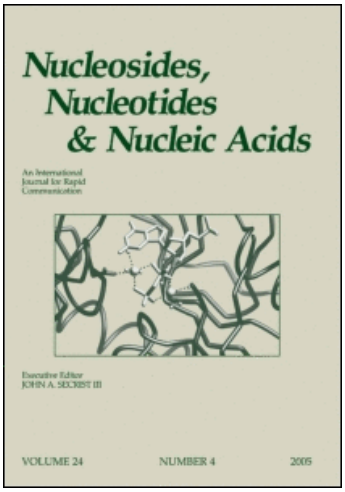

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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Online publication date: 31 March 2001

To cite this Article Eisenberg, Eugene J. , He, Gong-Xin and Lee, William A.(2001) 'METABOLISM OF GS-7340, A NOVEL PHENYL MONOPHOSPHORAMIDATE INTRACELLULAR PRODRUG OF PMPA, IN BLOOD', Nucleosides, Nucleotides and Nucleic Acids, 20: 4, 1091 — 1098
To link to this Article: DOI: 10.1081/NCN-100002496
URL: http://dx.doi.org/10.1081/NCN-100002496

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 20(4–7), 1091–1098 (2001)

METABOLISM OF GS-7340, A NOVEL PHENYL MONOPHOSPHORAMIDATE INTRACELLULAR PRODRUG OF PMPA, IN BLOOD

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ABSTRACT

PMPA, an acyclic nucleoside phosphonate analog, is a potent inhibitor of HIV. In the cells, PMPA is efficiently phosphorylated by intracellular kinases to produce PMPApp, the pharmacologically active metabolite. Despite its demonstrated antiviral potency, PMPA has limited cell permeability presumably resulting from the presence of two negative charges on the phosphonyl group. To enhance intracellular concentrations of PMPA, we developed a prodrug, selectively metabolized inside cells. GS-7340 (9-[(R)-2-[[[[(S)-1-(isopropoxycarbonyl)ethyl] amino] phenoxy-phosphinyl]-methoxy] propyl] adenine) is a prodrug which is orally bioavailable in dogs as the intact prodrug and has demonstrated anti-HIV activity in cell culture of over 1000-fold greater than that of PMPA. The metabolism of PMPA in peripheral blood mononuclear cells (PBMC), red blood cells (RBC) and plasma was examined following exposure of whole blood to PMPA or GS-7340 at concentrations similar to ones observed systemically following oral administration in dogs. Following 1 hour incubation with whole blood, GS-7340 was stable in plasma, produced high levels of PMPA and its phosphorylated metabolites in PBMC but not in RBC. No intact prodrug was present in PBMC. The only other species present in PBMC was monoalaninyl PMPA. The levels of PMPA and the phosphorylated metabolites were over 20 times greater than those after incubation with PMPA. The dog and human blood data were similar. The intracellular levels of PMPA and PMPApp were roughly proportional to GS-7340 over a 10-fold concentration range indicating a lack of saturability of uptake and phosphorylation. Since PMPApp is the species responsible for antiviral activity of PMPA, the high intracellular levels of PMPApp should be an important indicator of greater clinical efficacy of GS-7340.

SUMMARY

GS-7340 (9-[(R)-2-[[[[(S)-1-(isopropoxycarbonyl) ethyl] amino] phenoxy-phosphinyl]-methoxy] propyl] adenine) is a prodrug of PMPA which is orally bioavailable in dogs as the intact prodrug and has demonstrated anti-HIV activity in cell culture of over 1000-fold greater than that of PMPA. The purpose of the study was to examine distribution of PMPA and its metabolites into peripheral blood mononuclear cells (PBMCs), red blood cells (RBCs) and plasma following exposure of human or dog whole blood to GS-7340. The results were compared with those after the exposure to PMPA or bis-POC PMPA (tenofovir disoproxil), an oral prodrug of PMPA, currently in Phase III studies for the treatment of HIV. GS-7340 was stable in dog and human blood and produced the highest levels of PMPA and its phosphorylated metabolites (PMPAp and PMPApp) in PBMCs. No PMPA or PMPApp were observed after incubation of blood with PMPA or GS-7340. The PBMC levels of PMPA and the phosphorylated metabolites were over 10 and 30 times greater after incubation with GS-7340 than after incubation with bis-POC PMPA and PMPA, respectively. The dog and human blood data were essentially similar.

INTRODUCTION

PMPA is a potent inhibitor of both hepadnaviruses and retroviruses, including the human immunodeficiency virus (1). Inside the cells, PMPA is converted by

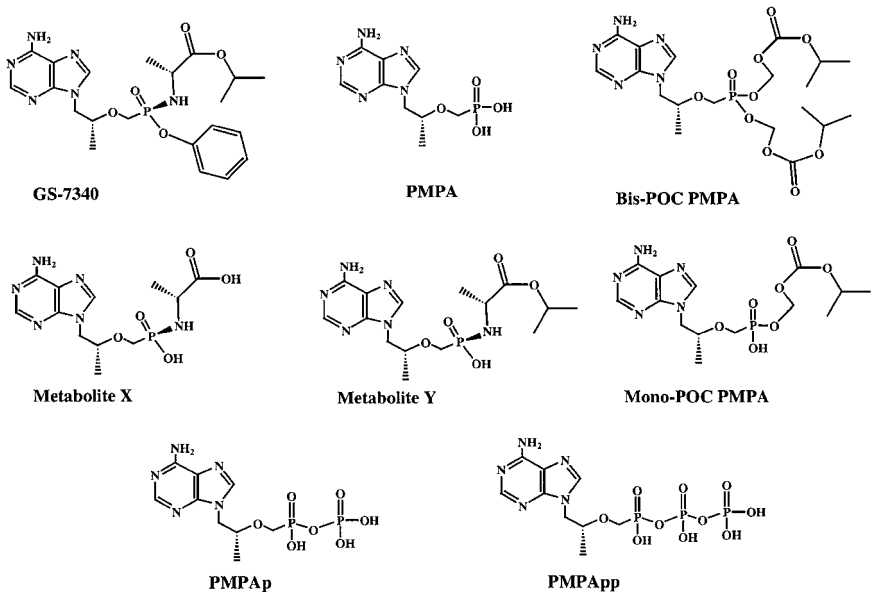

Figure 1. Structures of GS-7340 and its Intra- and Extracellular Metabolites.

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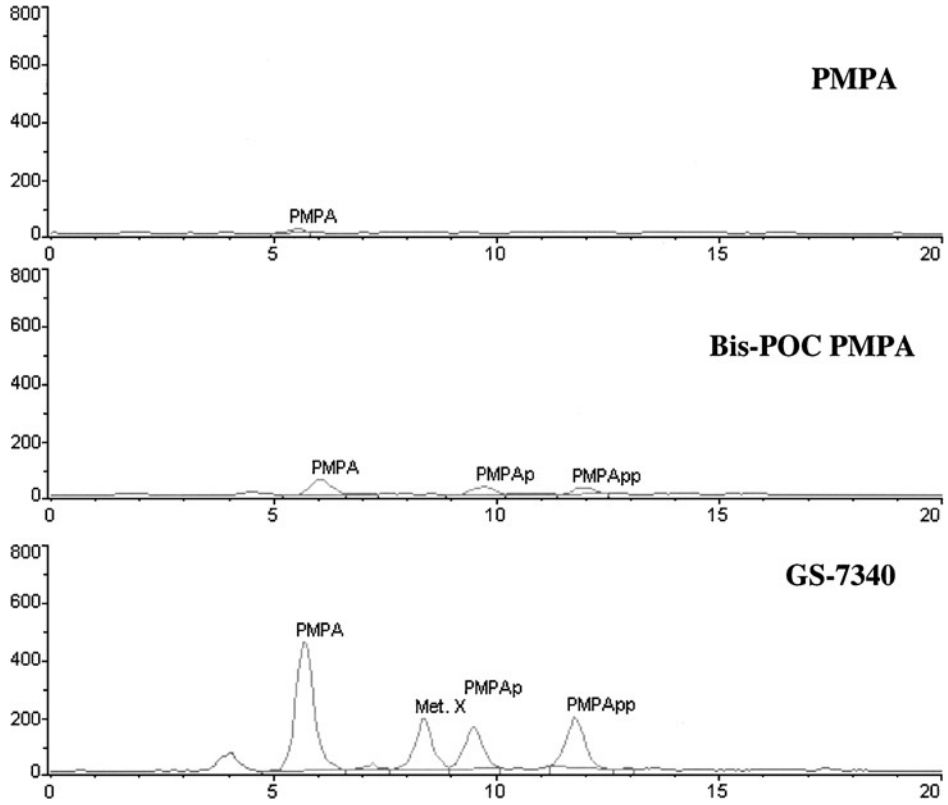

Figure 2. HPLC/C-14 Traces of PBMC Extracts from Human Blood Incubated for 1 hr at 37°C with GS-7340, Bis-POC PMPA or PMPA.

intracellular kinases to its phosphorylated metabolites. Diphosphorylated PMPA (PMPApp) is the species responsible for antiviral activity of PMPA. Despite its demonstrated antiviral potency, it has limited oral bioavailability in animals, resulting from the presence of two negative charges on the phosphonyl group. Isopropyl methyl carbonate ester group has been introduced to the molecule to mask the charges. The prodrug (bis-POC PMPA, tenofovir disoproxil) is currently in phase III clinical trials for the treatment of HIV. Preliminary results have shown that it is safe and well tolerated and caused a 1.1 log reduction of HIV RNA levels after only eight doses (2). It has been demonstrated *in vitro* that bis-POC PMPA provides greater intracellular levels of PMPA and PMPApp than the parent drug (3). However, the prodrug is highly susceptible to hepatic and blood esterases which limits its persistence in plasma and ability to interact directly with target cells.

We have sought to overcome this limitation by the development of a prodrug which is stable in blood but is selectively hydrolyzed in target cells to produce PMPA. A series of novel phosphoramidate prodrugs of PMPA were synthesized. GS-7340 (phenyl monoester isopropyl alaninyl phosphoramidate of PMPA) was

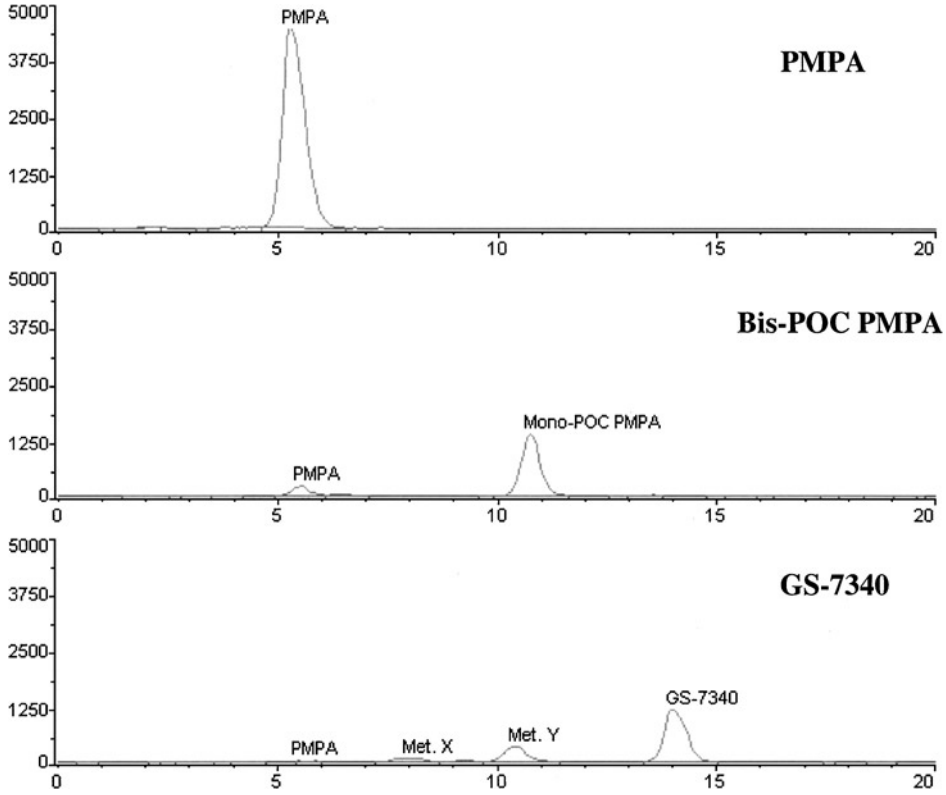

Figure 3. HPLC/C-14 Traces of Plasma Extracts from Human Blood Incubated for 1 hr at 37°C with GS-7340, Bis-POC PMPA or PMPA.

selected for further development. GS-7340 demonstrated potent anti-HIV activity in cell culture, was stable in blood and persisted in plasma up to 2 hr after oral administration in dogs. The purpose of the present study was to compare the relative distribution of the prodrug and its metabolites in plasma, RBCs and PBMCs after incubation with whole dog or human blood under the conditions similar to those *in vivo* after oral administration of the corresponding prodrug or the parent compound.

METHODS

Human or dog whole blood was incubated for 1 hr at 37°C with each of the 3 radiolabelled compounds: GS-7340, bis-POC PMPA and PMPA at concentration of 5 μg-equivalent PMPA per mL. The whole blood was subjected to treatment with the Ficoll-Paque sodium diatriozate solution. The treatment resulted in formation of layers containing plasma, PBMCs and red blood cells (RBCs) aggregated by Ficoll-Paque. Aliquots of the plasma PBMC and RBC layers (0.5 mL) were extracted

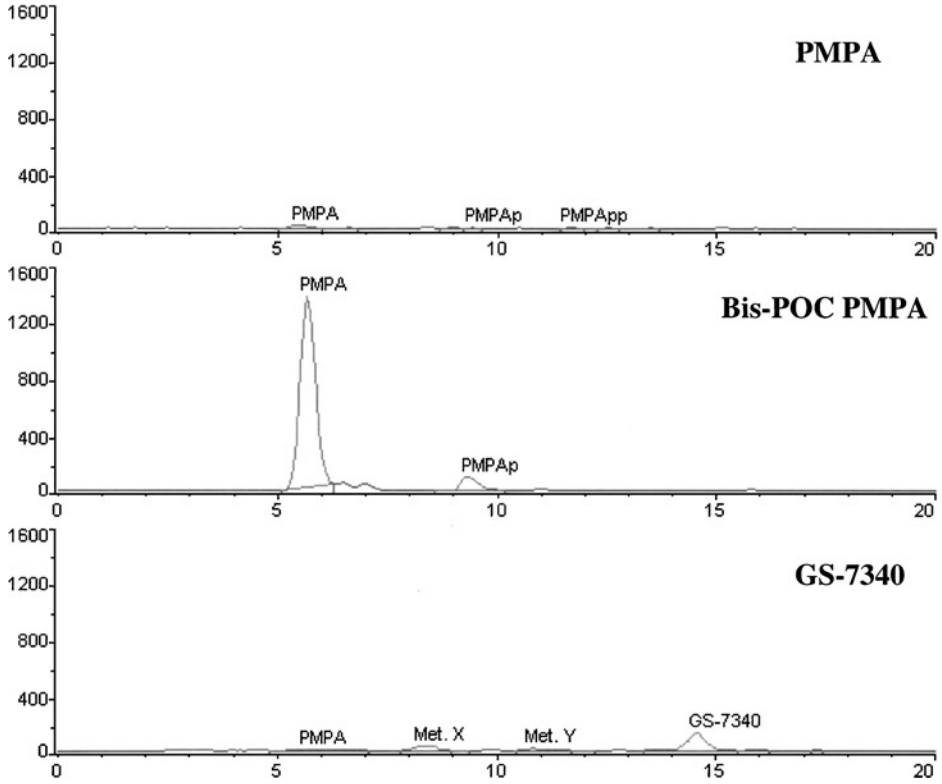

Figure 4. HPLC/C-14 Traces of Aggregated RBC Extracts from Human Blood Incubated for 1 Hr At 37°C with GS-7340, Bis-POC PMPA or PMPA.

with methanol. Radioactivity in all layers was measured by oxidation/scintillation counting and by comparing with radioactivity from the standard solutions. All extracts were reconstituted in water and analyzed using HPLC with radiometric flow detection.

HPLC CONDITIONS

HPLC System comprised a Model P4000 solvent delivery system with a Model AS3000 autoinjector (ThermoQuest, San Jose, CA). An analytical column was Inertsil ODS-2 (5 μm, 150 $\times$ 4.6 mm) (Phenomenex) maintained at 40°C. Mobile phases were A, 5% AcCN in 25 mM phosphate buffer with 5 mM tetrabutylammonium Br, pH 6 and B, 60% AcCN in 25 mM phosphate buffer with 5 mM tetrabutylammonium Br, pH 6.Gradient profile was 0–100% B over 20 minutes at 1.2 mL/min. For detection a Radiomatic FLO-ONE/Beta liquid scintillation detector was utilized (Packard Series A-500), with FLO-SCINT A (Packard) scintillation fluid at 1:2.5 HPLC eluant/fluid mixing ratio.

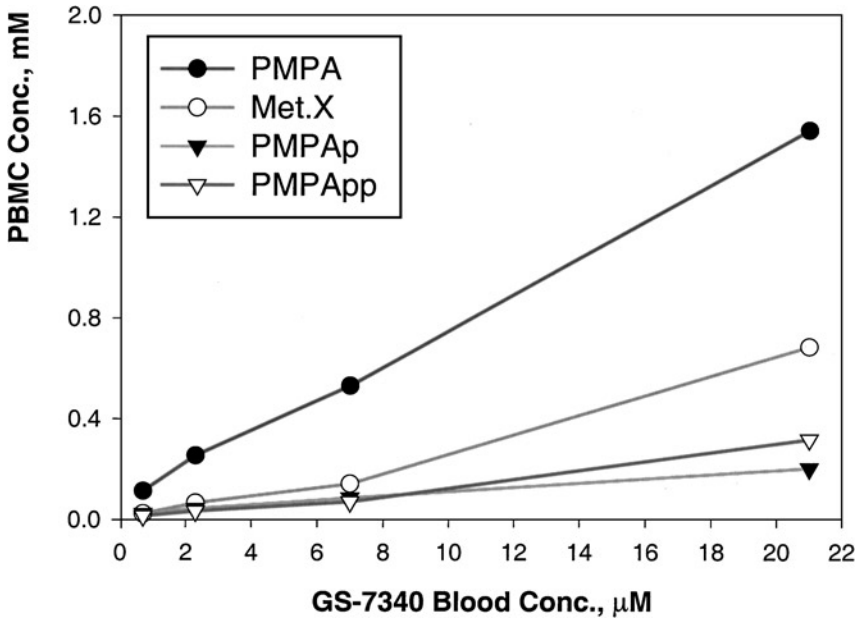

Figure 5. Concentrations of GS-7340 Metabolites in PBMC After 1 hr Incubation of Dog Blood with GS-7340 at 37°C.

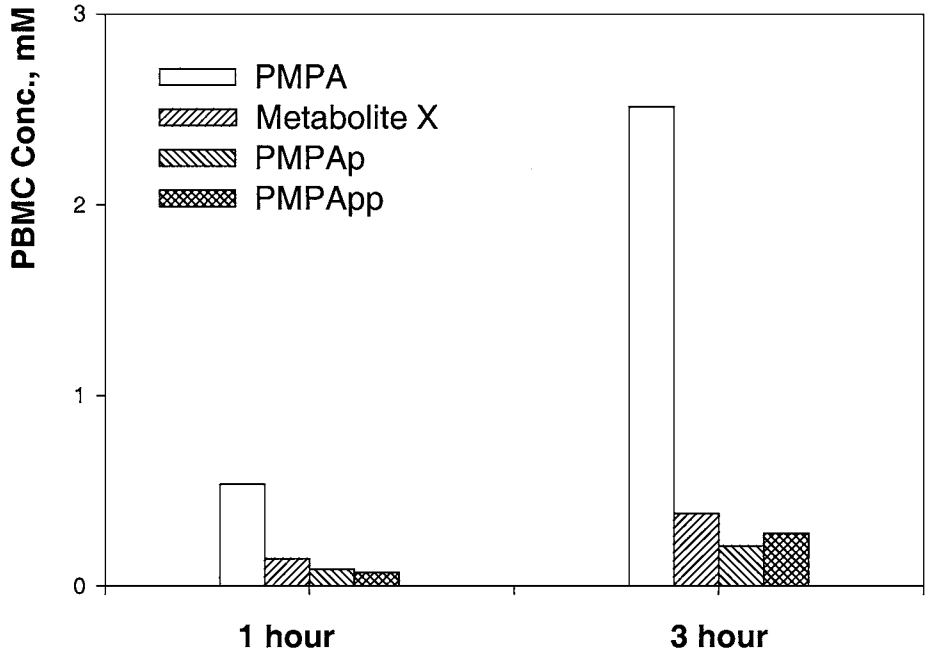

Figure 6. Concentrations of GS-7340 Metabolites in PBMC After Incubation of Dog Blood with 7 μM GS-7340 at 37°C for 3 hr.

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RESULTS

Table 1. PMPA Metabolites in Plasma, PBMCs and RBCs After 1 hr Incubation of 60 μg-eq GS-7340, Bis-POC PMPA or PMPA in 12 mL of Dog Blood

			Metabolites (μg-eq PMPA)						
Compound	Matrix	Total C-14 Recovered μg-eq PMPA	PMPA	PMPAp	PMPApp	Met. X	Met. Y	Mono-POC PMPA	Intact Prodrug
	Plasma	54.3	2.17	<LOD	<LOD	6.52	9.23		36.4
GS-7340	PBMC	1.97	0.93	0.14	0.51	0.39	<LOD		<LOD
	RBC	5.12	<LOD	<LOD	<LOD	<LOD	<LOD		5.12
	Plasma	54.2	21.7	<LOD	<LOD			32.5	<LOD
Bis-POC	PBMC	0.14	0.07	0.03	0.03			<LOD	<LOD
PMPA	RBC	12.2	9.76	1.10	<LOD			<LOD	<LOD
	Plasma	58.3	58.3	<LOD	<LOD				
PMPA	PBMC	0.04	0.01	0.01	<LOD				
	RBC	1.46	1.46	<LOD	<LOD				

CONCLUSIONS

GS-7340 was stable in whole blood and RBCs producing only about small quantities of metabolites Y and X. No intact GS-7340 or metabolite Y was detected in PBMC extracts. Only PMPA, its phosphorylated metabolites and metabolite X were present in the PBMC extracts. The PBMC levels of PMPA and the phosphorylated metabolites after incubation with GS-7340 were about 10-fold and 30-fold greater than those after incubation with bis-POC PMPA and PMPA, respectively.

Table 2. PMPA Metabolites in Plasma, PBMCs and RBCs After 1 hr Incubation of 60 μg-eq GS-7340, Bis-POC PMPA or PMPA in 12 mL of Human Blood

			Metabolites (μg-eq PMPA)						
Compound	Matrix	Total C-14 Recovered, μg-eq PMPA	PMPA	PMPAp	PMPApp	Met. X	Met. Y	Mono-POC PMPA	Intact Prodrug
	Plasma	43.0	0.43	<LOD	<LOD	0.860	5.59		36.12
GS-7340	PBMC	1.25	0.56	0.200	0.263	0.225	<LOD		<LOD
	RBC	12.6	1.01	<LOD	<LOD	3.02	1.39		7.18
	Plasma	48.1	5.29	<LOD	<LOD			42.8	<LOD
Bis-POC	PBMC	0.13	0.07	0.033	0.023			0.009	<LOD
PMPA	RBC	10.5	9.77	0.735	<LOD			<LOD	<LOD
	Plasma	55.7	55.70	<LOD	<LOD				
PMPA	PBMC	0.033	0.03	0.005	<LOD				
	RBC	3.72	2.75	0.372	0.595				

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The greater levels of PMPA and its phosphorylated metabolites in PBMCs after incubation with GS-7340 in comparison to PMPA suggest selective intracellular enzymatic activity and a lack of saturability of phosphorylation of PMPA. Proportional increases in PBMC levels of GS-7340 metabolites following incubation of whole dog blood with increasing levels of GS-7340 suggest a lack of saturability of GS-7340 uptake, hydrolysis and phosphorylation of PMPA.

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