Amino Acids (1993) 4: 177–185

Amino
Acids

Changes in plasma amino acids during conditioning therapy prior to bone marrow transplantation: their relevance to antioxidant status

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Accepted August 1, 1992

Summary. Bone marrow transplant (BMT) recipients undergo a bimodal regimen of conditioning therapy, the precise prescription being dependent upon the primary disease of the individual patient. Generally, this treatment consists of chemotherapy and total body irradiation prior to transplantation, although the latter may or may not be included in the regimen. We have investigated amino acid metabolism and oxidant status in in a small series of BMT recipients before and after conditioning therapy.

Plasma amino acids were measured by HPLC on 10 BMT recipients prior to commencing conditioning therapy, and again one week later before transplantation. In addition some general nutritional parameters and antioxidant components were measured. A marked decrease in the plasma concentration of a number of amino acids, especially those concerned with antioxidants, was observed over the 7 days of conditioning therapy.

There is also a significant reduction in antioxidant capability, as reflected by measurements of glutathione and erythrocyte glutathione peroxidase (GSHPx), which may have an influence upon post-transplant recovery and graft function. Such a reduction in antioxidant concentrations may also have an influence upon the erythrocyte and platelet support required post-grafting.

The data presented in this paper adds to the evidence for the conditional essentiality of some amino acids such as taurine and glutamine, and may support the case for specific antioxidant intervention treatment prior to, and/or after conditioning therapy together with monitoring antioxidant status during the post-grafting period.

Keywords: Amino acids – Antioxidants – Selenium – Magnesium – Vitamin B_6 – Bone marrow transplantation

Introduction

Bone marrow transplantation (BMT) has become an accepted form of treatment for haematological malignancy (both chronic and acute), immunodeficiency states and some congenital disorders (Storb et al., 1983).

As a prelude to BMT, patients undergo a regimen of conditioning, high-dose chemotherapy which may or may not be coupled with total body irradiation (TBI). This treatment takes place over the period of a week. The purpose of conditioning therapy is to destroy existing bone marrow and create space and immunosuppression for donor marrow development. Conditioning therapy also makes the graft recipients very susceptible to opportunistic infections which, in an immunosuppressed individual, may be life-threatening (Atkinson et al., 1979).

In addition, conditioning therapy is toxic per se, the complete regime approaching the tolerance limit of many tissues. The toxicity reactions, which occur in most tissues following such treatment, include radiation mucositis and mucosal ulceration of the gastrointestinal tract, liver damage and haemorrhagic cystitis. Nausea and vomiting occurs almost invariably with consequent adverse effects upon the general nutritional state of the patient (Donaldson et al., 1979).

Conditioning therapy may initiate tissue peroxidation reactions by a variety of mechanisms. Membrane peroxidation and other free-radical mediated damage may be a feature of both cyclophosphamide therapy (Gurtoo et al., 1981) and of TBI (Purohit et al., 1980), primarily through the generation of high concentrations of the hydroxyl radical. It may, therefore, be postulated that some of the toxicity of conditioning therapy may be due to free-radical mediated cell damage.

We proposed to investigate amino acid metabolism both pre and post conditioning therapy to assess the effect it may have on the body amino acid pool, as measured by plasma amino acid concentrations. In addition to the obvious nutritional value of amino acids, some act as antioxidants, e.g. taurine and glycine, or as precursors to antioxidant molecules, e.g. glutamine, glycine and methionine (Babior et al. 1983; Pangborn, 1985).

The degree of free-radical induced tissue damage may be reduced, or recovery enhanced, if antioxidant elements such as ascorbate, selenium etc. were included in the post-grafting support therapy. Amino acid supplementation, especially in the case of taurine, may also have an important role to play in this context.

Patients, material and methods

The study was approved by the ethical committee of the London Clinic.

Patients

Ten consecutive patients presenting for BMT were entered into this study (mean age 20y, range 6–46y; 4 males and 6 females). The original diagnoses of the patients varied and were acute myeloid leukaemia ($n = 7$), acute lymphoblastic leukaemia ($n = 1$), chronic granulocytic leukaemia ($n = 1$) and Non-Hodgkins lymphoma ($n = 1$). The leukaemic patients were all in remission at the time of transplantation.

The precise regimen of the conditioning therapy varied with each patient according to the original disease and were generally selected from current European Bone Marrow Transplantation (EBMT) protocols, and fall into 5 categories:

1. Cyclophosphamide, dounorubicin and TBI ($n = 6$).
2. Cyclophosphamide, dounorubicin, cytosine arabinoside and TBI ($n = 1$).
3. Cyclophosphamide, idarubicin and TBI ($n = 1$).
4. Vincristine, cytosine arabinoside, tenoposide and prednisolone ($n = 1$).
5. Nitrosourea (BCNU), etoposide, cytosine arabinoside and cyclophosphamide ($n = 1$).

Conditioning was usually given over the seven days prior to transplantation with fractionated TBI being given over the final 3 days, from day −3 up to the day of transplant (Day 0). No attempt is made in this study to differentiate the types of conditioning therapy in the analysis as the numbers are too small for statistical comparison. None of the patients received intravenous nutrition during the period of the study.

Normal control values for erythrocyte magnesium, erythrocyte glutathione peroxidase, plasma and erythrocyte glutathione and pyridoxine were obtained from a group of 10 volunteers (mean age 26y, range 9–50y; 6 males and 4 females) who were healthy, on an apparently adequate diet, and receiving no medication or nutritional supplementation at the time of sampling.

Analytical methods

Plasma amino acid profiles were measured on admission for conditioning therapy (Baseline; Day −7), and again at the end of conditioning, prior to transplantation (Day 0). Blood samples (10 ml) were taken into lithium heparin tubes ("Vacutainer", Becton-Dickinson (UK) Ltd., Between Towns Rd., Cowley, Oxon. OX4 3LY) early in the morning, prior to any food. The plasma was separated within one hour and then frozen (−20°C) until analysis, usually carried out within 48 hrs. The profiles, consisting of 39 amino acids as listed in Table 1, were measured using a fully automated gradient elution HPLC system with fluorimetric detection (Gilson Medical Electronics, Villiers-le-Bel, France), using the o-phthaldehyde-2-mercaptoethanol (OPA-MCE) derivatisation method as previously described by Turnell and Cooper (1982). Proline and hydroxyproline, which are not detected using this system, were measured using chloramine-T oxidation followed by OPA-MCE derivatisation (Cooper et al. 1984). The mean within-run CV was 6%, with a range of 3.2% (Serine) to 12.3% (Ornithine).

Blood was taken for assessment of intracellular magnesium and vitamin B_6 and the antioxidants glutathione and glutathione peroxidase at the same time intervals as for amino acids.

Red blood cell magnesium (RBCMg) concentrations were measured by atomic absorption spectrophotometry using a Pye Unicam PU9000 AA spectrophotomer (Phillips Scientific Ltd., York St., Cambridge. CB1 2PX. England.) according to the method previously described by Abraham and Lubran (1981). The mean intra and inter batch CV were 1.8% and 2.2% repectively. The laboratory reference range is 1.70–2.60 mM/l.

Vitamin B_6 concentrations were measured indirectly by functional analysis using the activation of erythrocyte glutamate oxaloacetate transaminase (EGOT), a B_6 dependant red cell enzyme (Vuilleuimier et al., 1983). The mean inter and intra-batch CV were 12% and 16% respectively. The laboratory reference ranges are:

(a) up to 15% activation – normal.
(b) 15%–25% activation – borderline.
(c) greater than 25% – functional vitamin deficiency.

These ranges are in line with other groups (Leklem, 1988; Bender, 1989).

Reduced glutathione concentrations were measured in both plasma and erythrocytes using the 5-5′-dithiobis(2-nitrobenzoic acid) (DTNB) reduction method (Adams et al., 1983). The mean intra and inter batch CV were 3.6% and 4.4% repectively. The reference ranges for glutathione are 3.8–8.2 uM/l (plasma) and 1.6–2.8 mM/l (RBC).

Red blood cell glutathione peroxidase (RBC-GSHPx) activity was measured according to the method previously described by Pleiban et al. (1982). The mean intra and inter batch CV were 3.0% and 4.8% respectively. The reference range expressed as Units per gram of haemoglobin (U/gHb) is 67–90.

Table 1. Plasma amino acid profiles (⇒ aminogram)

Essential amino acids	
Threonine	Methionine
Valine	Phenylalanine
Leucine	Lysine
Isoleucine	Tryptophan
Non-essential amino acids	
Arginine	Glutamine
Histidine	Glycine
Aspartic acid	Alanine
Serine	Asparagine
Glutamic acid	Cysteine
Tyrosine	Proline
"Metabolic" amino acids	
Phosphoserine	α-amino butyric acid
α-amino adipic acid	β-amino butyric acid
Homocysteine	γ-amino butyric acid
Ethanolamine	Phosphoethanolamine
1-methyl histidine	Hydroxyproline
3-methyl histidine	Hydroxylysine
Citrulline	Ornithine
β-alanine	Taurine
Cystathionine	Methionine sulphoxide
Carnosine	Anserine

Results

The overall results of the plasma aminograms are shown in Table 2, expressed as mean concentration (umol/l) and standard error of the mean (SEM). Due to the numbers of amino acids measured, only those where differences between the groups are evident are recorded. In all other cases there were no significant differences in amino acid concentrations between the groups.

There are some differences evident between the BMT baseline samples (Day −7) and the control group. The mean concentration of serine, alanine and the branched chain amino acids are lower than in the normal group, although not statistically so. Mean concentrations of tyrosine, phosphoserine, ethanolamine and ornithine are higher in the BMT baseline group compared with normals, although again this difference is not statistically significant.

There are marked differences in the aminograms between the BMT baseline samples and post-conditioning therapy (Day 0). Valine, leucine, isoleucine, tyrosine, phosphoserine and ornithine are all significantly higher post-conditioning ($p < 0.01$). Serine, histidine and alanine show a significant fall in concentration ($p < 0.01$). The most marked differences between Day −7 and Day 0 are the fall in methionine (40% fall; $p < 0.001$), glutamine (47% fall; $p < 0.001$), tyrosine (44% fall; $p = 0.001$) and cysteine (50% fall; $p < 0.001$). There

Table 2. Plasma amino acid concentrations (μmol/l) in the normal group and pre and post-conditioning therapy

	Normal (A) $n = 10$	Baseline (Day −7) (B) $n = 10$	Post-therapy (Day 0) (C) $n = 10$
Histidine	106 (7)	109 (5)	93 (6) *
Valine	276 (10)	240 (20)	305 (12) *
Leucine	196 (20)	178 (13)	210 (10) *
Isoleucine	105 (14)	96 (12)	115 (22) *
Methionine	29 (6)	22 (5)	13 (3) *
Cysteine	62 (9)	53 (8)	24 (5) †
Serine	122 (17)	115 (10)	90 (5) *
Glutamine	769 (34)	708 (40)	295 (30) †
Alanine	406 (21)	379 (29)	270 (13) *
Tyrosine	75 (6)	86 (10)	130 (20) *
Phosphoserine	4 (1)	6 (2)	11 (2) *
1-methylhistidine	ND	ND	5 (1) †
3-methylhistidine	3 (1)	3 (1)	20 (2) †
Taurine	78 (7)	75 (3)	42 (11) †
Ethanolamine	2 (1)	7 (2) **	8 (2)
Phosphoethanolamine	4 (1)	6 (2)	12 (2) *
Ornithine	93 (4)	100 (6)	150 (12) *
Methionine sulphoxide	ND	ND	13 (2) †

Results expressed as mean (SEM)
* $p < 0.01$ C vs B.
† $p < 0.001$ C vs B.
** $p < 0.01$ B vs A.

are also marked increases in the concentrations of both 1-methylhistidine and 3-methylhistidine (particularly with 3MH) ($p < 0.001$) and methionine sulphoxide ($p < 0.001$).

Table 3 shows the results obtained for RBCMg, vitamin B_6 and the antioxidants measured, shown as mean concentration and SEM.

Magnesium and pyridoxine concentrations, as measured by RBCMg and vitamin B_6 activation of erythrocyte GOT, are sub-optimal in the BMT recipients prior to conditioning compared with the normal group ($p < 0.001$ for RBCMg, $p < 0.001$ for vitamin B_6), and there is a deterioration in this state over the period of conditioning therapy. The measured concentrations of RBCMg and vitamin B_6 fall significantly between Day −7 and Day 0 ($p < 0.01$ between these points in time).

Post-conditioning, the concentrations of both plasma and red cell glutathione fall significantly ($p < 0.001$). The depletion of intracellular glutathione is most marked in comparison with extracellular glutathione (60% and 40% respectively, $p < 0.01$).

RBC GSHPx activity falls significantly between Day −7 and Day 0 ($p < 0.001$). Note that there are statistically significant differences between the base-

Table 3. General micronutrients and antioxidants in the normal group and pre and post-conditioning therapy

	Normal (A) $n = 10$	Baseline (Day −7) (B) $n = 10$	Post-therapy (Day 0) (C) $n = 10$
RBC Magnesium (mmol/l)	1.93 (0.07)	1.67 (0.02) ††	1.55 (0.02) *
Vitamin B_6 (% actn.)	12 (3)	25 (3) †	34 (4) *
Glutathione Plasma (uM/l)	5.92 (0.93)	4.97 (0.76)	2.96 (0.35) **
RBC (mM/l)	2.02 (0.22)	1.93 (0.13)	0.76 (0.24) **
RBC GSHPx (U/gHb)	79 (8)	67 (2) *	55 (3) **

line samples and the normal group ($p < 0.01$), reflecting either poor selenium absorption or increased utilisation of GSHPx with inadequate replacement in the BMT recipents prior to transplantation.

Discussion

The results of this study clearly show a decrease in the plasma concentration of a number of important amino acids and cellular antioxidants after conditioning therapy. Such deficiences may adversely affect the post-transplant course of the recipients.

The initial baseline aminograms agree, in part, with those reported previously (McMenamy et al., 1960). The current studies, however, employ a more sensitive method of amino acid measurement, increasing the sensitivity with which changes in amino acid concentrations can be detected. This may account for some of the observed differences between this and previous studies. BMT recipients show some initial mild abnormalities of amino acid status in comparison with a normal group. It has been demonstrated that BMT patients have deficiences of some essential nutrients, which may affect the absorption and interconversion of amino acids in normal metabolism (Moreno et al., 1990). Magnesium and vitamin B_6 (as pyridoxal phosphate) are central to the normal metabolism of amino acids, acting as enzyme cofactors. This study highlights some baseline deficiencies in these cofactors which may account for the slightly abnormal baseline aminograms.

There are some marked abnormalities in the concentration of plasma amino acids occuring over the week of conditioning therapy that are not directly associated with cofactor availability but in connection with antioxidants, an important area in this group of patients. This occurs most notably with glutamine (Gln) and taurine (Tau). Under the definition of Rose (1949), both of these amino acids are non-essential, i.e. can be synthesised by the body in the presence

of an adequate precursor supply. Therefore they are not considered as essential requirements of the diet. However, there is mounting evidence to suggest that some non-essential amino acids become conditionally essential in pathological states (Chipponi et al., 1982).

Evidence for increased free-radical stress in BMT patients is provided by:

(a) The increase of Methionine sulphoxide, seen in the aminogram, caused by the oxidation of methionine by superoxide radicals.
(b) Reduction in the activity of RBC-GSHPx, an enzyme which detoxifies peroxides produced as a result of free radical interaction with cell membranes.
(c) The marked fall in the concentrations of both intra and extracellular glutathione during the period of study.

Glutamine is the most abundant amino acid in plasma acting as a nitrogen shuttle. In situations where tissue damage occurs there is a marked increase in the loss of Gln from the muscle tissue pool, with muscle protein breakdown and loss of Gln (Johnson et al., 1984). The hypothesis of muscle catabolism is confirmed by the observed increases of both 1-methylhistidine and 3-methylhistidine in this study post-conditioning, the latter being an indicator of skeletal muscle catabolism (Pangborn, 1985). It is conceivable that this feature might contribute to post-operative weight loss in BMT recipients. In addition, within the gastrointestinal tract, Gln may be important for normal cell function and serve in a "damage-limitation" capacity for free radical mediated damage. This would be important in the amelioration of the effects of radiation mucositis. Supporting this hypothesis, it has been shown that the dramatic decrease in the Gln pool may be inhibited by Gln supplementation (Kapardia et al., 1985) and in animal experiments this has been shown to improve outcome and reduce toxicity in rats exposed to radiation (Soubra et al., 1990).

Taurine (Tau) is a sulphur-containing amino acid functioning as a membrane stabiliser and modulator of transmembrane calcium transport (Chesney, 1985). Importantly, due to its high concentration in tissues subject to high free radical activity, it is presumed to act as an antioxidant, particularly in the removal of hypochlorous acid (Koyama et al., 1990). In the body taurine may be synthesised from methionine via cysteine, a vitamin B_6 and vitamin B_2 dependant process, but there is some evidence to suggest that the in-vivo ability to do this may be limited (Vinton et al., 1986). It is known that the urinary loss of Tau is increased in patients undergoing chemotherapy (Dilley, 1972), but a combination of increased free-radical stress, vitamin deficiences and reduced precursor availability in the BMT patients could contribute to the observed Tau deficiency. The cysteine deficiency observed may be due to increased demand for both Tau synthesis and synthesis of the tripeptide glutathione (L-glutamyl-L-cysteinyl-glycine). Glutathione is the main vehicle for cysteine transport around the body, and a direct and indirect antioxidant (Meister et al. 1983). Early in the post-transplant period and at the time of conditioning therapy in BMT patients, glutathione is in high demand as an antioxidant. This increased demand coupled with reduced precursor availability could contribute to the deficit illustrated in this study. In addition, vitamin deficiency (especially vit B_2, a cofactor for

glutathione reductase in the regeneration of reduced glutathione) may also contribute to the observed glutathione deficit in these patients.

Glutathione may be of major importance later in the post-transplant course once engraftment has occurred. There is evidence to show that glutathione is important immunologically, being critical for the initiation/progression of lymphocyte activation and also for the function of natural killer cells (MacDermott et al., 1986). Attention to glutathione levels, or precursor supply, at this time may help with the more rapid development of normal white cell populations. Care must be taken, however, because if glutathione influences lymphocyte activation it may be involved in graft rejection and graft versus host disease (GVHD), which may therefore increase in incidence and/or severity if we are successful in increasing glutathione supply. This could be a disadvantage with allogeneic BMTs especially with matched unrelated donors where GVHD occurs almost invariably.

As a result of these studies, we propose an intervention trial to investigate the addition of "antioxidant therapy" to the standard post-operative TPN regimens of BMT patients and its effect on drug toxicity and patient/graft survival in the early post-grafting period.

Acknowledgements

The authors would like to thank Dr. Peter Gravett, Consultant Haematologist of the London Clinic for access to his patients and review of the manuscript.

The authors would also like to gratefully acknowledge financial support from the Biolab Research Fund.

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Received April 26, 1992