ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1995, p. 2511–2515 Vol. 39, No. 11
0066-4804/95/$04.00+0

Methionine Recycling Pathways and Antimalarial Drug Design

JANICE R. SUFRIN,[1]* STEVEN R. MESHNICK,[2] ARTHUR J. SPIESS,[1] JOANNE GAROFALO-HANNAN,[3] XING-QING PAN,[2] AND CYRUS J. BACCHI[3]

Grace Cancer Drug Center, Roswell Park Cancer Institute, Buffalo, New York 14263[1]; Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, Michigan 48109[2]; and Haskins Laboratories and Department of Biology, Pace University, New York, New York 10038[3]

Received 1 June 1995/Returned for modification 20 July 1995/Accepted 14 September 1995

5′-Deoxy-5′-(methylthio)adenosine (MTA) is an *S*-adenosylmethionine metabolite that is generated as a by-product of polyamine biosynthesis. In mammalian cells, MTA undergoes a phosphorolytic cleavage catalyzed by MTA phosphorylase to produce adenine and 5-deoxy-5-(methylthio)ribose-1-phosphate (MTRP). Adenine is utilized in purine salvage pathways, and MTRP is subsequently recycled to methionine. Whereas some microorganisms metabolize MTA to MTRP via MTA phosphorylase, others metabolize MTA to MTRP in two steps via initial cleavage by MTA nucleosidase to adenine and 5-deoxy-5-(methylthio)ribose (MTR) followed by conversion of MTR to MTRP by MTR kinase. In order to assess the extent to which these pathways may be operative in *Plasmodium falciparum*, we have examined a series of 5′-alkyl-substituted analogs of MTA and the related MTR analogs and compared their abilities to inhibit in vitro growth of this malarial parasite. The MTR analogs 5-deoxy-5-(ethylthio)ribose and 5-deoxy-5-(hydroxyethylthio)ribose were inactive at concentrations up to 1 mM, and 5-deoxy-5-(monofluoroethylthio)ribose was weakly active (50% inhibitory concentration = 700 μM). In comparison, the MTA analogs, 5′-deoxy-5′-(ethylthio)adenosine, 5′-deoxy-5′-(hydroxyethylthio) adenosine (HETA), and 5′-deoxy-5′-(monofluoroethylthio)adenosine, had 50% inhibitory concentrations of 80, 46, and 61 μM, respectively. Extracts of *P. falciparum* were found to have substantial MTA phosphorylase activity. Coadministration of MTA with HETA partially protected the parasites against the growth-inhibitory effects of HETA. Results of this study indicate that *P. falciparum* has an active MTA phosphorylase that can be targeted by analogs of MTA.

Inhibitors of polyamine metabolism have emerged recently as promising agents for the chemotherapy of cancer (16) and of parasitic diseases (1). Polyamine biosynthetic enzymes which serve as drug targets include ornithine decarboxylase, *S*-adenosylmethionine decarboxylase, spermidine synthase, and spermine synthase. In addition, enzymes which degrade the polyamine biosynthesis by-product, 5′-deoxy-5′-(methylthio)adenosine (MTA), are potentially exploitable for chemotherapy, since MTA metabolism in microorganisms (8, 13, 18) and tumor cells (20) differs in significant ways from MTA metabolism in normal mammalian cells.

In mammalian cells, MTA is rapidly cleaved by a highly specific MTA phosphorylase to yield adenine and 5-deoxy-5-(methylthio)ribose-1-phosphate (MTRP) (Fig. 1). Adenine is then salvaged, and MTRP is converted into methionine. MTA phosphorylase is absent in many solid tumors and leukemias (6). It is also absent in microorganisms such as *Giardia lamblia* (17) and *Klebsiella pneumoniae* (14, 15), which utilize an alternative pathway not present in mammalian cells for metabolism of MTA to MTRP. In this alternate pathway, MTA is cleaved to adenine and 5-deoxy-5-(methylthio)ribose (MTR) by MTA nucleosidase. MTR, once formed, is phosphorylated to MTRP by MTR kinase (Fig. 1). It has previously been reported that *Plasmodium falciparum* utilizes the MTA nucleosidase-MTR kinase pathway and, as a result, is sensitive to inhibition by MTR analogs which are presumed to be phosphorylated by the kinase to MTRP analogs (17, 18). In contrast, trypanosomes contain MTA phosphorylase, but this enzyme differs from the mammalian enzyme in that it has less stringent requirements for substrate activity (8).

* Corresponding author. Phone: (716) 845-4582. Electronic mail address: ROSUFRIN@UBVMS.cc.buffalo.edu.

We recently synthesized a series of 5′-alkyl-substituted analogs of MTA (19) and found that they were excellent substrates for MTA phosphorylase in trypanosomes and had significant trypanocidal activity in vitro (2). The most active of these analogs, 5′-deoxy-5′-(hydroxyethylthio)adenosine (HETA), was curative in mice infected with *Trypanosoma brucei brucei*. As predicted, the trypanocidal activity of HETA was reversed by exogenous methionine.

In this paper, we have compared the antimalarial activities of a series of MTR and MTA analogs (shown in Fig. 2) as a means to determine the relative importance of the MTA phosphorylase pathway, which was previously reported to be absent in plasmodia (7, 17, 18).

MATERIALS AND METHODS

Parasites. *P. falciparum* was cultured by the method of Trager and Jensen (22). The FCR-3 strain was used for all experiments. For enzyme measurements, parasites were isolated from infected erythrocytes by saponin lysis (5) and stored at −70°C.

Measurement of antimalarial activity in vitro. Infected erythrocytes and various concentrations of drugs (5, 10, 20, 50, 75, and 125 μM) were aliquoted in triplicate wells of a 96-well microtiter plate (Corning). Each well contained a total volume of 200 μl with a final hematocrit of 1.5% and parasitemia of 0.5 to 1%. The microtiter plates were incubated at 37°C in candle jars. Drug effects on parasite viability were next analyzed by one or both of the following methods. The effects of MTR analogs were determined by method 1; the effects of MTA analogs were determined by method 1 and by method 2. Fifty percent inhibitory concentrations (IC_{50}s) represent the averages of triplicate determinations and are within 10% of the standard error of the mean.

Method 1. [^{3}H]hypoxanthine incorporation was measured by a modification of the procedure of Desjardins et al. (4). [^{3}H]hypoxanthine (0.5 μCi) was added to each well at 24 h following treatment with various concentrations of drug (5, 10, 20, 50, 75, and 125 μM). After an additional 24 h of incubation, the parasites were harvested with a PHD cell harvester (Cambridge Technologies). Filters were immersed in Aquasol (New England Nuclear), and radioactivity was counted with an LKB rack beta scintillation counter.

Method 2. Lactate dehydrogenase (LDH) activity was measured by the method of Makler et al. (12). Reagents for the assay were supplied in kit form by

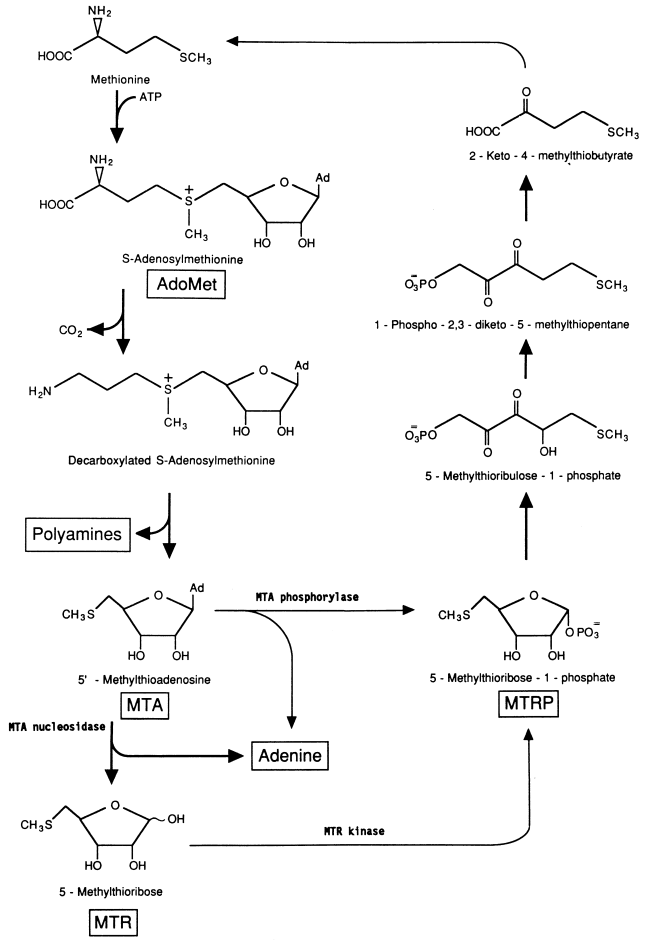
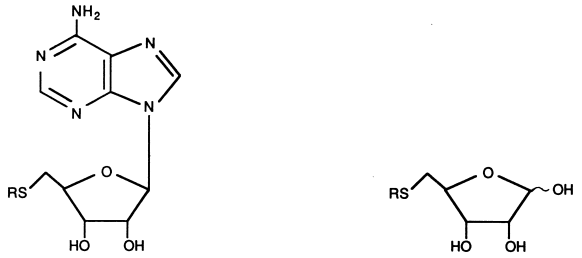


FIG. 1. Pathways of methionine recycling from MTA.



Compound	R	Compound
MTA	CH_3	MTR
ETA	CH_3CH_2	ETR
MFETA	FCH_2CH_2	MFETR
HETA	$HOCH_2CH_2$	HETR

FIG. 2. Analogs of MTA and MTR.

Flow Incorporated (Portland, Oreg.). At 36 h after the addition of drug at various concentrations (5, 10, 20, 50, 75, 125, 250, 500, and 1,000 μM), 20 μl of erythrocyte suspension was withdrawn from each culture well, mixed with 100 μl of Malstat (from the assay kit) in a 96-well enzyme-linked immunosorbent assay plate, and covered with aluminum foil. Then 20 μl of an aqueous mixture (1:1 [vol/vol]) of nitroblue tetrazolium (2 mg/ml) and phenazine ethosulfate (0.1 mg/ml) was added to each well, and the plate was kept at room temperature for 20 min. A 100-μl aliquot of 5% acetic acid was then added to each well to stop color development. The optical density in each well was read at 630 nm in an MR 600 Microplate Reader (Dynatech Laboratories). Optical density measurements were used to construct curves from which IC_{50}s were determined.

Effect of MTA on the antimalarial activity of HETA. Infected erythrocytes were treated with various concentrations of HETA in the presence or absence of a fixed concentration of MTA (10 μg/ml) as described above. This was the highest concentration of MTA that did not inhibit parasite growth. The effects of HETA, with and without MTA, were determined by method 2.

Enzyme preparation and assays. *P. falciparum* was grown as described above, and parasitized cells were isolated as described by Fairfield et al. (5). Cell pellets (1 mg) were frozen and thawed three times in breakage buffer (0.05 M Tris-HCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol [pH 7.4]), cleared at 3,000 × *g* for 10 min, and dialyzed twice against two changes of breakage buffer (1 h). Enzyme assays were done as described by Bacchi et al. (2).

MTA analogs. MTA, 5′-deoxy-5′-(ethylthio)adenosine (ETA), and HETA were synthesized according to the method of Kikugawa et al. (10). 5′-Deoxy-5′-(monofluoroethylthio)adenosine (MFETA) was prepared as described by us previously (19). The stability of MFETA and HETA has been demonstrated under conditions which are relevant to in vitro and in vivo biological assays (19).

MTR analogs. 5-Deoxy-5-(ethylthio)ribose (ETR) was synthesized according to the method of Kuhn and Jahn (11). 5-Deoxy-5-(hydroxyethylthio)ribose (HETR) was synthesized by our published procedure (2). The preparation of 5-deoxy-5-(monofluoroethylthio)ribose (MFETR) is described below.

MFETR. A solution of methyl 5-deoxy-5-hydroxyethylthio-2,3-*O*-isopropylideneribofuranoside (2) (2.0 g) in dry methylene chloride (50 ml) was cooled to −74°C. Diethylaminosulfur trifluoride (3.0 ml) was injected under argon, and after 2.5 h, the reaction mixture was cautiously treated with saturated sodium bicarbonate solution and then extracted with methylene chloride. The organic extract was washed with saturated aqueous NaCl, dried over $MgSO_4$, filtered, and evaporated under vacuum to give 1.9 g of crude product. This material was dissolved in a minimum amount of diethyl ether and applied to a silica gel column which was eluted with diethyl ether-petroleum ether (1:1). Methyl 5-deoxy-5-monofluoroethylthio-2,3-*O*-isopropylidene-ribofuranoside (677 mg) was the first component to be eluted from the column: ^{19}F nuclear magnetic resonance ($CDCl_3$) δ −208.6 to −210.2 (triplet of triplets, centered at −209.4, $J = 48$ and 21 Hz). The protected riboside (215 mg) was dissolved in a solution containing 0.1 N sulfuric acid (2875 μl) and dioxane (1400 μl) and heated at 80°C for 2.75 h. The solution was cooled to room temperature, neutralized with solid barium hydroxide, filtered, and evaporated to dryness under vacuum. The 167-mg residue was dissolved in a minimum amount of methanol and applied to a Florisil column. The column was eluted with ethyl acetate, and the first component which emerged was the desired product (19 mg). MFETR: ^{19}F nuclear magnetic resonance (D_2O) δ −211 to −213.4 (multiplet). Thin-layer chromatography on silica gel (ethyl acetate–methanol [23:2], visualized with iodine) was performed to determine that no appreciable decomposition of MFETR occurred (i) upon long-term storage below freezing temperatures, (ii) in a solution of dimethyl sulfoxide kept at room temperature for several days, or (iii) in aqueous solution kept at 37°C for 24 h.

RESULTS

Three MTR analogs were tested for in vitro antimalarial activity (Fig. 3). Neither ETR nor HETR had any antimalarial

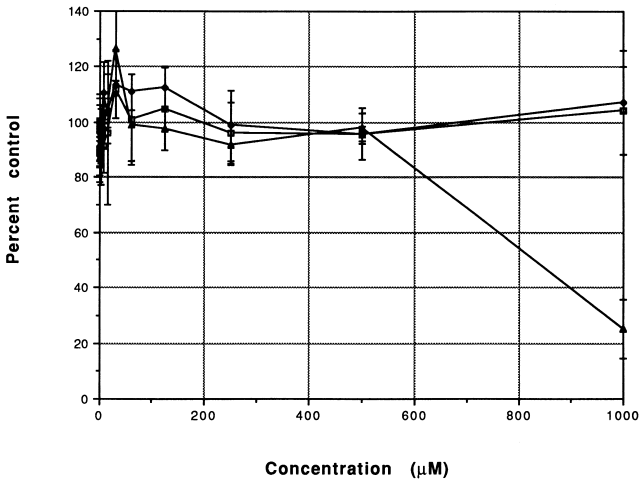


FIG. 3. Effects of MTR analogs on in vitro growth of *P. falciparum*. Growth at various concentrations of compounds is expressed as percentages of the growth observed in untreated control cultures. ⊡, ETR; ◆, HETR; ▲, MFETR.

TABLE 1. Susceptibility of *P. falciparum* FCR-3 to MTA analogs in vitro

Analog	IC_{50} (μM) as determined from[a]:	
	[^{3}H]hypoxanthine incorporation	LDH activity
MTA	ND[b]	80
ETA	30	80
HETA	22	46
MFETA	30	61

[a] Antiproliferative activity of MTA analogs was determined by two different methods. One assay measured [^{3}H]hypoxanthine incorporation, and the second assay measured LDH activity, under conditions described in the text. IC_{50}s (averages of triplicate determinations) are within 10% of the standard error of the mean and indicate the concentrations of analog that produced levels of [^{3}H]hypoxanthine incorporation or LDH activity equal to 50% of those for control cells in the respective assays.

[b] ND, not determined.

activity at concentrations up to 1 mM. MFETR was weakly active (IC_{50} = 700 μM). In comparison, the three MTA analogs, ETA, HETA, and MFETA, were more effective than their corresponding carbohydrate analogs (Table 1). Antiproliferative activity of these nucleosides was determined by two different methods. The assay which measures [^{3}H]hypoxanthine incorporation is a widely used method for determining drug activity. However, for the nucleoside analogs ETA, HETA, and MFETA, which are presumed to generate adenine by enzyme cleavage, it is possible that the observed inhibitory effects reflect competition for uptake between hypoxanthine and a nucleoside inhibitor or are simply a dilution effect on [^{3}H]hypoxanthine incorporation. For this reason, the growth-inhibitory effects of the MTA analogs were also determined by using LDH activity as a measure of their ability to affect proliferative activity. This assay for screening antimalarial activity has shown good correlation with the [^{3}H]hypoxanthine assay (12). When the LDH assay was used as a measure of the antiproliferative activity of these analogs, ETA, HETA, and MFETA were found to have IC_{50}s of 80, 46, and 61 μM, respectively (Table 1). These IC_{50}s were closely comparable to those obtained by using [^{3}H]hypoxanthine incorporation as a measure of viability (Table 1). The greater activity of the nucleosides ETA, HETA, and MFETA versus the corresponding MTR analogs, ETR, HETR, and MFETR, is consistent with the idea that these three MTA analogs are metabolized by MTA phosphorylase in *P. falciparum*. The slight differences in the IC_{50}s of the nucleoside analogs may reflect differential behavior by their corresponding 5-methylthioribose-1-phosphate analogs, presumed to form in situ.

The observation that each of the MTA analogs inhibited growth more effectively than its corresponding MTR analog suggested that the malarial parasites used in this study lack MTR kinase and preferentially utilize MTA phosphorylase to produce MTRP. Indeed, malarial extracts were found to have substantial MTA phosphorylase activity (37 nmol/mg of protein/30 min with 200 μM MTA as the substrate). This activity, as expected, was enhanced 175% (to 65 nmol/mg of protein/30 min) by added phosphate (50 mM PO_4). Interestingly, HETA (200 μM) was also a good substrate for this enzyme (activity = 34.5 nmol/mg of protein/30 min) but was cleaved at about 65% of the MTA rate. The K_m for MTA (plus PO_4) in this system was 27.4 μM, and that for HETA (plus PO_4) was 18.4 μM. Enzyme activity with HETA as the substrate was also increased (to 42.5 nmol/mg of protein/30 min) in the presence of phosphate (50 mM PO_4).

Since the parasites used in these studies were isolated by methods that retain small numbers of erythrocytes as contaminants, there exists the possibility that these blood cells, rather than the parasites, were the source of MTA phosphorylase activity. However, our previous studies (19) of HETA in cultured murine L5178Y cells suggest otherwise: HETA is not only poorly metabolized by mammalian MTA phosphorylase, but it is only modestly growth inhibitory, with an IC_{50} of 500 μM, in the presence of a full cellular complement of the mammalian enzyme. Thus, the significant antimalarial effects that we observed for HETA cannot reasonably be attributed to the contaminating presence of MTA phosphorylase from erythrocytes. We cannot rule out the possibility that the growth-inhibitory effects of ETA, HETA, and MFETA result from interactions of these nucleosides with a nucleosidase and a phosphorylase that are both derived from *P. falciparum*. This notion is supported by the data presented above which show significant MTA cleaving activity of *P. falciparum* extracts in the absence of added phosphate.

To examine the possibility that HETA was acting by interfering with recycling of methionine from MTA, we looked for possible competitive inhibition of its activity by MTA and determined that to a small but appreciable extent, MTA can protect the parasites from the effects of HETA (Fig. 4).

DISCUSSION

Polyamine pathway inhibitors exhibit considerable activity as antitumor and antiparasitic agents, but these effects are mediated by two distinct biochemical mechanisms. Polyamine inhibitors such as α-difluoromethylornithine exert their antitumor effects by lowering endogenous levels of cellular polyamines (16). In contrast, the antiparasitic effects of α-difluoromethylornithine and related inhibitors may be mediated primarily through interference with methionine and *S*-adenosylmethionine (AdoMet) metabolism (3, 25).

Methionine is an essential amino acid which is a constituent of proteins. In addition, it has a unique role in cellular growth and function, originating in its metabolic conversion to AdoMet and the subsequent participation of this key biochemical intermediate in polyamine biosynthesis and methylation reactions. The ultimate conservation of methionine is an integral part of AdoMet metabolism and underscores the importance of main-

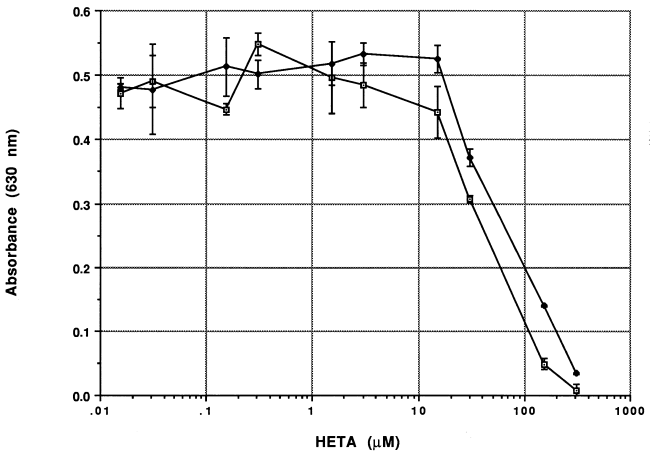


FIG. 4. Effects of HETA on in vitro growth of *P. falciparum* in the absence and presence of MTA. Growth at various concentrations of HETA in the absence of added MTA is compared with growth at various concentrations of HETA in the presence of a fixed concentration of MTA (10 μg/ml). Growth inhibition was confirmed by an LDH assay as described in the text. ⊡, HETA; ◆, HETA plus MTA.

taining adequate supplies of this amino acid for its diverse participation in physiological functions. Thus, when AdoMet is utilized in methyl transfer reactions, the product formed, *S*-adenosylhomocysteine, is further metabolized to homocysteine and adenosine. In mammalian cells, homocysteine can then be converted to methionine via enzymatic catalysis by betaine-homocysteine methyltransferase or 5-methyltetrahydrofolate-homocysteine methyltransferase. When AdoMet is utilized in polyamine biosynthesis, it is first converted to decarboxylated AdoMet, which subsequently donates its aminopropyl group to form spermidine and spermine and concomitantly the nucleoside by-product MTA. Methionine is then regenerated via the multistep metabolism of MTA (Fig. 1). It is of interest that maintenance of methionine recycling is essential for the viability of *P. falciparum*, despite the presence of methionine in both hemoglobin and parasite cultivation media (RPMI 1640). It may be that methionine is poorly transported into the parasite.

Riscoe et al. (18) highlighted methionine recycling from MTA in protozoa as a target for drug development based on the existence of the two-step metabolism of MTA to MTRP, unique to certain protozoa. However, their particular strategy excludes those protozoa which metabolize MTA via MTA phosphorylase. Indications that MTA phosphorylase may also be a selective therapeutic target for interfering with methionine recycling in parasites have come from observations of potentially exploitable differences in the substrate specificities of the mammalian and trypanosomal enzyme forms (8, 13). These indications have been substantiated in our recent studies of the MTA analog HETA, which is trypanocidal in vivo and yet nontoxic to the infected or cured animal (2). The apparent basis for this selectivity is the excellent activity of HETA as a substrate of trypanosomal MTA phosphorylase (2) and its poor activity against the mammalian enzyme (19).

The ability to exploit the pathways of methionine recycling from MTA as selective chemotherapeutic targets in protozoa will ultimately require a more extensive, systematic classification of microorganisms as MTA phosphorylase utilizing or MTA nucleosidase and MTR kinase utilizing, with respect to their metabolism of MTA. In this regard, it remains to be determined whether pathogens contain either MTA phosphorylase or MTA nucleosidase-MTR kinase activities, exclusive of each other, or whether both enzyme pathways to MTRP can be present but with substantially different degrees of activity. Whereas previous studies (7, 17, 18) have been supportive of the either/or classification, our present studies utilizing *P. falciparum* suggest otherwise. Thus, *P. falciparum* has been reported to contain significant MTA nucleosidase and MTR kinase activity but to be essentially devoid of MTA phosphorylase activity (7, 17, 18); consistent with the presence of MTA nucleosidase and MTR kinase, all four strains tested were sensitive to the effects of ETR, with IC_{50}s in the range of 100 to 200 μM. It was therefore entirely unexpected when we found that ETR and the related analogs MFETR and HETR were inactive in a concentration range of 100 to 500 μM against *P. falciparum* (strain FCR-3) (Fig. 3) and instead that the corresponding nucleoside analogs, ETA, MFETA, and HETA, were the more active species (Table 1). These results suggested that MTA phosphorylase was present in *P. falciparum*, and this conclusion was confirmed by an enzyme assay. Since ETR and the FCR-3 strain of *P. falciparum* were common components of both studies, this discrepancy between our results and those of Fitchen et al. (7) may reflect differences in culture conditions and/or parasite staging. It is noteworthy that Tower and coworkers (21) have found that the specific activity of MTR kinase changes in *K. pneumoniae* when the concentration of L-methionine is varied. Thus, it is possible that MTR kinase activity is similarly controlled in *P. falciparum* and that the growth-inhibitory activities of MTR and/or MTA analogs may be affected in part by the methionine concentration in the medium.

Trager et al. (23, 24) examined the antimalarial effects of the MTA analogs 5′-deoxy-5′-*S*-isobutyladenosine (SIBA) and 5′-deoxy-5′-*S*-isobutyl-3-deazaadenosine (deaza-SIBA). SIBA was not significantly inhibitory at concentrations below 300 μM in *P. falciparum* cultures (23), and deaza-SIBA was only slightly more active (24). These earlier studies, done when possible interactions of these analogs with enzymes of MTA metabolism were not considered important, did not encourage further pursuit of MTA analogs as antimalarial agents. More recently, Houston et al. (9) examined the effects of several fluorinated intermediates of the methionine salvage pathway on *P. falciparum* (strain FCB1) and, consistent with our results, found that growth-inhibitory activity was displayed by MTA analogs rather than MTR analogs.

Our results, together with those of three other studies (7, 9, 21), suggest to us the possibility that both pathways of MTA-to-MTRP metabolism can be present in *P. falciparum*, that their relative levels in different strains may vary, and that these variations may be regulated in part by the end product, methionine. If this is indeed the case, in vivo studies will be invaluable in further defining the potential of MTR and MTA analogs as antimalarial agents.

ACKNOWLEDGMENTS

This work was funded in part by Public Health Service grants CA 37606 (J.R.S.), AI 32975 (J.R.S), and AI 26848 (S.R.M.) and by the UNDP/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases (C.J.B., S.R.M, and J.R.S).

We thank Allen Ranz for excellent technical assistance. We especially thank Michael Riscoe for helpful discussions and for his interest in our related scientific studies.

REFERENCES

1. **Bacchi, C. J., and P. P. McCann.** 1987. Parasitic protozoa and polyamines, p. 317–344. *In* P. P. McCann, A. E. Pegg, and A. Sjoerdsma (ed.), Inhibition of polyamine metabolism. Academic Press, Inc., Orlando, Fla.
2. **Bacchi, C. J., J. R. Sufrin, H. C. Nathan, A. J. Spiess, T. Hannan, J. Garofalo, K. Alecia, L. Katz, and N. Yarlett.** 1991. 5′-Alkyl-substituted analogs of 5′-methylthioadenosine as trypanocides. Antimicrob. Agents Chemother. **35:**1315–1320.
3. **Byers, T. L., T. L. Bush, P. P. McCann, and A. J. Bitonti.** 1991. Antitrypanosomal effects of polyamine biosynthesis inhibitors correlate with increases in *Trypanosoma brucei brucei S*-adenosyl-L-methionine. Biochem. J. **274:**527–533.
4. **Desjardins, R. E., C. J. Canfield, J. D. Haynes, and J. D. Chulay.** 1979. Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique. Antimicrob. Agents Chemother. **16:**710–718.
5. **Fairfield, A. J., S. R. Meshnick, and J. W. Eaton.** 1983. Malaria parasites adopt host cell superoxide dismutase. Science **221:**764–766.
6. **Fitchen, J. H., M. K. Riscoe, B. W. Dana, H. J. Lawrence, and A. J. Ferro.** 1986. Methylthioadenosine phosphorylase deficiency in human leukemias and solid tumors. Cancer Res. **46:**5409–5412.
7. **Fitchen, J. H., M. K. Riscoe, and A. J. Ferro.** 1988. Exploitation of methylthioribose kinase in the development of antiprotozoal drugs, p. 199–210. *In* V. Zappia and A. E. Pegg (ed.), Progress in polyamine research. Plenum Press, New York.
8. **Ghoda, L. Y., T. M. Savarese, C. H. Northrup, R. E. Parks, Jr., J. Garofalo, L. Katz, B. B. Ellenbogen, and C. J. Bacchi.** 1988. Substrate specificities of 5′-deoxy-5′-methylthioadenosine phosphorylase from *Trypanosoma brucei brucei* and mammalian cells. Mol. Biochem. Parasitol. **27:**109–118.
9. **Houston, M. E., Jr., D. L. Vander Jagt, and J. F. Honek.** 1991. Synthesis and biological activity of fluorinated intermediates of the methionine salvage pathway. Bioorg. Med. Chem. Lett. **1:**623–628.
10. **Kikugawa, K., K. Iizuka, Y. Higuchi, H. Hirayama, and M. Ichino.** 1972. Platelet aggregation inhibitors. 2. Inhibition of platelet aggregation by 5′-, 2-, 6- and 8-substituted adenosines. J. Med. Chem. **15:**387–390.
11. **Kuhn, R., and W. Jahn.** 1965. Vom Adenosin abgeleitete Thioather, und

S-oxide. Chem. Ber. **98:**1699–1704.
12. **Makler, M. T., J. M. Ries, J. A. Williams, J. E. Bancroft, R. C. Piper, B. L. Gibbons, and D. J. Hinrichs.** 1993. Parasite lactate dehydrogenase as an assay for *Plasmodium falciparum* drug sensitivity. Am. J. Trop. Med. Hyg. **48:**739–741.
13. **Miller, R. L., and D. P. Toorchen.** 1988. MTA phosphorylase in protozoa: a potential target for chemotherapeutic attack, p. 211–218. *In* V. Zappia and A. E. Pegg (ed.), Progress in polyamine research. Plenum Press, New York.
14. **Myers, R. W., and R. H. Abeles.** 1989. Conversion of 5-*S*-ethyl-5-thio-D-ribose to ethionine in *Klebsiella pneumoniae*. J. Biol. Chem. **264:**10547–10551.
15. **Myers, R. W., and R. H. Abeles.** 1990. Conversion of 5-*S*-methyl-5-thio-D-ribose to methionine in *Klebsiella pneumoniae*. J. Biol. Chem. **265:**16913–16921.
16. **Pegg, A. E.** 1988. Polyamine metabolism and its importance in neoplastic growth and as a target for chemotherapy. Cancer Res. **48:**759–774.
17. **Riscoe, M. K., A. J. Ferro, and J. H. Fitchen.** 1988. Analogs of 5-methylthioribose, a novel class of antiprotozoal agents. Antimicrob. Agents Chemother. **32:**1904–1906.
18. **Riscoe, M. K., A. J. Ferro, and J. H. Fitchen.** 1989. Methionine recycling as a target for antiprotozoal drug development. Parasitol. Today **5:**330–333.
19. **Sufrin, J. R., A. J. Spiess, D. L. Kramer, P. R. Libby, J. T. Miller, R. J. Bernacki, Y. Lee, R. T. Borchardt, and C. W. Porter.** 1991. Targeting 5′-deoxy-5′-(methylthio)adenosine phosphorylase by 5′-haloalkyl analogues of 5′-deoxy-5′-(methylthio)adenosine. J. Med. Chem. **34:**2600–2606.
20. **Toohey, J. I.** 1978. Methylthioadenosine phosphorylase deficiency in methylthio-dependent cancer cells. Biochem. Biophys. Res. Commun. **83:**27–35.
21. **Tower, P. A., D. B. Alexander, L. L. Johnson, and M. K. Riscoe.** 1993. Regulation of methylthioribose kinase by methionine in *Klebsiella pneumoniae*. J. Gen. Microbiol. **139:**1027–1031.
22. **Trager, W., and J. B. Jensen.** 1976. Human malaria parasites in continuous culture. Science **193:**673–675.
23. **Trager, W., M. Robert-Gero, and E. Lederer.** 1978. Antimalarial activity of *S*-isobutyl adenosine against *Plasmodium falciparum* in culture. FEBS Lett. **85:**264–266.
24. **Trager, W., M. Tershakovec, P. K. Chiang, and G. L. Cantoni.** 1980. *Plasmodium falciparum*: antimalarial activity in culture of sinefungin and other methylation inhibitors. Exp. Parasitol. **50:**83–89.
25. **Yarlett, N., and C. J. Bacchi.** 1988. Effect of DL-α-difluoromethylornithine on methionine cycle intermediates in *Trypanosoma brucei brucei*. Mol. Biochem. Parasitol. **27:**1–10.