



# Gut mucosa barrier preservation by orally administered IgA-IgG to patients undergoing bone marrow transplantation: a randomised pilot study

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## Summary:

**Intensive cytotoxic therapy with bone-marrow transplantation (BMT) allows a potential cure for haematological malignancies. Protective strategies to minimise haematological toxicities have been successful and currently toxicity to the gastro-intestinal tract is the major cause of treatment-related morbidity and the dose-limiting factor that prevents further dose escalation. In a randomised, placebo-controlled trial we investigated whether an oral immunoglobulin preparation (IgA-IgG) can diminish intestinal toxicity with autologous BMT. IgA-IgG ( $n = 6$ ) and placebo ( $n = 7$ ) were orally administered from 1 day prior to the start until 1 week after the termination of the cytotoxic treatment (a total of 14 days). Intestinal toxicity was assessed by a <sup>51</sup>Cr-EDTA absorption test for intestinal permeability and by the clinical criteria laid down by the WHO for the period before the start of the cytotoxic treatment, 1 day prior to stem-cell infusion and 4, 7, 10 and 14 days after stem-cell infusion. In the placebo group there was a significant increase in intestinal permeability on day 4 ( $P < 0.005$ ) and on day 7 ( $P < 0.05$ ) after stem-cell infusion, compared with the baseline, which was not seen for IgA-IgG. In addition, patients receiving IgA-IgG had significantly less intestinal permeability on day 4 ( $P < 0.05$ ) and on day 7 ( $P < 0.05$ ), compared with the placebo group. No significant, positive effect as regards clinical toxicity was observed. Oral administration of IgA-IgG to patients undergoing intensive cytotoxic therapy prior to BMT seems to have a protective effect on the gut mucosa barrier which is normally disrupted by this therapy.**

**Keywords:** gastro-intestinal; toxicity; cytotoxic therapy; immunoglobulins; prophylactic therapy; randomised trial

therefore limits the application of the treatment. Up to now, there have been no convincing therapies to circumvent gastro-intestinal toxicity due to the intensive cytotoxic treatment given in connection with BMT.

Immunoglobulin A (IgA) is the principal immunoglobulin on mucosal surfaces.<sup>2</sup> Although its protective effect, primarily through the inhibition of antigen adherence to the intestinal mucosa (immune exclusion), is well documented,<sup>3,4</sup> the exact mechanisms involved are not entirely clear. In clinical trials the oral administration of several different immunoglobulin preparations has revealed that it can survive passage through the gastrointestinal tract of bone marrow transplantation recipients<sup>5</sup> and has protective effects against infectious diarrhoea and necrotizing enterocolitis.<sup>6–9</sup>

It may be anticipated that production and function of IgA in the gastro-intestinal tract is disrupted by the preparative regimen preceding BMT. The aim of this placebo-controlled, randomised trial was therefore to investigate whether the oral administration of an IgA-IgG preparation made from human serum would reduce gastro-intestinal toxicity or infectious complications related to autologous BMT.

## Patients and methods

### Patients

Fourteen consecutive patients referred for autologous BMT with no history of prior gastro-intestinal disease or present symptoms from the gastro-intestinal tract were randomly assigned to treatment ( $n = 7$ ) or placebo ( $n = 7$ ) between 30 May and 13 November 1997. Patient characteristics are described in Table 1. The study protocol was approved by the Ethical Committee and the Isotope Committee of Sahlgrenska University Hospital and all patients gave informed, written consent before randomisation.

### Methods

The trial was a randomised, double-blind, placebo-controlled trial. A schematic description of the transplantation procedure and study design is presented in Figure 1.

The treatment group received orally 45 ml of an IgA-IgG preparation made from human serum (IgAbulin; Immuno AG, Vienna, Austria), described in detail earlier

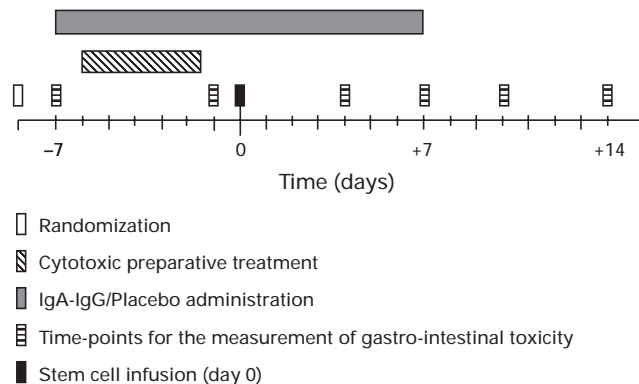
Bone marrow transplantation has improved the prognosis for patients with haematological malignancies, and encouraging results have been achieved with some solid tumours.<sup>1</sup> However, transplantation-related toxicity, especially to the gastro-intestinal tract, prevents further dose escalation and

**Table 1** Baseline clinical characteristics and chemo/radiotherapy of the patients in the two study groups

Characteristic	Placebo (n = 7)	IgA-IgG (n = 6)
Age (years)	50.9 ± 9	41.5 ± 16
Weight (kg)	80.7 ± 12.4	83.4 ± 12.7
Male sex (percent of group)	71	100
GFR (ml/min/1.73 m <sup>2</sup> )	80.9 ± 21.7	95.3 ± 10.8
s-protein (g/l)	61.9 ± 6.2	62.2 ± 6.2
Interval since latest received cytostatics (days)	31.9 ± 8.1	24.0 ± 7.1
Disease (n)		
CLL	1	0
NHL	4	6
Myeloma	2	0
Transplant (n)		
ABSCT	4	5
ABMT	0	1
ABMT/ABSCT	3	0
Chemo/radiotherapy (n)		
Cyclo/TBI	3	1
BCNU/Mel/TBI	3	0
BEAM	1	5
TBI (n/percent of group)	6/86	1/17*

Mean values ± s.d. are shown. \**P* < 0.05 (Fisher's exact test).

GFR = glomerular filtration rate; CLL = chronic lymphocytic leukaemia; NHL = non-Hodgkin lymphoma; ABSCT = autologous blood stem cell transplantation; ABMT = autologous bone marrow transplantation; ABSCT/ABMT = combined ABSCT and ABMT; Cyclo = cyclophosphamide; TBI = total body irradiation (10 Gy fractionated on four occasions); Mel = melphalan; BEAM = BCNU/etoposide/Ara-C/melphalan.

**Figure 1** Schematic description of the transplantation procedure and the study design.

by others<sup>6</sup> daily, divided into three doses. Treatment was given from the day prior to the start of the cytotoxic treatment until 7 days after the infusion of the stem cells (totalling 14 days). The dose was estimated to correspond to the daily amount of IgA synthesised and secreted throughout the bowel in man.<sup>10</sup> The placebo group received an equivalent volume of Haemaccel (Behringwerke AG, Marburg, Germany) three times daily during the same period. This substance was used because it was regarded as inert and had a colour, taste and consistency very similar to the immunoglobulin preparation. Inert flavourings (Apoteket AB, Production Department, Gothenburg, Sweden) were added to both the substances. If any of the

test substances were vomited within an hour after intake, this was regarded as a dropout of a dose.

The principal end-points of the study were the change in intestinal permeability and cumulative clinical toxicity within the study period (during the period of drug administration and 1 week after).

Gastro-intestinal permeability was assessed by a <sup>51</sup>Cr-EDTA-absorption test<sup>11,12</sup> before the start of the cytotoxic treatment, 1 day prior to stem-cell infusion (the day after termination of the cytotoxic treatment) and 4, 7, 10 and 14 days after stem-cell infusion. In four patients (two from each group) urine was collected in 24 h portions for the whole study period.

During the same period, gastro-intestinal toxicity according to the WHO criteria<sup>13</sup> was graded (grades 0–4) by a nurse and by a dentist, both unaware of the permeability data and of what treatment the patients had received. All enteral and parenteral nutrition was monitored daily for each patient, except for one patient in the treatment group (due to insufficient data). Blood, urine and other appropriate specimens were obtained for culture when the temperature exceeded 38°C. All patients received the same prophylactic antibiotics (ciprofloxacin, aciclovir and fluconazole) throughout the study period and anti-emetic therapy (ondansetron) during the preparative cytotoxic therapy.

All data were stored and prepared in a computer and the treatment code was broken 1 month after inclusion of the last patient.

## Statistics

Data are expressed as means ± s.d. The Mann–Whitney *U* test was used for significance calculations of differences in 24-h urinary excretion between and within the two study groups. A *P* value < 0.05 was considered statistically significant.

## Results

### Acceptance of the treatment and patient characteristics

One patient accepted only one dose of the test substance (IgA-IgG) and was therefore excluded. One hundred and six out of 294 doses (36%) were missed in the placebo group (predominantly due to nausea and/or vomiting) and 80 of these (75%) were missed during the second week of treatment. The corresponding data for the IgA-IgG group were 123 missed doses out of 252 (49%) of which 101 (82%) were during the second week. Apart from the disagreeable taste of both the substances, no other side-effects were recorded. There were no significant differences between the two study groups as regards baseline clinical characteristics, but a significantly greater proportion of the patients assigned to the placebo group received total body irradiation in their cytotoxic treatment (86 vs 17%, *P* < 0.05, Fisher's exact test) compared with the treatment group (Table 1).

**Table 2** Clinical outcome according to treatment assignment

Variable	Placebo (n = 7)	IgA-IgG (n = 6)
Administered doses (n)	25.7 ± 12.8	21.5 ± 8.7
Fever ≥38°C (days)	6.4 ± 3.8	6.3 ± 2.8
Antibiotic treatment (days)	7.6 ± 3.6	8.0 ± 3.5
Positive blood cultures (n/percent of group)	2/29	1/17
CRP ≥100 (days)	2.4 ± 3.8	0.4 ± 0.6
TPN (days)	7.1 ± 5.3	9.5 ± 3.3
G-CSF treatment (days)	6.1 ± 3.3	5.6 ± 1.3
Infused stem cells (CD34 × 10 <sup>6</sup> /kg bw)	2.7 ± 2.4	3.5 ± 1.6
Time to engraftment (LPK ≥1.0 × 10 <sup>9</sup> /l; days)	12.0 ± 3.2	13.7 ± 9.0
Discharge (day)	22.3 ± 10.3	18.7 ± 6.7

Mean values ± s.d. are shown. TPN = total parenteral nutrition; CRP = C-reactive protein; G-CSF = granulocyte colony-stimulating factor.

*Oral intake of food and non-intestinal clinical outcome*

The mean, daily, oral intake of energy and protein did not differ as between the two study groups throughout the study period (data not shown).

A slight, but insignificant dominance regarding the number of days with C-reactive protein exceeding 100 in the placebo group was observed but otherwise, infectious complications and non-intestinal, clinical outcome were very similar in the two study groups (Table 2).

*Intestinal permeability*

In the placebo group, but not in the IgA-IgG group, there was a significant increase in intestinal permeability on day 4 ( $P < 0.005$ ) and on day 7 ( $P < 0.05$ ) after stem-cell infusion, compared with the baseline. In a comparison with the placebo group, patients who received IgA-IgG had significantly less intestinal permeability on day 4 ( $P < 0.05$ ) and on day 7 ( $P < 0.05$ ) after stem-cell infusion (Table 3). In four patients from whom urine was collected during the whole study period no significant radioactivity was detected beyond 48 h after the intake of <sup>51</sup>Cr-EDTA. Permeability

**Table 3** Intestinal permeability (<sup>51</sup>Cr-EDTA resorption; % ± s.d.) in the two study groups

Day	Placebo (n = 7)		IgA-IgG (n = 6)		P <sup>b</sup>
	<sup>51</sup> Cr-EDTA	n <sup>a</sup>	<sup>51</sup> Cr-EDTA	n <sup>a</sup>	
Baseline	2.0 ± 1.1	6	1.3 ± 0.8	6	NS
-1	2.4 ± 1.6	5	1.5 ± 0.5	5	NS
+4	7.3 ± 2.0 <sup>c</sup>	6	4.4 ± 2.7	5	<0.05
+7	3.6 ± 0.7 <sup>d</sup>	4	2.4 ± 0.5	4	<0.05
+10	3.4 ± 2.6	5	2.2 ± 0.6	4	NS
+14	3.0 ± 1.7	3	1.3 ± 0.8	5	NS

<sup>a</sup>The number of permeability assessments for each group.  
<sup>b</sup>Compared with the placebo group.  
<sup>c</sup> $P < 0.005$  compared with the baseline for the placebo group.  
<sup>d</sup> $P < 0.05$  compared with the baseline for the placebo group.

data were obtained on 29 out of 36 measurement days in the IgA-IgG-group and on 29 out of 42 days in the placebo group. Missed values were due to discharge of the patient before day 14 (one patient in the IgA-IgG group), vomiting (IgA-IgG = 4, placebo = 7), technical problems (usually inadvertent loss of urine sample; IgA-IgG = 2, placebo = 3) and in three cases because of very severe, oral toxicity, making the test substance impossible to swallow (all from the placebo group).

*Clinical toxicity*

The cumulative, clinical toxicity during the entire transplantation course did not differ between the two study groups for either oral or non-oral clinical toxicity (nausea/vomiting and diarrhoea taken together (data not shown)).

**Discussion**

Intestinal barrier function has implications for the aetiology and pathogenesis of various intestinal diseases and intestinal permeability is an accepted parameter for evaluating gut damage.<sup>14</sup> There is a disruption of the intestinal barrier in bone marrow transplantation,<sup>12,15,16</sup> during remission-induction therapy for acute myeloid leukaemia<sup>17</sup> and also in inflammatory bowel disease.<sup>18</sup> Preservation of the gut mucosa barrier is known to be of importance in preventing the passage of viable enteric bacteria or endotoxins through the intestinal epithelial barrier (bacterial translocation) in mice<sup>19</sup> and maybe in humans.<sup>20-22</sup> Therefore, the main finding in this randomised pilot study – a protection of the intestinal barrier function among BMT patients receiving IgA-IgG – seems to be of some relevance despite not modifying clinical toxicity.

The small number of patients in this trial was a consequence of the fact that IgA-IgG made from human serum is a very expensive treatment (£4200 per patient) and at the time of the study there was also limited access to the drug. Therefore it seemed reasonable to test this novel approach in a small pilot study before initiating a larger randomised trial.

Potential factors that may influence gut morphology and subsequent disruption of the intestinal barrier (primarily enteral feeding)<sup>23,24</sup> were comparable between the two study groups. No differences between the groups as regards factors which may influence <sup>51</sup>Cr-EDTA excretion, eg renal function or intestinal transit time (evaluated indirectly by the incidence of vomiting or by the absence of delayed urinary excretion in four patients), were identified. There was an imbalance between the groups regarding the number of patients receiving total body irradiation (TBI) in their preparative cytotoxic treatment. However, currently there is no evidence that preparative regimens with TBI are associated with aggravated intestinal toxicity<sup>12,15</sup> and thus, it is unlikely that this imbalance could have been responsible for the difference, as regards intestinal permeability between the groups.

The question is how IgA may contribute to the preservation of the intestinal barrier. Secretory IgA (sIgA), con-

sisting predominantly of the dimeric form of IgA, is known to have the ability to prevent bacterial translocation *in vitro*.<sup>25</sup> By preventing translocation, secondary inflammation and subsequent barrier disruption may also be prevented. However, the exact mechanisms involved are not entirely clear and since most of the IgA in the immunoglobulin preparation studied was prepared from serum and was therefore predominantly monomeric, other mechanisms may be conceivable. For example, one may speculate that IgA exerts a direct, anti-inflammatory effect, not mediated through its anti-infectious properties. This hypothesis may be supported by the fact that human serum IgA *in vitro* downregulates the release of inflammatory cytokines in human monocytes.<sup>26</sup> A recently published case report which showed that orally administered IgA (IgAbulin) diminished intestinal permeability in two patients with severe Crohn's disease who had failed to respond to traditional medical therapy may give additional support to this hypothesis of a direct, anti-inflammatory effect.<sup>27</sup> In the present, study there was a tendency to a more pronounced, inflammatory response (days with C-reactive protein  $\geq 100$ ) in the placebo group, but this difference did not reach statistical significance.

We failed to demonstrate any significant effect on clinical, intestinal toxicity according to the WHO criteria. This may be explained by the fact that significant differences with respect to a qualitative, clinical scoring system may be difficult to demonstrate in a small study. An alternative explanation may be that the intestinal-barrier preservation is subclinical. Furthermore, despite the addition of flavourings, the acceptance was low (about 50% of the planned doses) in both the treatment groups. Duration of the treatment was based on the experience that clinical toxicity and disruption of the intestinal barrier seem to peak 4–7 days after stem cell infusion. It is true that the optimal duration of the treatment is uncertain, but the fact that about 80% of the dose drop-outs occurred during this period may be important when evaluating the efficacy of the treatment.

A variety of systems, including gut-protective nutrients, growth factors and the local, gastro-intestinal, immune system regulate the proliferative response of the gut.<sup>28</sup> Intestinal injury induced by cytotoxic therapy may disturb all of these protective and proliferative systems. In such a complex system, a major protective effect cannot be expected to be attained with a single-drug intervention concept and therefore further intervention trials of the intestinal toxicity induced by cytotoxic drugs should perhaps combine different protective strategies.

In conclusion, gut damage, irrespective of how it is induced, seems to be associated with a disruption of the gut mucosal barrier. Orally administered immunoglobulins to patients undergoing intensive cytotoxic therapy prior to BMT in this randomised pilot study seemed to have a protective effect on the gut mucosa barrier. However, further and larger randomised trials are warranted in order to evaluate the clinical significance of this finding. In such a trial other immunoglobulins, not extracted from serum may be used.

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