Levels of the Candida Metabolite D-Arabinitol in Sera of Steroid-Treated and Untreated Patients with Sarcoidosis

BRIAN WONG,1 ROBERT P. BAUGHMAN,2 AND KAREN L. BRAUER1

Divisions of Infections 1 and Pulmonary 2 Diseases, Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267

Received 21 February 1989/Accepted 5 May 1989

We measured the Candida metabolite D-arabinitol and its enantiomer L-arabinitol in 42 serum samples from 33 patients with sarcoidosis and compared the results with those from 27 healthy adults and 4 patients with candidiasis. The D- and L-arabinitol concentrations and the D- and L-arabinitol/creatinine ratios did not differ significantly in the sarcoidosis patients and the controls; the D-arabinitol concentrations and the D-arabinitol/creatinine ratios were much higher in the patients with candidiasis. Among the patients with sarcoidosis, the D- and L-arabinitol levels in the steroid recipients did not differ significantly from those in patients not receiving steroids. Higher D-arabinitol/creatinine ratios were associated with roentgenographic evidence of pulmonary fibrosis and low forced vital capacities, but not with disease activity as determined by the proportion of lymphocytes to total nucleated cells in bronchoalveolar lavage fluid or the CD4/CD8 ratio in bronchoalveolar lymphocytes. We conclude that neither sarcoidosis nor corticosteroid treatment is associated with high levels of D-arabinitol in serum.

D-Arabinitol is a major metabolic product of the medically important Candida species, and animals and humans with invasive candidiasis have more arabinitol in their body fluids and tissues than uninfected controls (3, 4, 7, 9, 12, 13, 15, 18, 20, 21). It has thus been proposed that D-arabinitol is a quantitative diagnostic marker for candidiasis. The usefulness of this diagnostic approach clearly depends on the specificity of elevated levels of D-arabinitol in body fluid for candidiasis. Many groups have reported that renal failure causes high levels of arabinitol in serum (4, 7, 9–13, 15, 18–21). However, this does not limit the usefulness of arabinitol as a diagnostic marker because the effects of renal failure are predictable and can be corrected for by calculating the arabinitol/creatinine ratios (7, 19).

Two other conditions have also been associated with high levels of arabinitol in serum. Karam et al. reported high levels of arabinol in serum from patients with sarcoidosis (8), and de Repentigny et al. reported high arabinitol levels in serum from uninfected rabbits (5) and humans (6) receiving corticosteroids. We studied an additional group of steroid-treated and untreated sarcoidosis patients because of concerns about the methods used in the earlier studies and because a method for quantifying the fungal metabolite D-arabinitol enantioselectively in human serum is now available (21).

(Part of this research was presented at the 28th Interscience Conference on Antimicrobial Agents and Chemotherapy, Los Angeles, Calif., 1988.)

MATERIALS AND METHODS

Blood was collected from 33 patients with sarcoidosis during 43 separate evaluations in the University of Cincinnati General Clinical Research Center, and the serum was stored at −70°C until studied. Patients studied more than once were evaluated at least 2 months apart because of changes in clinical status or treatment. Chest roentgenograms were evaluated by the criteria of Scadding (14), corticosteroid dosages were recorded, and forced vital lung capacities were determined by spirometry. Bronchoscopy with bronchoalveolar lavage was performed as previously described (1, 2), and the ratios of bronchoalveolar lymphocytes to total nucleated cells were determined by microscopy. Bronchoalveolar lymphocytes bearing surface CD4 and CD8 were enumerated by flow cytometry whenever 4 × 106 lymphocytes were available for study, and the CD4/CD8 ratios were calculated.

Concentrations of D-arabinitol and L-arabinitol in serum were measured by a combined enzymatic-gas chromatographic method as previously described (21). Briefly, α-methylmannoside and α-methylglucoside were added as internal standards to two 0.2-ml portions of serum, and one portion was treated with the Klebsiella pneumoniae NAD: oxidoreductase D-arabinitol dehydrogenase, rabbit muscle lactate dehydrogenase, NAD, and sodium pyruvate to remove D-arabinitol. Arabinitol was quantified as its trimethylsilyl ether derivative by capillary GC. The retention times of arabinitol and the internal standards were determined daily from a standard solution, and the relative detector response factors and efficiency of enzymatic D-arabinitol degradation were determined daily by analyzing normal serum to which 20 µg of D-arabinitol per ml was added. Quantitation was by comparison of peak areas of arabinitol and α-methylmannoside. The concentrations of D- and L-arabinitol were derived from the arabinitol concentrations in the enzyme-treated and untreated portions of each sample. Creatinine was measured with an autoanalyzer, and the D- and L-arabinitol/creatinine ratios were calculated to correct for the effects of renal function (19, 21).

The serum arabinitol results in the patients with sarcoidosis were compared with those obtained previously from 27 healthy adults and 4 patients with Candida fungemia or histologically proven invasive candidiasis (21). Means were compared by using the Student t test, and linear regression analyses were by least-squares methods.

RESULTS

Characteristics of the patients studied. All 33 sarcoidosis patients had typical clinical and laboratory features, and
extensive evaluations revealed no alternative explanations for the findings. The diagnosis was confirmed histologically in 29 patients; noncaseating granulomata were demonstrated in biopsies of lung in 19 patients, skin in 4 patients, lymph nodes in 3 patients, liver in 2 patients, and mediastinal tissue in 1 patient.

One patient had a normal chest roentgenogram (grade 0), 5 had enlarged hilar lymph nodes alone (grade 1), 16 had enlarged hilar lymph nodes and a pulmonary infiltrate (grade 2), and 11 had pulmonary fibrosis (grade 4). Spirometry was performed during 40 evaluations, and the mean ± standard deviation (SD) forced vital capacity was 71.2% ± 19.6% of the predicted values. Bronchoalveolar lavage fluid was obtained during 38 evaluations, and the mean ± SD ratio of lymphocytes to total nucleated cells was 0.289 ± 0.152. Twenty bronchoalveolar lavage fluid samples contained sufficient lymphocytes for flow cytometric analysis; the mean ± SD CD4/CD8 ratio was 4.87 ± 4.83.

Nineteen serum samples were from patients who were receiving 5 to 40 mg of prednisone per day, and 23 serum samples were from patients who were not receiving steroids. The serum D- and L-arabinitol concentrations and the D- and L-arabinitol/creatinine ratios in the 42 serum samples from the patients with sarcoidosis, the 27 healthy adults, and the 4 patients with candidiasis are summarized in Table 1. There were no significant differences between the values for the sarcoidosis patients and those for the healthy adults; the D-arabinitol concentrations and the D-arabinitol/creatinine ratios were much higher in the patients with candidiasis.

Among the sarcoidosis patients who were receiving or not receiving corticosteroid therapy, there were no significant differences in the D- and L-arabinitol concentrations or the D- and L-arabinitol/creatinine ratios. Moreover, there was no significant correlation by linear regression analysis between daily prednisone dosage and the D- or L-arabinitol concentrations or the D-arabinitol/creatinine ratios.

Roentgenographic evidence of advanced pulmonary sarcoidosis was associated with higher serum D-arabinitol/creatinine ratios but not with higher L-arabinitol/creatinine ratios. The serum D-arabinitol/creatinine ratios were higher in 18 patients with grade 4 chest roentgenograms (mean ± SD = 0.029 ± 0.011) than in 24 patients with grade 0 to 2 chest roentgenograms (0.016 ± 0.0039, P = 0.0002). Whether these higher D-arabinitol/creatinine ratios were associated with past or current disease activity was examined by correlating the D-arabinitol/creatinine ratios with forced vital lung capacity and with two parameters of current disease activity (1, 2, 16). There was a significant negative correlation between the serum D-arabinitol/creatinine ratios and percentage of predicted forced vital lung capacity (r = −0.357, P = 0.0239, n = 40), but there were no significant correlations between the serum D-arabinitol/creatinine ratios and either the ratios of bronchoalveolar lymphocytes to total nucleated cells (r = −0.137, P = 0.412, n = 38) or the bronchoalveolar lymphocyte CD4/CD8 ratios (r = −0.200, P = 0.397, n = 20).

**DISCUSSION**

In 1984, Karam and co-workers reported serum arabinitol levels in 53 patients with sarcoidosis, 35 patients with *Candida* species colonization or disease, 25 patients with other fungal infections, 25 patients with chronic obstructive pulmonary disease, 25 patients with end-stage renal disease who were receiving hemodialysis, and 25 healthy subjects (8). The principal new finding was that 27 (51%) of the sarcoidosis patients had elevated arabinitol levels in serum (0.5 μg/ml or more) compared with none of 25 healthy subjects. Those authors found no association between arabinitol levels in serum and either severity of disease as determined by chest roentgenograms or administration of corticosteroids, and they suggested that tissue injury associated with sarcoidosis may have resulted in increased arabinitol production by host tissues (8).

A careful reading of that report reveals several potential methodologic problems. First, the GC analyses were performed with a low-resolution packed column, and the chromatographic conditions were such that the trimethylsilyl derivative of arabinitol eluted after only 3 min. The ade-
quacy of the chromatographic separations cannot be assessed directly because representative chromatograms were not shown. However, we have analyzed trimethylsilylated human serum using many different packed and capillary GC columns, and we believe that arabinitol cannot be resolved from other serum components in 3 min with a packed OV-1 column (1.8 m by 2 mm). Second, the minimum detection limit of the method used was too high (0.5 µg of arabinitol per ml of serum) to permit quantitative comparisons between groups; instead, any serum in which arabinitol was detectable was considered abnormal. Third, serum samples from 13 of 25 hemodialysis patients contained no detectable arabinitol; arabinitol should have been detectable in all of these samples because arabinitol accumulates in serum directly in proportion to creatinine (7, 19). Fourth, none of 25 healthy subjects had detectable amounts of arabinitol in the urine, whereas several groups have shown that arabinitol is a normal constituent of human urine (3, 10, 11, 17, 19). Lastly, arabinitol/creatinine ratios were not used to correct for the effects of renal function.

Because of these problems, we reexamined the issue of arabinitol levels in serum of sarcoidosis patients using a sensitive and accurate combined enzymatic and GC method. The method used in this study clearly separates arabinitol from all other serum constituents, and it is sensitive enough (minimum detection limit, <0.08 µg/ml [21]) to yield quantitive results in all sera and urines studied to date. In addition, enzymatic depletion of specimens of D-arabinitol permits differentiation of fungal D-arabinitol from nonfungal L-arabinitol. Lastly, we corrected for the effects of renal function on the serum arabinitol concentrations by calculating the arabinitol/creatinine ratios.

We found that the serum D- and L-arabinitol concentrations and the D- and L-arabinitol/creatinine ratios in 42 serum samples from 33 patients with sarcoidosis were not significantly different from the corresponding values in samples from healthy controls. Although the patients with more advanced pulmonary sarcoidosis (grade 4 chest roentgenograms and low forced vital lung capacities) had significantly higher D-arabinitol levels in serum than patients with less severe pulmonary disease, these differences were too small to influence the diagnostic value of D-arabinitol as a marker for invasive candidiasis: the serum D-arabinitol/creatinine ratios in the subgroup of sarcoidosis patients with grade 4 chest roentgenograms were not significantly higher than the corresponding values in the healthy controls.

We also found no significant differences in the levels of D- or L-arabinitol in serum between the sarcoidosis patients who were receiving corticosteroids and those who were not. This confirms the results reported by Gold et al. (7), who found no differences in the serum arabinitol/creatinine ratios of steroid-treated and untreated cancer patients without candidiasis. In contrast, de Repentigny et al. reported markedly higher arabinitol concentrations and arabinitol/creatinine ratios in serum from uninfected rabbits given cortisone acetate than in saline controls (5) and in uninfected cancer patients receiving steroids than in those not receiving steroids (6). We have previously shown that the aldono-nitrile-peracetate derivatization method used by de Repentigny et al. results in conversion of a small proportion of glucose to arabinitol as a reaction by-product, and we suggested that this artifact or the derivatization reaction was responsible for the findings of de Repentigny (B. Wong, E. M. Bernard, D. Armstrong, J. Roboz, R. Suzuki, and J. F. Holland. Letter. J. Clin. Microbiol. 21:478–479, 1985). The results of the current study support this conclusion.

In summary, the present study indicates that neither steroid-treated nor non-steroid-treated sarcoidosis patients have more D- or L-arabinitol in their serum than healthy controls. If arabinitol/creatinine ratios are used to correct for effects of renal function, invasive candidiasis remains the only condition known so far to cause high serum DL- or D-arabinitol levels.

ACKNOWLEDGMENTS

This study was supported by Public Health Service grants AI-23938 from the National Institute of Allergy and Infectious Diseases and RR-00068 from the National Institutes of Health, General Clinical Research Center.

LITERATURE CITED


