Erythrocyte Spermidine Levels in IDDM Patients

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OBJECTIVES — To evaluate whether erythrocyte levels of polyamines spermidine and spermine (expressed in nmol/ml packed erythrocytes [PRBCs]) are modified in insulin-dependent diabetes mellitus (IDDM) and are associated with the presence of retinopathy or nephropathy.

RESEARCH DESIGN AND METHODS — We studied erythrocyte spermidine and spermine levels in 38 IDDM patients with or without persistent microalbuminuria (urinary albumin excretion rate [AER] between 20 and 200 μg/min), macroalbuminuria (AER >200 μg/min), or retinopathy compared with 60 sex- and age-matched control subjects.

RESULTS — Mean ± SD erythrocyte spermine content was similar in both diabetic (9.7 ± 5.5 nmol/ml PRBCs) and control (8.8 ± 3.5 nmol/ml PRBCs) subjects, whereas spermidine was higher in diabetic (19.1 ± 7.2 nmol/ml PRBCs) than in control (14.5 ± 4 nmol/ml PRBCs, $P = 0.0007$) subjects. Moreover, spermidine was significantly higher in the groups with microalbuminuria ($n = 11$, 22.5 ± 9.2 nmol/ml PRBCs) and macroalbuminuria ($n = 4$, 22.2 ± 5.7 nmol/ml PRBCs) than in both normoalbuminuric ($n = 23$, 16.9 ± 5.6 nmol/ml PRBCs) and control ($F = 9.78$, $P = 0.0001$) subjects, and correlated with log AER ($r = 0.41$, $P = 0.009$). Similarly, proliferative retinopathy was associated with a significant increase in spermidine ($n = 5$, 20 ± 7 nmol/ml PRBCs compared with control subjects [$P = 0.0009$]).

CONCLUSIONS — Our data suggest that erythrocyte spermidine content is increased in IDDM patients associated with both diabetic nephropathy and advanced retinopathy.

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Most eukaryotic cells contain significant amounts of the polyamines spermidine and spermine and their precursors (1). Moreover, studies have shown that both cell growth and differentiation require polyamines (2,3). In blood, these amines are mainly transported by erythrocytes and their erythrocyte content is a reliable index of cell proliferation (4). Insulin-dependent diabetic (IDDM) patients with persistent microalbuminuria or overt nephropathy are characterized by enhanced cell growth of fibroblasts in vitro (5). Furthermore, sera of IDDM patients with proliferative retinopathy, a condition closely related to nephropathy (6), have been reported as having a cell growth–promoting activity (7). This study was designed to test whether erythrocyte content of polyamines is related to the presence of renal disease or retinal lesions in IDDM.

RESEARCH DESIGN AND METHODS

— Thirty-eight IDDM patients and 60 healthy nondiabetic control subjects (blood donors), strictly matched for age, sex, and body mass index (Table 1), participated in the study. According to the urinary albumin excretion rate (AER), assayed as the mean value of three samples (by radioimmunoassay, Biodata, Rome, Italy), patients were divided into three groups: *1*) those with a normal AER (<20 μg/min, $n = 23$), 2) those with microalbuminuria (AER 20–200 μg/min, $n = 11$), and 3) those with macroalbuminuria (AER >200 μg/min, $n = 4$). Both spermidine and spermine erythrocyte concentrations were measured in erythrocyte pellets obtained and hemolyzed according to Moulinoux et al. (4). Samples were alkalinized with 1 M NH_4OH and underwent a first elution by a Bond-Elut C_{18} 500-mg column (Analytichem, Harbor City, CA), activated by a solution of methanol (10 vol) and 0.5 N HCl (1 vol). Derivatization was conducted with a dansyl-chloride solution saturated with sodium carbonate at 70°C for 15 min. After a second elution on Bond-Elut 100-mg columns (8), samples

Table 1—*Clinical details of diabetic and control patients*

	DIABETIC	CONTROL	*P*
N (M/F)	22/16	35/25	
AGE (YR)	46 ± 13	44 ± 10	NS
DURATION (YR)	14 ± 9		
BODY MASS INDEX (KG/M^2)	25 ± 4	24 ± 6	NS
BLOOD GLUCOSE (MM)	11.5 ± 4.5	4.7 ± 0.6	0.0001
SYSTOLIC BLOOD PRESSURE (MMHG)	143 ± 22	128 ± 15	0.001
DIASTOLIC BLOOD PRESSURE (MMHG)	83 ± 10	75 ± 8	0.03
HbA_{1c} (%)	8.3 ± 1.7	5.8 ± 0.7	0.0001
DAILY INSULIN DOSE (U/KG)	0.59 ± 0.3		
SPERMINE (NMOL/ML PACKED ERYTHROCYTES)	9.7 ± 5.5	8.8 ± 3.5	NS
SPERMIDINE (NMOL/ML PACKED ERYTHROCYTES)	19.1 ± 7.2	14.5 ± 4	0.0007
SPERMIDINE/SPERMINE	2.3 ± 1.1	1.8 ± 0.7	0.01

Values are means ± SD.

were injected onto the liquid chromatograph (655 A-11, Hitachi, Tokyo) equipped with a 4 × 70 3-μm column (Beckman, Berkeley, CA) and a spheriguard C_{18} column with a time-variable gradient elution with acetonitrile and water at a flow rate of 1.2 ml/min. Effluent fluorescence was detected with a 340/515 nm excitation/emission wavelength (8). Results are expressed as nanomoles per milliliter packed erythrocytes (PRBCs). Coefficients of variation within and between assays were <12%. Plasma glucose and creatinine were measured by standard methods and HbA_{1c} by high-performance liquid chromatography. Retinal damage, evaluated by direct ophthalmoscopic examination and fluorescein angiography was scored as absent, background retinopathy (≥1 microaneurysm, hard exudates, cotton-wool spots, hemorrhages, venous beadings), or proliferative retinopathy (new vessels, vitreous hemorrhages). Differences between groups were tested by two-tailed *t* test and one-way analysis of variance. Correlations were evaluated by the least-squares method. AER was log transformed due to its nonnormal distribution. $P < 0.05$ was significant. Results are means ± SD.

RESULTS— Mean spermidine and spermine erythrocyte concentrations and spermidine-spermine ratio of a healthy nondiabetic control group were similar to the mean values found by others (9). Both spermidine and spermidine/spermine were significantly increased in diabetic patients compared with control subjects, although spermine levels were similar in both groups (Table 1). Spermidine erythrocyte content was higher in the groups with micro- or macroalbuminuria compared with diabetic patients with a normal AER and in control subjects, whereas, spermidine/spermine was significantly increased only in the group of macroalbuminuric patients (Table 2). Erythrocyte spermidine (x) was significantly related with log AER (y) ($y = 0.456 + 0.036x$, $r = 0.41$, $P = 0.009$), whereas, there was no correlation with age, duration of diabetes, blood glucose, daily insulin dose, blood pressure, and serum creatinine. The correlation between AER and spermidine remained significant also after processing the data with a multiple regression analysis model where age, duration of diabetes, HbA_{1c}, daily insulin dose, creatinine, presence of retinopathy, and blood pressure were covariates ($F = 7.35$, $P = 0.011$). As to the presence of retinal damage, only proliferative retinopathy was associated with an increase in both spermidine erythrocyte content and spermidine/spermine (Table 3).

CONCLUSIONS— In cell cultures, there is a direct correlation between spermidine content and/or spermine-spermadine ratio and growth rate, suggesting that spermidine accumulation is primarily associated with cell replication processes (1–3). According to this study,

Table 2—*Erythrocyte spermine and spermidine concentrations in control and diabetic patients stratified according to albumin excretion rate (AER)*

	AER (μG/MIN)			
	<20	20–200	>200	CONTROL
N	23	11	4	60
AER (μG/MIN)	8 (2–16.5)	36.4 (22–130)	295.2 (200–574)	2.8 (0.8–12.7)
CREATININE (μM)	70 ± 14.1	68.2 ± 14.1	122.3 ± 71.7*	66.4 ± 9.7
SYSTOLIC BLOOD PRESSURE (MMHG)	137 ± 19	144 ± 24*	170 ± 8*	128 ± 15
DIASTOLIC BLOOD PRESSURE (MMHG)	81 ± 9	83 ± 10*	94 ± 12*	75 ± 8
SPERMINE (NMOL/ML PACKED ERYTHROCYTES)	9.3 ± 4.8	10.8 ± 7.4	8.6 ± 4.4	8.8 ± 3.5
SPERMIDINE (NMOL/ML PACKED ERYTHROCYTES)	16.9 ± 5.6	22.5 ± 9.1*	22.2 ± 5.7*	14.5 ± 4
SPERMIDINE/SPERMINE	2.1 ± 0.8	2.4 ± 0.9	3.3 ± 2.3*	1.8 ± 0.7

Values are means ± SD with ranges in parentheses. *$P < 0.05$ vs. other groups (Duncan's test after analysis of variance).

Table 3—*Erythrocyte spermine and spermidine concentrations in control and diabetic patients stratified according to severity of retinopathy*

	Retinopathy			
	None	Background	Proliferative	Control
N	22	11	5	60
Spermine (nmol/ml packed erythrocytes)	10.4 ± 8.3	10.7 ± 4.9	8 ± 4	8.8 ± 3.5
Spermidine (nmol/ml packed erythrocytes)	18.1 ± 7.8	19.6 ± 7.1	20 ± 7*	14.5 ± 4
Spermidine/spermine	2.2 ± 0.7	2.2 ± 1.2	3 ± 2*	1.8 ± 0.7

Values are means ± SD. *$P < 0.05$ vs. control (Duncan's test after analysis of variance).

erythrocyte spermidine content is significantly higher in IDDM patients with raised AER or proliferative retinopathy, and such increases could simply mirror a greater cell growth's trend, as seen in patients with diabetic nephropathy (5), or be the response to growth stimulation exerted by diabetic sera as observed in patients with proliferative retinopathy (7). Furthermore, the activity of serum spermidine oxidase, a key enzyme involved in the catabolism of polyamines, was described to be positively related with urinary albumin excretion and the presence of proliferative retinopathy in a group of IDDM patients (10), raising some indirect evidence that both diabetic nephropathy and proliferative retinopathy could be associated with an activation of cell polyamines turnover. In conclusion, our results suggest that an increased spermidine erythrocyte content might be specifically associated with both diabetic nephropathy and proliferative retinopathy in IDDM. The exact significance of such an increase and whether it can be considered the cause or the effect of diabetic microvascular disease, however, remains to be elucidated.

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