Plasma adenosine deaminase isoform 2 in cancer patients undergoing chemotherapy

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ABSTRACT

Adenosine deaminase (AD), a purine salvage enzyme, exists as AD isoform 1 (AD1) and AD isoform 2 (AD2). Plasma AD has been advocated for the screening and monitoring of cancer, as AD2 activity is increased in conditions associated with tumour growth. Plasma AD2 was measured before and seven to 10 days after the first dose of chemotherapy in patients with different tumours. A 'tumour regression score' was assessed independently based on radiological changes seen in the tumour following completion of chemotherapy. Changes in plasma AD2 were then compared with the tumour regression score. Following first-dose chemotherapy, plasma AD2 decreased on average from 22.7±10.5 U/L to 15.0±4.6 U/L. The percentage decrease in plasma AD2 correlated with the tumour regression score (r=0.5, P=0.028). These data suggest plasma AD2 may have a role in determining tumour response to treatment.

KEY WORDS: Adenosine deaminase.
Drug therapy.
Neoplasms.
Plasma.

Introduction

Adenosine deaminase (AD, EC 3.5.4.4) is a widely distributed metaloenzyme that catalyses the hydrolysis of adenosine or 2′ deoxyadenosine to inosine or 2′ deoxyinosine and ammonia (Fig. 1).[1] It has an important role in human monocyte differentiation and tumour cell toxicity.[2]

Two isoforms have been reported: AD1 is found in all tissues but levels are highest in the thymus and other lymphoid tissues, while AD 2 originates from the monocyte-macrophage system as a response to infection.[3] The AD2 isoform has been reported as the main isoform in plasma (10.7±3.2 U/L), with lower levels (3.1±0.8 U/L) seen for AD1.[4]

As long ago as 1957, Straub *et al*. recommended the measurement of plasma AD as a screening test for cancer.[5] Ho and Gabeshaguru[6] advocated the estimation of the enzyme in plasma for adult T-cell leukaemia, while Walia *et al*.[7] suggested that plasma AD be assayed for the monitoring of patients with breast cancer. However, Zavialov *et al*.[8] reported that it is AD2 isoform activity that is increased in conditions associated with tumour growth.

Recently, fresh interest in AD has been generated by reports of increased activity of the enzyme in tissues from different cancers.[9–12] A report by Bukulmez *et al*.[13] showed that AD is decreased in patients with psoriasis after treatment with methotrexate, which is a drug also used in cancer chemotherapy. Furthermore, work by Chan and Cronstein[14] found that the use of methotrexate to alleviate the symptoms of rheumatoid arthritis resulted in a decrease in plasma AD.

The present study aims to determine the activity of plasma AD2 in cancer patients undergoing treatment to ascertain the value of measuring any change in the isoform as a tool in monitoring patient response to chemotherapy.

Materials and methods

Blood samples were collected from 21 patients suffering from different types of cancer, selected from those attending the chemotherapy clinic and who would be investigated radiologically before and after chemotherapy. The blood samples were collected before chemotherapy (pre-dose) and between seven and 10 days after the first dose of chemotherapy had been given (post-dose).

The patients were being treated with various drugs and combinations (Table 1). In all cases drug half-life was such that it would have been cleared from the blood before collection of the post-dose sample, and thus would not be present in the sample to inhibit the AD assay.[15]

Two volunteers had blood collected at the start of the project and again one week later; one exhibited a plasma AD2 around the mean of the reference range, while the other showed plasma AD2 activity above the reference mean (>17.1 U/L).

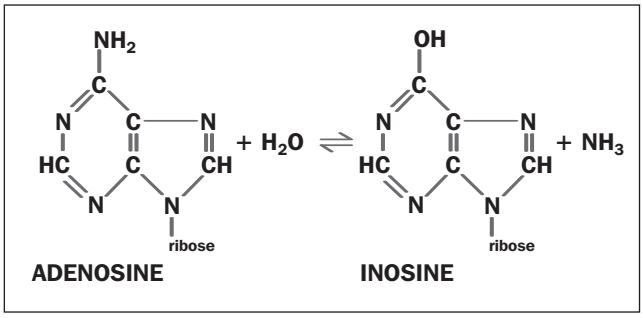

Fig. 1. Hydrolysis of adenosine or 2'deoxyadenosine to inosine or 2'deoxyinosine and ammonia.

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Table 1. Patient information including cancer type and drug regime with evidence (key phrases) of tumour shrinkage or regression. The key phrases were scored according to the data obtained from the radiographic scan. The scan scores were compared to the percentage change in plasma activity of AD2.

Patient code (cancer type) drug	AD2 pre U/L	AD2 post U/L	AD2 pre-post U/L	% AD2 decrease	Tumour regression score	Evidence (key phrases)
A (Breast) Tax	24	15.1	8.9	37	4	No signs of secondary in lung. No abnormality in bones/liver.
B (Breast) Tax	29	15.5	13.5	46	3	Appearance unchanged (chest) since last examination.
C (Breast) Tax	35.2	28.3	6.9	20	3	Little normal liver (May). Numerous malignant secondaries (August).
D (Breast) ECF/2M	25.6	13.6	12	47	4	No change in chest. Osteolytic lesion in pelvis now treated.
E (Breast) ECF	15.1	10.9	5.2	34	4	Secondary mass in lung looks considerably smaller now (CT).
F (Breast) ECF	15.4	12.8	2.6	17	5	Clearly a dramatic response to treatment (Ultrasound).
G (Breast) FEC	24.7	17.4	7.3	30	2	Now two/three liver lesions found.
H (Breast) FEC	21	13.8	7.2	34	5	No mammographic evidence (now) of breast pathology.
I (Breast) Tax	18.9	14.2	4.7	25	5	No sign of recurrence.
J (Gastric) ECF	18	13.3	4.7	26	3	No further focal abnormality. No evidence of spread outside gastric wall.
K (Gastric) ECF	20.7	17.2	3.5	16.9	3	No evidence of metastasis.
L (Gastric) ECF	15.1	11.6	3.5	23	3	Although a pelvic mass and bladder tumour suspected, this was ruled out.
M (Gastric) ECF	12.6	9.6	3	24	3	Large area of gastro-oesophageal junction involved. No spread or metastasis.
N (Gastric) ECF	24	16.6	7.4	31	3	The (?) liver metastases show no change. No other abnormality.
O (Colon) I/5FU	24	21.3	2.7	11	2	Similar to last examination. Enlarged lymph node. New lung lesion.
P (Colon) I/5FU	13.3	9.9	3.4	26	3	No further abnormality.
Q (Colon) I/5FU	20.7	15.3	5.4	26	3	No significant change in size or appearance of liver metastases.
R (Colon) I/5FU	20.7	20.0	0.7	3	2	Duodenum/part of stomach now obstructed? Prostate cancer.
S (Colon) I/55U	15.7	13.9	1.8	11	4	Tumour size in the right upper lobe reduced since last chest X-ray.
T (Lymphoma) MVP	20.6	6.6	14	68	5	Now no sign of spread of bladder base mass into pelvic lymph nodes.
U (Lymphoma) MVP	61.8	18.0	43.8	71	6	Now no convincing sign of lymphadenopathy (CT).

ECF: epirubicin, cisplatin and fluorouracil, FE: fluorouracil, epirubicin and cyclophosphamide, CMF: cyclophosphamide, methotrexate and fluorouracil, Tax: taxotere, 2Ms: mitoxantrone and methotrexate, I/5FU: irinotecan and fluorouracil, MVP: mitomycin, vinblastin and cisplatin and prednisolone, CHOP: cyclophosphamide, adriamycin (hydroxodaunorubicin), vincristine (Oncovin) and prednisolone.

All chemicals were purchased from Merck (VWR International, Magma Park, Lutterworth, UK) unless otherwise specified. All chemicals were at least Analar grade. Erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA) was obtained from Tocris Cookson (Avonmouth, UK) and adenosine from Sigma (Poole, Dorset, UK). Plastic tubes (12 x 75 mm) were obtained from Sarstedt (Leicester, UK). These were found to be ammonia-free and were used in the laboratory for the routine estimation of plasma ammonia.

Colorimetric measurement of AD2

The method used to measure AD2 was as described previously.[16] Briefly, 0.1 mL plasma was incubated with 0.15 mL adenosine (18.5 mmol/L) buffered at pH 6.8 with phosphate buffer (66 mmol/L) containing EHNA (0.1 mmol/L) for 30 min at 37°C. Phenol/nitroprusside solution (1.5 mL) was then added, followed by 1.5 mL alkaline hypochlorite solution. Plasma blanks and reagent blanks were also prepared. After incubation for a further 35 min at 37°C, the blue colour was measured at 625 nm and compared to dilutions of a 0.86 mmol/L (equivalent to 43.2 U/L) ammonium standard prepared by diluting a 17.2 mmol/L ammonia stock standard. One unit of enzyme activity is defined as the amount required to produce 1 µmol ammonia per min under the conditions described.

Tumour regression score

The effect of chemotherapy on each patient's tumour (tumour regression score) was assessed independently by the Lead Specialist Practitioner (Ultrasound) in the radiology department at Bronglais Hospital, based on key phrases contained in the patient's notes. These contained the changes in tumour progression reported by the radiologist utilising scans taken before and after the course of

chemotherapy. The key phrases abstracted from the notes were taken in context and given a score of 1 to 6 (1 being the poorest outcome while 6 the best outcome) by the Lead Specialist Practitioner.

Statistical analysis

Regression analysis was used to investigate the relationship between a percentage decrease in plasma AD2 following chemotherapy and the tumour regression score. Owing to the non-parametric nature of the tumour regression scores, two-tailed Spearman regression analysis was used to calculate the correlation coefficient (r) and the probability (P). This non-parametric analysis makes no assumption about the distribution of values or their normal distribution. All statistical tests were performed using GraphPad Prism 3 for Windows (version 5.1.2600; Prism Statistical Computer Package, GraphPad Software, San Diego, USA).

Results and discussion

The percentage increase in seven days for the two reference samples were 2.8% and 1.4% for the subjects with plasma AD2 activity of 13.8 U/L and 24.2 U/L, respectively. In the patient group, following the first dose of chemotherapy, plasma AD2 decreased on average from 22.7+10.5 U/L to 15.0+4.6 U/L (34%).

The results of the AD2 assay and tumour regression scores, together with patient information, are shown in Table 1. Cancer types and drug regimes for each patient are also included. A significant correlation between the percentage decrease in plasma AD2 and tumour regression score was found (r=0.50, P=0.028), as shown in Figure 1.

Drug treatment resulted in decreased plasma AD2 activity compared to the slight increase of AD2 seen in the reference group. The decrease in AD2 activity appears to correlate with the effectiveness of the drug and so the measurement of plasma AD2 before and after the first dose of chemotherapy could predict the efficacy of the drug being used.

Although the largest decreases in AD2 appeared to be found in the patients suffering from lymphoma, the authors of this small study recommended that these findings be confirmed on a larger number of subjects. □

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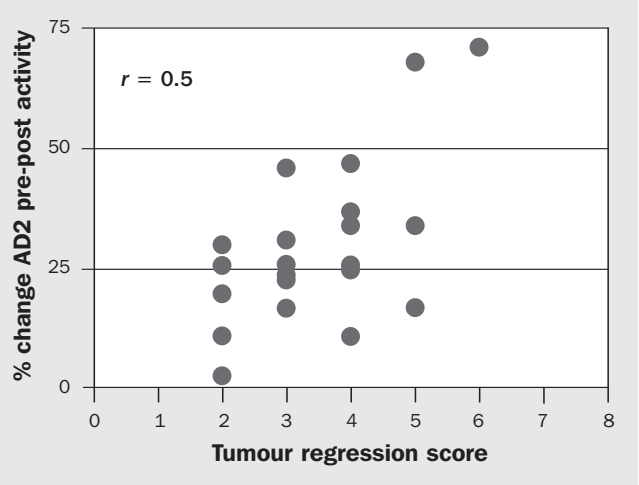

Fig. 2. Spearman's non-parametric correlation between tumour regression score and the difference between pre-dose and post-dose AD2 found for each patient during the course of treatment expressed as a percentage of the pre-dose AD2 at that time.

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