Rev. Inst. Med. trop. São Paulo
29(5): 312-316, *setembro-outubro,* 1987

RADIOMETRIC STUDIES ON THE OXIDATION OF (U-^{14}C) L-AMINO ACIDS BY DRUG-SUSCEPTIBLE AND DRUG-RESISTANT MYCOBACTERIA

Edwaldo E. CAMARGO, Teresa M. KOPAJTIC, Glinda K. HOPKINS, Nancy P. CANNON & Henry N. WAGNER JR.

SUMMARY

A radiometric assay system has been used to study oxidation patterns of (U-^{14}C) L-amino acids by drug-susceptible and drug-resistant mycobacteria.

Drug-susceptible **M. tuberculosis** (H_{37}Rv TMC 102 and Erdman) along with the drug-resistant organism **M. tuberculosis** (H_{37} Rv TMC 303), **M. bovis, M. avium, M. intracellulare, M. kansasii** and **M. chelonei** were used. The organisms were inoculated into a sterile reaction system with liquid 7H9 medium and one of the (U-^{14}C) L-amino acids.

Each organism displayed a different pattern of amino acid oxidation, but these patterns were not distinctive enough for identification of the organism. Complex amino acids such as proline, phenylalanine and tyrosine were of no use in identification of mycobacteria, since virtually all organisms failed to oxidize them. There was no combination of substrates able to separate susceptible from resistant organisms.

KEY WORDS: Radiometric assay; **M. tuberculosis;** (U-^{14}C) L-Amino Acids; Drug resistance.

INTRODUCTION

Preliminary experiments reported from this laboratory showed differences in the oxidation rates of fatty acids by mycobacteria and led to the hypothesis that oxidation patterns of fatty acids might provide a basis for differentiation of these organisms. For example, a comparison of oxidation patterns of **M. lepraemurium, M. bovis, M. tuberculosis** (H_{37}Rv and Erdman) showed significant differences[2, 3].

In addition, investigation of isoniazid-resistant and isoniazid-susceptible strains of **M. tuberculosis** suggested that oxidation patterns of fatty acids and amino acids might help to identify susceptible and resistant organisms[4].

The present study describes a series of experiments in which oxidation of (U-^{14}C) L-amino acids was used in an attempt to differentiate susceptible from resistant strains of mycobacteria.

MATERIALS AND METHODS

Preparation of Bacilli: The Trudeau Mycobacterial Culture Collection (Saranac Lake, N. Y.)

Division of Nuclear Medicine, The Johns Hopkins Medical Institutions. Baltimore, Maryland 21205 — U.S.A.
Supported in part by USPHS Grant GM 10548.
Offprint requests to: Dr. Edwaldo E. Camargo. Centro de Medicina Nuclear da Universidade de São Paulo. Caixa Postal 22022. CEP 01499 São Paulo, SP, Brasil.

supplied the mycobacteria include in this investigation: two fully susceptible strains (**M. tuberculosis** H_{37}Rv TMC 102, **M. tuberculosis** Erdman TMC 107) and six drug-resistant strains (**M. tuberculosis** H_{37}Rv TMC 303, **M. bovis** TMC 602, **M. avium** TMC 706, **M. kansasii** TMC 1201, **M. intracellulare** TMC 1403, **M. chelonei** TMC 1542). All organisms were grown in liquid 7H9 medium with 10% albumin-dextrose-catalase (ADC, BBL) enrichment and 0.05% polysorbate 80. The organisms were incubated at 37°C for 6 to 11 days. The bacteria were then homogeneized with a Sorvall Omnimixer (8.5 speed scale) twice for 30 seconds. The number of bacteria was estimated using MacFarland barium sulfate standards[1]. The final suspension was diluted with sterile 7H9 medium to yield from 1 x 10^8 to 6 x 10^8 bacteria per ml.

Experimental Media: Liquid 7H9 medium with 10% ADC enrichment and no polysorbate was used with all the organisms tested.

Reaction System: The reaction system for detection of $^{14}CO_2$ consisted of 0.8 ml medium in a 5.0 ml multidose sterile glass vial with 0.1 ml of bacterial suspension and 0.1 ml (1.0 uCi) of ^{14}C-substrate.

The following (U-^{14}C) L-amino acids (Amersham Corporation) were used in aqueous solution: glycine, alanine, serine, aspartate, glutamate, threonine, leucine, isoleucine, methionine, arginine, proline, phenylalanine, tyrosine, histidine, and (1-^{14}C) L-valine. Leucine, isoleucine, methionine and histidine were omitted with **M. kansasii, M. intracellulare** and **M. chelonei**. As indicators of metabolic activity of the organisms, ^{14}C-formate (25 mCi/mM) and (U-^{14}C) acetate (25 mCi/mM) were used[7]. All vials were prepared at least in triplicate. Control vials were prepared in the same way, but with autoclaved bacteria added. Each experiment was repeated at least twice.

Radiometric Measurement: All vials were incubated at 37°C. An ion chamber device (Bactec R-301, Johnston Laboratories) was used to measure bacterial metabolism. The vials were sampled daily for 3 days. Results were obtained as nanocuries of ^{14}C activity. Mean and standard deviation of the cumulative $^{14}CO_2$ production of combined experiments for each substrate over the entire experimental period were calculated. Results were expressed as percent formate oxidation.

Sterility Testing: Sterility tests were performed on positive samples and consisted of subculture on chocolate-agar plates and radiometric sterility testing with (U-^{14}C) glucose[5, 6].

RESULTS

When the organisms were exposed to the series of (U-^{14}C) L-amino acids, different patterns of oxidation were observed (Figs. 1 and 2).

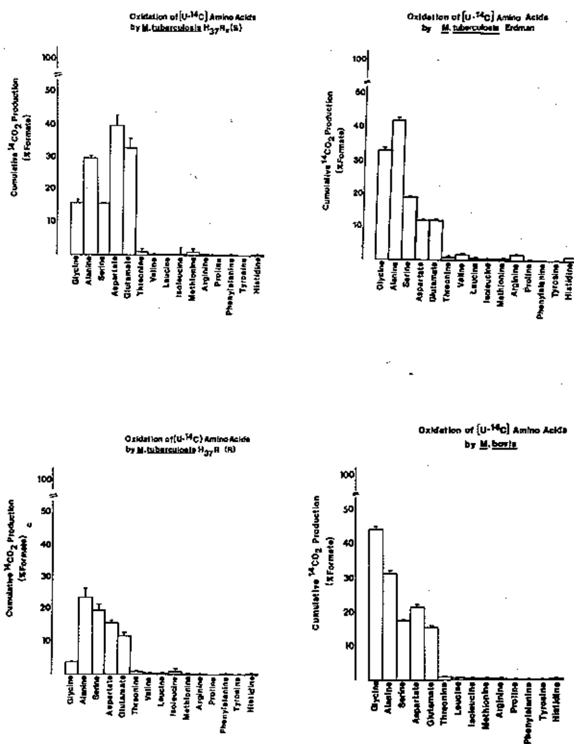

Fig. 1 — Pattern of (U-^{14}C) L-amino acid oxidation by **M. tuberculosis** H_{37}Rv TMC 102 drug susceptible (upper left), **M. tuberculosis** Erdman TMC 107 (upper right), **M. tuberculosis** H_{37}Rv TMC 303 resistant to isoniazid (lower left), and **M. bovis** TMC 602 (lower right). All organisms were grown in liquid 7H9 medium. Formate oxidation rates by these organisms were respectively 324, 753, 628 and 1,145 nanocuries of ^{14}C activity.

To simplify the results presented in Figures 1 and 2, an arbitrary threshold set at > 20% formate oxidation was chosen to eliminate poorly oxidized substrates (Table 1). An attempt was then made to differentiate the organisms using

the smallest possible number of substrates in Table 1. With glycine, glutamate, aspartate and alanine, the majority of the organisms could be identified. However, it was not possible to separate **M. tuberculosis** Erdman from **M. bovis** and to separate **M. tuberculosis** H_{37}Rv-R from **M. intracellulare.** Arginine and serine were of no use differentiating mycobacteria (Table 2).

TABLE 1

(U-^{14}C) L-amino acids with significant oxidation rates

	M. tuberculosis (H_{37}Rv-S)	M. tuberculosis (Erdman)	M. tuberculosis (H_{37}Rv-R)	M. bovis	M. kansasii	M. chelonei	M. intracellulare	M. avium
Glycine	0	+	0	+	+	+	0	0
Alanine	+	+	+	+	+	+	+	0
Serine	0	0	0	0	+	+	0	0
Aspartate	+	0	0	0	0	+	0	0
Glutamate	+	0	0	0	+	+	0	0
Arginine	0	0	0	0	0	+	0	0

\+ = > 20% formate oxidation
0 = < 20% formate oxidation
S = Susceptible
R = Resistant

TABLE 2

Oxidation patterns of selected (U-^{14}C) L-amino acids by micobacteria*

	Glycine	Glutamate	Aspartate	Alanine
M. tuberculosis (H_{37}Rv-S)	0	+	+	+
M. tuberculosis (Erdman)	+	0	0	+
M. tuberculosis (H_{37}Rv-R)	0	0	0	+
M. bovis	+	0	0	+
M. kansasii	+	+	0	+
M. chelonei	+	+	+	+
M. intracellulare	0	0	0	+
M. avium	0	0	0	0

* From Table 1, the smallest possible number of (U-^{14}C) L-amino acids was chosen on a trial and error basis to attempt distinction of each individual organism. However, **M. tuberculosis** H_{37}Rv resistant could not be distinguished from **M. intracellulare** and **M. tuberculosis** Erdman was indistinguishable from **M. bovis.**

S = Susceptible
R = Resistant

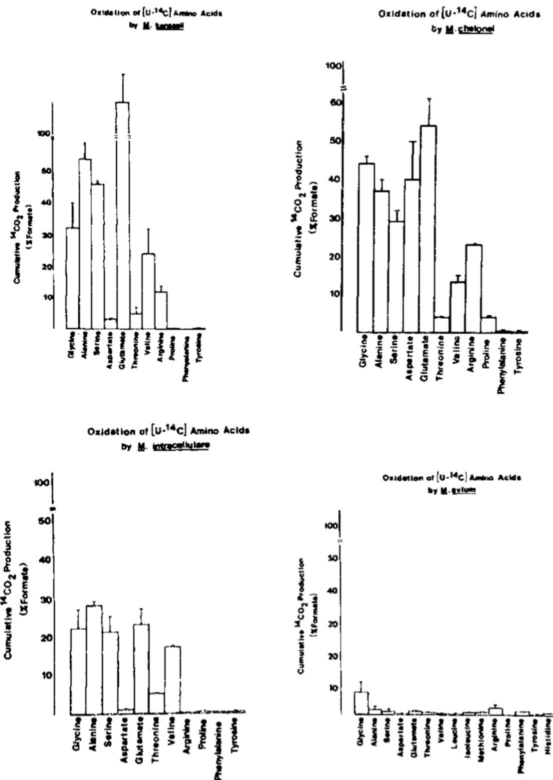

Fig. 2 — Pattern of (U-^{14}C) L-amino acid oxidation by **M. kansasii** TMC 1201 (upper left), **M. chelonei** TMC 1542 (upper right), **M. intracellulare** TMC 1403 (lower left) and **M. avium** TMC 706 (lower right). All organisms were grown in liquid 7H9 medium. Formate oxidation rates by these organisms were respectively 150, 746, 763 and 599 nanocuries of ^{14}C activity.

As expected, $^{14}CO_2$ production was not observed in the control vials and all sterility tests were negative.

DISCUSSION

Radiometric studies on the oxidation of the amino acids series by live mycobacteria have not been reported. The enzyme systems of the various organisms used in this study did not oxidize the (U-^{14}C) L-amino acids series at the same rates. In general, the simpler amino acids were better oxidized than the more complex molecules. Also, the different oxidation rates for the various amino acids varied from one organism to another. To some extent, differential oxidation patterns could be recognized.

In our preliminary studies comparing the susceptible strain **M. tuberculosis** $H_{37}Rv$ TMC 102 to the isoniazid-resistant strain **M. tuberculosis** $H_{37}Rv$ **TMC** 303 and **M. avium**, we noticed an inverse relationship between drug resistance and ability to oxidize amino acids[4]. However, with the inclusion of other drug-resistant organisms in our study, high oxidation rates were also noted with **M. bovis, M. kansasii, M. chelonei** and **M. intracellulare.** Although each individual organism displayed a different pattern of amino acids oxidation, these patterns were not distinctive enough for identification of the organism and no separation between susceptible and resistant organisms was found.

Our results have demonstrated that identification of all organisms included in our investigation is not feasible, using amino acids. Since virtually all organisms in our study have failed to oxidize the more complex amino acids such as proline, phenylalanine and tyrosine, these substrates are likely to be of no use in identification of mycobacteria.

RESUMO

Estudos radiométricos sobre a oxidação de (U-^{14}C) L-aminoácidos por micobactérias sensíveis e resistentes a drogas

Um sistema radiométrico foi utilizado para estudar os padrões de oxidação dos (U-^{14}C) L-aminoácidos por micobactérias sensíveis e resistentes a drogas. Foram usadas duas cepas do **M. tuberculosis** sensíveis a todas as drogas, $H_{37}Rv$ e Erdman. As micobactérias resistentes foram **M. tuberculosis** $H_{37}Rv$ resistente a 5 ug/ml de hidrazida, **M. bovis, M. avium, M. intracellulare, M. kansasii** e **M. chelonei.** As micobactérias foram inoculadas em frascos estéreis contendo o meio líquido 7H9 e um dos (U-^{14}C) L-aminoácidos.

Cada micobactéria apresentou um padrão de oxidação de aminoácidos, mas estes padrões não foram suficientemente diferentes para identificá-la. Aminoácidos complexos como a prolina, fenilalanina e tirosina não tiveram utilidade na identificação das micobactérias, pois praticamente todos os microorganismos foram incapazes de oxidá-los. Nenhuma combinação de aminoácidos foi capaz de separar as micobactérias sensíveis das resistentes a drogas.

CAMARGO, E. E.; KOPAJTIC, T. M.; HOPKINS, G. K.; CANNON, N. P. & WAGNER JR., H. N. — Radiometric studies on the oxidation of (U ^{14}C) L amino acids by drug susceptible and drug resistant mycobacteria. **Rev. Inst. Med. trop. São Paulo, 29**:312 316, 1987.

REFERENCES

1. BAILEY, W. R. & SCOTT, E. G. — **Diagnostic microbiology.** 2nd. ed. St. Louis, C. V. Mosby, 1970. p. 368.

2. CAMARGO, E. E.; KERTCHER, J. A.; LARSON, S. M.; TEPPER, B. S. & WAGNER JR., H. N. — Radiometric measurement of differential metabolism of fatty acids by **Mycobacterium lepraemurium. Int. J. Leprosy, 47**: 126 132, 1979.

3. CAMARGO, E. E.; KERTCHER, J. A. & LARSON, S. M. — Radiorespirometry in identification of mycobacteria. In: HORST, W.; WAGNER JR., H. N. & BUCHANAN, J W., ed. — **Frontiers in nuclear medicine.** Heildelberg, Springer Verlag, 1980a. p. 286 298.

4. CAMARGO, E. E.; MILKOWSKI, T. A. & WAGNER JR., H. N. — Relationship between amino acid metabolism and drug resistance of **M. tuberculosis** (Abstract). **J. nucl. Med., 21**: 59, 1980b.

5. DeBLANC JR., H. J.; DeLAND, F. H. & WAGNER JR., H. N. — Automated radiometric detection of bacteria in 2,967 blood cultures. **Appl. Microbiol. 22**: 846 849, 1971.

6. DeLAND, F. H. & WAGNER JR., H. N. — Automated radiometric detection of bacterial growth in blood cultures. **J. Lab. clin. Med., 75:** 529 534, 1970.

7. KERTCHER, J. A.; CHEN, M. D.; CHARACHE, P.; HWANGBO, C. C.; CAMARGO, E. E.; McINTYRE, P. A. & WAGNER JR., H. N. — Rapid radiometric susceptibility testing of **Mycobacterium tuberculosis. Amer. Rev. resp. Dis., 117**: 631 637, 1978.

Recebido para publicação em 03 05 87.