA, made during 5-min pulses, is profoundly depressed soon after exposure to rifampicin; but synthesis of the ribosomal type of RNA, formed during the longer 30 min period of labeling, is suppressed later. The inhibition of the latter type of RNA by actinomycin D relates it to transcription of ribosomal genes that are GC rich and particularly sensitive to actinomycin D. The observed reduction in protein synthesis after 3 hr of rifampicin treatment is consistent with the idea that inhibition of the requisite PHA transcription precedes a reduction in translation.

Inhibition of PHA-stimulated RNA synthesis in lymphocytes indicates that the primary site of rifampicin action, by analogy with bacterial and certain animal viruses (1, 2, 19–22), is on the induced RNA polymerases, particularly polymerase II. However, when assayed in vitro, the mammalian enzymes are unaffected below a concentration of 200 μg (1). The discrepancy between the in vitro and in vivo effects may result from (*a*) an intracellular structural modification of rifampicin, making it more active: this is the case of manmade chemical modifications of the antibiotic which result in very potent inhibitors of in vitro transcription, for example, the compound PR19 (3′-acetyl-1′-benzyl-2′-methylpyrrole [3,2-C]-4-desoxyrifamycin SV) (W. Keller, personal communication); or (*b*) altered configurational relationship between the complex of template-enzyme plus some factor(s) required for the initiation of transcription; they may be appropriate for in vivo drug action, but are disturbed after cell disruption. It is worth mentioning that induction of RNA polymerase activities by PHA in lymphocytes does not require protein synthesis, indicating that the suppression of induced polymerase activities by rifampicin is not the result of

synthesis of proteins involved in the regulation of the enzyme (13).

The 1 hr interval required for the onset of anti biotic action may be related to the time required to establish a critical intracellular concentration of the drug. It can be shown that uptake of ^{14}C-labeled rifampicin by PHA-treated lymphocytes occurs at a linear rate for about 2 hr and then ceases (B. G. T. Pogo, unpublished results). The effect of rifampicin may be related to its capacity to enter the cell since incubation with PHA enhances the permeability of lymphocytes to a variety of macromolecular precursors (23), and also elevates threefold the uptake of rifampicin-^{14}C.

Recent reports have claimed that derivatives of rifampicin inhibit an RNA-dependent DNA polymerase of lymphocytes (5). Such an activity is detected about 24 hr after exposure of cells to PHA (24), i.e., at a much later time than the initial stimulation of both RNA synthesis and enzyme induction. There appears to be no direct relationship between the action of rifampicin observed here and inhibition of reverse transcriptase reported by others.

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