Polyamine Levels in Normal Human Serum. Comparison of Analytical Methods

by

Frantisek Bartos, Dagmar Bartos, Donald P. Grettie, Robert A. Campbell
Pediatric Renal Metabolic Laboratory, Department of Pediatrics,
University of Oregon Health Sciences Center, Portland, Oregon 97201

Laurence J. Marton
Departments of Neurological Surgery and Laboratory Medicine,
University of California, San Francisco, California 94143

Ronald G. Smith and G. Doyle Daves, Jr.
Department of Chemistry, Oregon Graduate Center
Beaverton, Oregon 97005

Received February 2,1977

Summary

A comparison of levels of the polyamines, putrescine, spermidine and spermine, in individual and pooled normal human serum, as determined by three independent methods, high pressure cation-exchange chromatography (HPCC), radioimmunoassay (RIA) and gas chromatography-mass spectrometry (GC-MS), has been made. Generally good correlations were found for values derived by the three different methods.

An increased interest in the biochemistry of polyamines and in their potential use as indicators of human disease, especially malignancies (1), has created a demand for sensitive and specific methods for their analysis. As a result, several procedures for polyamine analysis have been developed and Bachrach, in a recent review (1), has commented on the relative merits of some of them. In this report, we compare results obtained in analysis of normal human serum using three independent methods for polyamine analysis. The three methods selected for this study are high pressure cation-exchange chromatography (HPCC) with fluorescence (o-phthalaldehyde) detection (2); radioimmunoassay (RIA) (3,4) and gas chromatography-mass spectrometry (GC-MS) using deuterated polyamine analogs as internal standards (5).

ISSN 0006-291X

Table I

Putrescine, Spermidine and Spermine Levels in Pooled Normal Human Serum. Comparison of Results Obtained by High Pressure Cation-Exchange Chromatography (HPCC), Radioimmunoassay (RIA) and Gas Chromatography-Mass Spectrometry (GC-MS) Methods

Method	Serum Required (per determination)	Analysis Time	Preanalysis Sample Preparation	Levels[a] of Putrescine	Spermidine	Spermine
				nmol/ml serum		
HPCC	1 ml	60 min	Silica column[b]	0.13±0.015	0.49±0.085	0.057±.004
RIA	20 µl	[d]	none	-	0.39±0.057	0.122±0.008 (0.036)[e]
GC-MS	1 ml	60 min	Silica column[b], derivatization[c]	0.13±0.008	0.34±0.016	0.041±0.002

[a]Average (± standard deviation) of analyses of six aliquots of serum pool.

[b]For details, see ref. 6.

[c]For details, see ref. 5.

[d]Not applicable, using standard, non-automated laboratory equipment 60-80 samples per day can be analyzed.

[e]Value obtained following correction [0.122-(0.22 x 0.39)] for 22% cross reactivity with spermidine (3).

Materials and Methods

Details of the HPCC (2), RIA (3,4) and GC-MS (5) methods of polyamine analysis have been reported elsewhere. The buffers utilized for the HPCC analysis have been modified from those originally described. A stock buffer (Buffer IV), 2.40 N potassium chloride and 0.09 N potassium citrate (pH 5.56) to which 12 ml/l of thiodiglycol has been added, is diluted as follows: Buffer I - 1 part stock to 4 parts water, Buffer II - 1 part stock to 1 part water, and Buffer III - 8 parts stock to 2 parts water. These four buffers are used sequentially to elute the polyamines.

A pool (600 ml) of human serum was obtained from the blood of five healthy volunteers (5 male, age 20 to 22) and stored at -15° until used. Six aliquots (10 ml) of this pooled serum, each containing ~10,000 dpm of an approximately 1:1 mixture of ^{14}C-putrescine and ^{14}C-spermine added to allow correction for losses during preanalysis manipulation, were subjected to the silica gel chromatographic procedure of Grettie et al. (6) for polyamine isolation. Each of these samples was then analyzed (single determination) for putrescine, spermidine and spermine by HPCC; the data are recorded in Table 1.

Table 2

Spermidine and Spermine Levels in Serum of Five Normal Human Males. Comparison of Results Obtained by High Pressure Cation-Exchange Chromatography (HPCC), Radioimmunoassay (RIA) and Gas Chromatography-Mass Spectrometry (GC-MS) Methods

Serum Sample	Spermidine (nmol/ml)			Spermine (nmol/ml)		
	HPCC	RIA	GC-MS	HPCC	RIA[a]	GC-MS
1	0.39	0.22	0.22	0.04	0.03	0.02
2	0.55	0.60	0.62	0.04	0	0.05
3	0.10	0.17	0.13	0.03	0.02	0.02
4	0.30	0.20	0.23	0.07	0.04	0.05
5	0.67	0.61	0.64	0.08	0.04	0.06

[a]As corrected for 22% cross reactivity with spermidine (level determined by RIA).

To each of a second set of six aliquots (10 ml) of the serum pool was added a mixture of 11 nmol of $^{2}H_{4}$-putrescine, 20 nmol of $^{2}H_{6}$-spermidine and 4 nmol of $^{2}H_{8}$-spermine (5). Following polyamine isolation (6), the samples were trifluoroacetylated and analyzed (triplicate determinations) by GC-MS as described (5).

Aliquots (20 µl) of the serum pool were analyzed by RIA (3,4) directly. The values in Table 1 represent the averages of triplicate determinations.

The data (Table 2) for polyamine levels in the sera of each of the five healthy male donors (age 20 to 22) were derived similarly.

Results and Discussion

Table 1 compares several parameters of the three analytical methods and results of analysis of aliquots from a single pool of unhydrolyzed normal human serum. In considering these data, perhaps most important is the fact that each of the three independent methods used possesses adequate sensitivity to permit polyamine analysis using readily available quantities of serum. This has not been possible using previously available, less

sensitive procedures (1). The levels of individual polyamines as determined by the different methods (Table 1) are in general agreement. The largest discrepancy - the 2-3 fold higher value for spermine obtained by RIA as compared to values obtained by HPCC or GC-MS - is ascribed to the cross reactivity of the spermine antibody (3) with spermidine (22%). Approximate correction for the effect of this cross reactivity was made by subtraction of 22% of the RIA-determined spermidine level from the value obtained using the spermine antibody. This correction appears to be valid for situations in which spermine and spermidine levels differ by no more than an order of magnitude (i.e., for normal serum levels) because of the symmetry of cross reactivity curves of the spermine antibody with spermine and spermidine (3); whether it will prove reliable for more extreme situations encountered in diseased states remains to be established. Following this correction of the spermine level determined by RIA, the results (Table 1) determined by the three methods are in agreement.

Comparison of spermidine and spermine levels in serum of normal males as determined by the three analytical methods are shown in Table 2. Again, the correlations are generally good. It is clear, based on the data contained in Tables 1 and 2 that each of the methods used is reliable and suitable for use in polyamine analyses when quantities of the individual polyamines present in individual analytical samples are of the order of 10 picomoles or greater.

In considering the utility of the individual analytical methods for specific purposes it is important to note that the HPCC, GC-MS and other chromatographic (7) methods are similar in important respects. Each requires preanalysis sample manipulation, utilizes complex instrumentation and is limited in the number of analyses which can be performed in a given time. In contrast, the RIA method has greater sensitivity, requires no prior sample manipulation, utilizes widely available instrumentation and is capable of large numbers of determinations. Admittedly, the RIA method is

dependent on the availability of specific antibodies for each of the polyamines. The spermidine antibody used in this study is quite specific (4) and preliminary attempts to purify the spermine antibody in order to eliminate undesirable cross reactivity are encouraging (F. Bartos and D. Bartos, unpublished results).

Acknowledgments

Appreciation is expressed for grants from the National Institutes of Health (CA-15515, CA-13525 and CA-16328), the M. J. Murdock Charitable Trust, the American Cancer Society and the Doernbecher Guild which provided financial support for this study. LJM is the recipient of NCI Research Career Development Award CA-00112.

References

1. Bachrach, U. (1976) Ital. J. Biochem., 25, 77-93.

2. Marton, L. J. and Lee, P. L. Y. (1975) Clin. Chem., 21, 1721-1724.

3. Bartos, D., Campbell, R. A., Bartos, F. and Grettie, D. P. (1975) Cancer Res., 35, 2056-2060.

4. Bartos, D., Bartos, F., Campbell, R. A., Grettie, D. P. and Dolney, A. M., Northwest Polyamine Conference, Portland, Oregon, 4-5 November, 1976.

5. Smith, R. G. and Daves, G. D., Jr. (1977) Biomed. Mass Spec., 4, in press.

6. Grettie, D. P., Bartos, D., Bartos, F. and Campbell, R. A., Northwest Polyamine Conference, Portland, Oregon, 4-5 November, 1976.

7. Samejima, K., Kawase, M., Sakamoto, S., Okada, M. and Endo, Y. (1976) Anal. Biochem., 76, 392-406.