

Plasma spermidine concentrations as early indication of response to therapy in human myeloma

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SUMMARY Eighteen patients with melphalan refractory myeloma were treated with vindesine and prednisone. Plasma spermidine concentrations were measured by radioimmunoassay before and after a single vindesine injection. Seven patients showed a significant rise of plasma spermidine after vindesine and five of these showed a clinical response on further evaluation. Of the 11 patients who did not show raised spermidine concentrations, 10 did not respond to the therapy. The correlation between clinical response/rise of spermidine and between non-response/no rise of spermidine was statistically significant ($p < 0.05$). Pretreatment spermidine concentrations did not distinguish those who responded to treatment nor did they differ in patients and controls.

Measurement of polyamines, mainly spermidine and putrescine, has been shown to be useful in experimental research especially concerning tumour growth and response to treatment.

Cell death is reflected in an increase of the spermidine concentration in biological fluids—for example, urine, serum, or cerebrospinal fluid—possibly because spermidine is released by the dying cell.^{1,2} In recent years a considerable amount of work has been done to establish practical clinical utility for polyamine measurements. In 1975 Russell *et al* showed that an increase of spermidine excretion in urine after cytostatic treatment correlated with clinical response³ in solid and haematological tumours. These results were extended and confirmed.⁴ Since 1977, however, the only clinical application for measurement of polyamines has been monitoring recurrence of medulloblastoma by measuring putrescine concentrations in CSF.¹ One of the possible reasons for a decline in interest¹ is that most polyamine determinations were done on 24-hour urine. Samples are cumbersome to obtain and their collection may be incomplete. Recently, a radioimmunoassay for spermidine measurement in plasma became available, and prompted by the studies of Russell *et al*^{3,4} we investigated the possibility of drug sensitivity testing in vivo.

In this paper we present the results of plasma spermidine measurements before and after an injection of vindesine in patients with melphalan-resistant multiple myeloma. The correlation of the spermidine concentration with the clinical response is investigated.

Patients, material and methods

Spermidine concentrations were measured by a radioimmunoassay.⁵ Antibodies to spermidine were raised in rabbits by immunisation with spermidine covalently bound to bovine thyroglobulin. Plasma spermidine concentrations were estimated by using a double-antibody technique. Only free spermidine is measured, the plasma is not pretreated by acid hydrolysis. Cross reaction with spermine or putrescine is less than 1%. Spermidine concentrations are expressed in nmol/l. Venous blood samples were taken in heparinised vacuum glass tubes. Directly after venepuncture the blood was centrifuged (3000 rpm for 10 min) and plasma was frozen and stored at -20° . Haemolytic samples were discarded. The spermidine assay took place within 14 days after sampling.

PATIENTS

All patients were treated with vindesine and prednisone for multiple myeloma resistant to melphalan/prednisone treatment. The preliminary results of this have been published.⁶ Eighteen patients participated in the present study.

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Vindesine was given as an intravenous bolus injection (2 mg/m^2) at weekly intervals for three consecutive weeks, followed by a three week therapy-free interval. After each vindesine injection prednisone was given orally in daily doses of 100 mg for five days. Evaluation of response took place after three months of treatment, response was measured according to the Southwest Oncology Group (SWOG)-criteria⁷ which differentiate between "responders," "improved patients" and "non-responders". In this paper the improved patients as well as the responders are called "responders." All patients were classified according to Durie⁸ as stage III disease (high tumour load).

PROTOCOL

Patients were admitted to hospital for their first vindesine injection. This injection was not followed by prednisone because of the probable stimulating affect of prednisone on polyamine metabolism.⁹ The baseline plasma sample was taken at 0800 h and vindesine was administered immediately afterwards. Further samples were taken at 2, 4, 6 and 8 h after the vindesine injection and daily at 0800 h for the next five or six days. Patients were free from bacterial infections. As controls, samples were taken from 12 healthy volunteers at 0800, 1100, 1300 and 1600 h.

STATISTICS

For correlation of response and spermidine concentration the 2×2 contingency tables were used.

Results

Pretreatment values of the patients are shown in Fig. 1 (range 50–920 nmol/l, mean 290 nmol/l) and they are compared with the 0800 h values of the 12 controls (range 90–550, mean 270.5 nmol/l).

Although two of the patients had very high pretreatment values it seems that these initial values do not discriminate between patients and controls, nor do they discriminate between responders to treatment and non-responders (open v closed circles, Fig. 1). Spermidine concentrations of the 12 healthy volunteers over the day are shown in Fig. 2. Values are expressed as a percentage of the 0800 h sample.

A diurnal fluctuation of $\pm 50\%$ was observed. The spermidine values, after the vindesine injection expressed as a percentage of the pretreatment value, are shown in Fig. 3. Because of the diurnal fluctuation observed in the controls we considered that a change in spermidine values of $\geq 100\%$ in the patients after treatment was significant. The patients were classified according to (a) response versus non-response to treatment and (b) significant versus

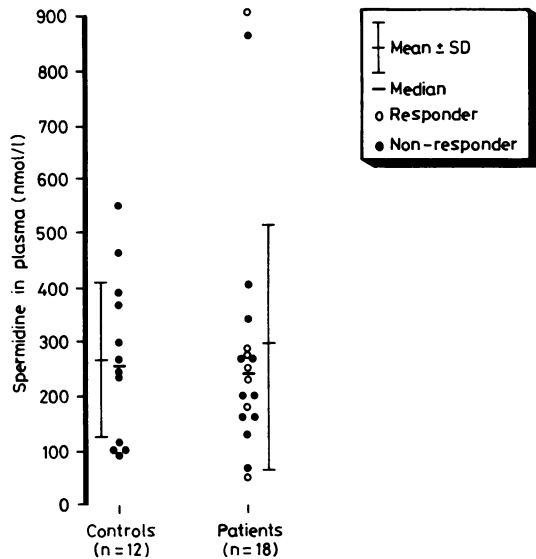


Fig. 1 Baseline values of controls (left) and patients (right). Responders are indicated by open circles.

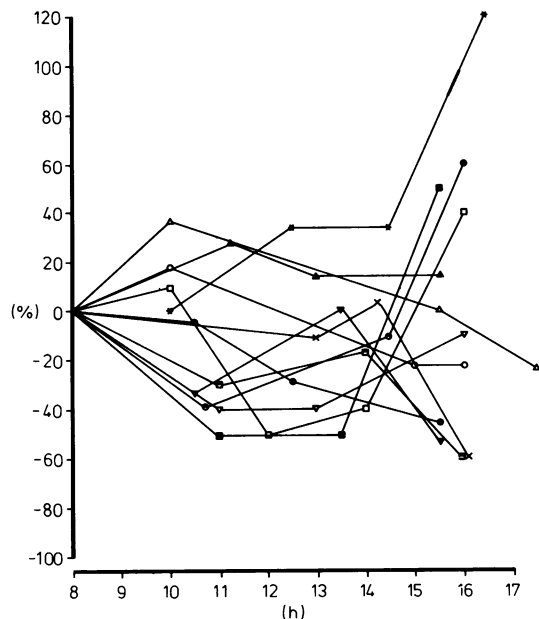


Fig. 2 Spermidine values (expressed as a percentage of the 0800 h values) at approximately 1100 h, 1300 h and 1600 h of 12 controls. One curve (*) starts at 1000 h.

the non-significant rise of spermidine concentration.

Five of the six responders showed a spermidine rise of $> 100\%$ while ten of the twelve non-responders did not show a significant rise (Table).

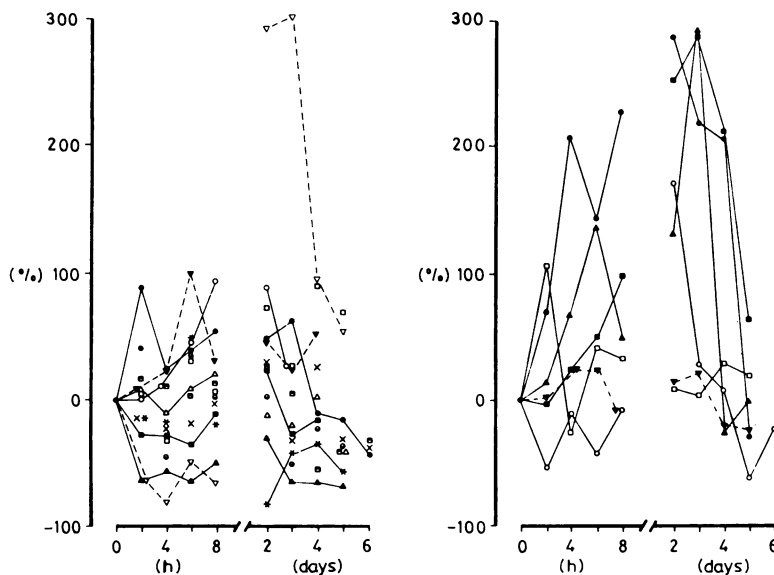


Fig. 3 Spermidine values (expressed as a percentage of the baseline values) of responding (right) and non-responding (left, not all curves drawn) patients. Dashed lines indicate two non-responders with a spermidine rise and one (right) responder without a spermidine rise.

Table Correlation between response/non-response and spermidine rise/non-spermidine rise ($p < 0.05$)

	Significant spermidine rise		
	+	-	
Response	5	1	Sensitivity 83%*
Non-response	2	10	Specificity 83%†
Total	7	11	
PV pos 71%‡PV neg 91%§			

*Sensitivity: true positive ratio.

†Specificity: true negative ratio.

‡PV pos: predictive value of a positive test result.

§PV neg: predictive value of a negative test result.

The correlation between response/non-response and significant/non-significant spermidine rise was statistically significant ($p < 0.05$).

From the same figures a decision matrix was calculated (Table). Sensitivity and specificity were both 83%. The predictive value of a significant spermidine rise was 71%; the predictive value of absence of a spermidine rise was 91%.

Discussion

Recently a radioimmunoassay for plasma spermidine concentration became available to us. In patients with melphalan-resistant multiple myeloma who were treated with vindesine and prednisone we studied plasma spermidine concentrations after vindesine administration. Although all patients had

stage III disease, only two patients showed extremely high pretreatment values, probably indicating increased spontaneous cell death. This could indicate different tumour kinetics in individual patients and is in accordance with the view of Durie¹⁰ who demonstrated different tumour doubling times within one group of patients with the same stage of disease. The spermidine concentrations of the responding patients showed a rise within 24 h indicating cell death quite soon after the vindesine administration, which agrees with *in vitro* experiments.¹¹ It also correlates with our clinical observation of rapidly decreasing skeletal pain in several patients. Both observations suggest that plasma spermidine concentrations can be used for research on drug and tumour kinetics.

Pretreatment spermidine concentrations did not distinguish patients from controls, nor between those who responded to treatment or those who did not. A statistically significant correlation was shown between a significant rise of the plasma spermidine value (after drug administration) and a clinical response. With the spermidine values obtained in this particular protocol, response and especially non-response could be predicted with reasonable accuracy (decision matrix, see Results). If this (or similar) protocol could be used to predict response or non-response to other cytostatic drugs, the possibility arises to test tumour drug sensitivity *in vivo* in the individual patient. In analogy to tumour drug sensitivity testing *in vitro* (tumour stem cell assay) treatment could then be selected on an individual basis.

This could be especially useful in malignancies in which response or non-response to treatment is not always readily apparent for example, multiple myeloma, where the prolonged half life of the M-protein may delay response evaluation. The radioimmunoassay for spermidine is a rapid method, so drug sensitivity testing for one or more drugs should enable the physician to select proper treatment shortly after his decision to treat the patient.

Since the present study is limited to a small group of patients, in which only one drug is tested for one disease, further studies are needed to confirm the usefulness of plasma spermidine measurement in tumour drug sensitivity testing.

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