EFFECT OF MONOMERIC IMMUNOGLOBULIN G (IGG) ON THE CLEARANCE OF SOLUBLE AGGREGATES OF IGG IN MAN


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SUMMARY Serum concentrations of IgG may influence Fc receptor-mediated clearance of immune complexes. For instance, when 123I-labeled aggregates of human IgG (123I-AIgG), used as a model for soluble immune complexes, are administered to patients with systemic lupus erythematosus (SLE), there is an inverse correlation between the serum concentrations of IgG and the clearance and volume of distribution in steady state (Vss) of 123I-AIgG. To answer the question whether IgG has a direct effect on the clearance of immune complexes, we measured the elimination of 123I-AIgG in eight patients with hypogammaglobulinemia, before and after substitution with intravenous gammaglobulin (IVIG). As expected, raising IgG concentrations in these patients (by 6 g/l) caused a significant decrease of the Vss of 123I-AIgG. However, clearance of 123I-AIgG remained unchanged by IVIG. Thus, the results of this study offer no experimental evidence that raising concentrations of IgG influences the clearance of soluble immune complexes.

Key words: Immunoglobulin G, aggregates of immunoglobulin G, Fc receptor function, immune complex endocytosis, systemic lupus erythematosus

INTRODUCTION

Impairment of Fc receptor-mediated clearance may play an important role in immune complex disease, as for instance systemic lupus erythematosus (SLE) (1). Fc receptor-dependent clearance is thought to be influenced by the plasma concentration of monomeric IgG. This is based on the observation that the elimination of erythrocytes coated with IgG (E–IgG) decreases with increasing plasma concentrations of IgG (2). Furthermore, administration of intravenous immunoglobulin G (IVIG) reduces the clearance of E–IgG (3). E–IgG is a particle-like probe and clearance of E–IgG is largely a measure of splenic Fc receptor function (4). The effect of the serum concentration of IgG on hepatic Fc receptor-mediated clearance is unknown.

Hepatic Fc receptor function can be assessed by evaluating the disappearance of aggregates of IgG from the circulation (5, 6). These aggregates have biologic activities similar to soluble immune complexes: they activate complement, subsequently bind to C3b receptors (CR1) on erythrocytes, and are cleared by hepatic Fc receptor-mediated mechanisms. Using soluble 123I-labeled aggregates of human IgG (123I-AIgG) as a model

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for immune complexes in patients with SLE, we found that clearance of $^{123}$I-AlgG was strongly and inversely correlated with plasma IgG concentration (7). From this study the question rose whether plasma IgG interferes directly with the clearance of soluble immune complexes, similarly to its effect on the elimination of E–IgG (3). To answer this question, we measured the clearance of $^{123}$I-AlgG in patients with hypogammaglobulinemia, before and after administration of IVIG.

**Materials and Methods**

**Patients**

Eight patients with hypogammaglobulinemia (7M, 1F, aged 20–34 years) participated in the study. Diagnoses were X-linked agammaglobulinemia or late onset hypogammaglobulinemia. The patients were on regular substitution therapy with intravenous or subcutaneous IgG, and all were free of infection at the time of study. They were studied before and after substitution with IVIG (Central Laboratory of the Blood Transfusion Service, Amsterdam, The Netherlands). IVIG was given twice: 18 g were given immediately following the first clearance experiment, and this was repeated on the day before the second clearance experiment. The time between the two clearance studies was seven days.

To prevent thyroid uptake of $^{123}$I, all subjects were given NaI orally 12 and 2 hours prior to the experiments. Blood pressure and pulse rate were monitored during the experiment.

The study protocol was approved by the local Ethical Committee. All patients gave informed consent, before participating in the study.

**Preparation of Human $^{123}$I-AlgG**

Aggregates of human IgG were prepared as previously described and stored at $-70^\circ$ (6). On the day of the study 1 ml of aggregates was thawed and mixed with 15 MBq (1 Megabecquerel = 0.027 mCi) Na$^{123}$I in a sterile tube, coated with Iodogen (8). After 15 min the radio-labeled solution was passed over a 0.22 μm filter. The filter was flushed with saline and an aliquot of the final solution was given to each subject. The amount of radioactivity administered was 0.1 MBq/kg body weight, of which 50% was associated with aggregates of IgG (as assessed by PEG precipitation, see below).

**Clearance of $^{123}$I-AlgG**

The clearance of $^{123}$I-AlgG was assessed as described previously (7). The subjects received approximately 1 mg of $^{123}$I-AlgG intravenously over 30 sec. From the opposite forearm serial blood samples were collected into chilled tubes, containing heparin. The samples were kept on ice until processed, 150 min after injection. Radioactivity was counted in 0.5 ml samples of whole blood and plasma. Aggregate-bound radioactivity was assessed by treating plasma samples with 3% (final concentration) polyethylene glycol (PEG) for 30 min at 0°, followed by centrifugation and counting of the precipitate. At this PEG concentration at least 95% of IgG aggregates and less than 5% of monomeric IgG is precipitated (6). Erythrocyte-bound radioactivity was measured after washing 0.5 ml samples of whole blood twice with 2 ml of PBS at 0°. Radioactivity was counted in a Minaxi Auto-Gamma 5530 Counter (Packard, Downers Grove, IL, USA).

The amount of PEG-precipitable radioactivity in plasma per ml of whole blood was calculated as:

$$(\text{whole blood} - \text{E}) \times (\text{PEG/plasma}) \text{ (in cpm/ml)}.$$

In this formula E stands for erythrocyte-bound cpm; PEG for PEG-precipitable cpm. The total amount of aggregate-bound radioactivity per ml of whole blood was then calculated by adding the PEG-precipitable cpm/ml, obtained with this formula, to the erythrocyte-bound cpm per ml. This total amount of $^{123}$I-AlgG (PEG-precipitable cpm in plasma and erythrocyte-bound cpm) per ml of whole blood was subsequently used in all calculations.

Concentrations of serum IgG were measured by radial immunodiffusion. Anti-IgG antibodies were obtained by immunisation of rabbits with purified IgG and rendered gamma-chain specific by absorption with kappa and lambda light chains. As a standard human serum of a known IgG concentration was used.

As $^{123}$I-AlgG is heterogeneous in size, disappearance of aggregates from the circulation was assessed in more detail by sucrose density gradient ultracentrifugation of plasma samples, as previously described (6).

**Organ Uptake of $^{123}$I-AlgG**

Radioactivity over hepatic and splenic areas was registered continuously with a gamma camera (GCA 40 A, Toshiba, Tokyo, Japan) for 1 h after the injection, using regions of interest (7). Organ counts were corrected for background. Background counts were obtained from a standard area below the aortic bifurcation. Organ uptakes were calculated as (organ minus background)/background ratio. Maximal uptake ratios and the time of maximal uptake were determined.

**Pharmacokinetic Analysis and Statistics**

Residual radioactivity-time curves of the total amount of aggregates per ml of blood (PEG-precipitable and
E-bound, calculated according to the formula described above) were plotted. The disappearance curves were analyzed by non-linear regression analysis, according to standard pharmacokinetic methods (9), using the computer programme SIPHAR (Sciméd, Créteil, France). A biexponential model was used. The area under the curve (AUC) was calculated by trapezoidal rule, with extrapolation to infinity. Clearance was calculated as dose/AUC. Volume of distribution in steady state (Vss) was calculated as (dose x AUMC)/AUC^2, in which AUMC is the area under the curve of the products of time and concentration. The dose of radioactivity entered in the calculations was the number of PEG-precipitable cpm administered. Clearance and Vss were normalized for body weight.

Group means were compared using Student's t-test for paired data. The disappearance curves before and after IVIG in the hypogammaglobulinemic patients were compared by applying the paired t-test at each time point, with correction for multiple testing. To compare the effect of IgG concentrations on the pharmacokinetics of 123I-AIgG in the hypogammaglobulinemic patients with the results obtained previously in patients with SLE (7), data from this study were reanalyzed. In 22 patients with SLE significant correlations were present between levels of IgG (range 1–25.4 g/l) and various pharmacokinetic parameters of 123I-AIgG (clearance, Vss and first halflife). With these data regression analysis was performed. The concentration of IgG was taken as the independent variable, and the pharmacokinetic parameters (clearance, Vss, and halflives) were taken as dependent variables. In the resulting regression equations, we substituted the values of the IgG concentrations obtained in the patients with hypogammaglobulinemia (4.6 and 11.0 g/l, see below) to predict the various pharmacokinetic parameters in the SLE patients for these given IgG concentrations. The predicted changes (percent increase or decrease from the value at the lower IgG concentration) in these parameters in the SLE patients were compared with the changes observed in the patients with hypogammaglobulinemia, before and after IVIG. Software used was SPSS. Values are given as mean ± SEM.

RESULTS

Administration of 123I-AIgG was well tolerated by the subjects. The disappearance curves could be described very well with the biexponential model. Correlation coefficients were >99% for all curves. Thus, the disappearance curves had two components: the first having a short halflife (T_1), the second having a longer halflife.

After administration of 2 × 18 g IVIG serum IgG concentrations rose from 4.6 ± 1.1 to 11.0 ± 1.2 g/l (Table). Analysis of the disappearance curves of 123I-AIgG showed that after treatment with IVIG residual aggregate-bound radioactivity was consistently higher at 30 min and later time points after injection (Figure 1). When the amount of residual 123I-AIgG at various time-points after injection was compared before and after administration of IVIG, this difference reached statistical significance at 60 min (p < 0.005).

Results of the pharmacokinetic analysis are shown in the Table and in Figure 2. The data shown in Figure 2 (a, b, d) are those of seven (of eight) subjects. In one

<table>
<thead>
<tr>
<th>Serum IgG</th>
<th>Before IVIG</th>
<th>After IVIG</th>
<th>Observed change (hypog.)</th>
<th>Expected change (SLE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IgG</td>
<td>4.6 ± 1.1</td>
<td>11.0 ± 1.2</td>
<td>139%*</td>
<td>139%*</td>
</tr>
<tr>
<td>Clearance</td>
<td>4.5 ± 0.4</td>
<td>4.6 ± 0.7</td>
<td>2%*</td>
<td>21%*</td>
</tr>
<tr>
<td>Vss</td>
<td>786 ± 105</td>
<td>540 ± 65</td>
<td>31%*</td>
<td>24%*</td>
</tr>
<tr>
<td>First T₁</td>
<td>547 ± 0.5</td>
<td>537 ± 0.63</td>
<td>2%*</td>
<td>20%*</td>
</tr>
<tr>
<td>Second T₁</td>
<td>174 ± 18</td>
<td>118 ± 14</td>
<td>32%*</td>
<td>3%*</td>
</tr>
</tbody>
</table>

Changes were expressed as the percentage of the value at the lower IgG concentration, which was taken to be 100%.

Observed change: hypogammaglobulinemic patients. Expected change: from data observed in SLE patients (7) and calculated by regression analysis (see Methods). * denotes an increase, † a decrease.
AFTER

Discussion

Raising IgG concentrations did not change the clearance of $^{123}$I-AIgG in patients with hypogammaglobulinemia, although a $20\%$ decrease was expected, based on the results obtained in a transversal study of SLE patients (7). In the SLE patients a highly significant inverse correlation was found between serum IgG on the one hand and clearance and Vss of $^{123}$I-AIgG on the other hand. First halflife was weakly and positively correlated with the concentration of IgG (7). Since administration of IVIG had no effect on clearance of $^{123}$I-AIgG, the decreased clearance of this probe observed in patients with SLE and elevated concentrations of serum IgG is probably not causally related to the high levels of IgG.

Of course, it cannot be excluded that continuous exposure of the MPS to a sustained high concentration of IgG, as present in the SLE patients, may affect clearance of immune complexes in a way different from the intermittently high levels of IgG in the hypogammaglobulinemic patients receiving IVIG. However, it seems likely that high IgG concentrations and decreased clearances of $^{123}$I-AIgG in this subgroup of SLE patients are independent parameters, possibly related to the activity of the disease (7).

As expected, the volume of distribution of $^{123}$I-AIgG decreased with higher levels of IgG, similarly to the relationship seen in patients with SLE. The pharmacokinetic parameter halflife is derived from Vss and clearance (the second, terminal halflife correlating with the quotient Vss/clearance) (9). Accordingly, as Vss decreased and clearance remained unchanged, the second halflife also decreased after IVIG treatment. In contrast, in the patients with SLE, the expected second halflife shows no change with higher concentrations of IgG, as both Vss and clearance are decreased proportionally at these higher IgG concentrations ($T_1 \sim \text{Vss/clearance}$).

In physiological terms, the decreased Vss of $^{123}$I-AIgG after IVIG suggests that IgG impairs the binding of $^{123}$I-AIgG to Fc receptors, by occupying available Fc receptors on Kupffer cells (10). However, the lack of an effect of IVIG on clearance of $^{123}$I-AIgG seems to indicate that endocytosis (as opposed to binding) of immune complexes is not influenced by plasma IgG. This is in accordance with in vitro studies in which the binding of affinity cross-linked IgG oligomers (used as model immune complexes) to Fc receptors was investigated. Monomeric IgG retarded the rate of association of these IgG oligomers with Fc receptors (11) but did not affect endocytosis (12).

It has been shown that IVIG prolongs the clearance of E–IgG by the spleen (3). Splenic Fc receptor blockade

Patient no statistically reliable fit was possible for the second phase. Therefore, the first halflife is the only pharmacokinetic parameter used in this patient.

Pharmacokinetic analysis revealed that clearance and first halflife of $^{123}$I-AIgG did not change after IVIG (Table, Figure 2A, 2C). However, Vss (Figure 2B) and second halflife (Figure 2D) decreased significantly ($p=0.04$ and $p=0.049$, respectively). The changes in pharmacokinetic parameters secondary to IVIG therapy were compared with the changes predicted from the data obtained in 22 patients with SLE (7). A similar increase of IgG concentration in the SLE patients should have resulted in a decrease in clearance and Vss, an increase in the first halflife and no change in the second halflife (Table).

Organ uptakes did not change significantly. Maximal liver uptake ratio was $2.7 \pm 0.2$ before and $2.6 \pm 1.0$ after IVIG. Maximal splenic uptake ratio was $1.4 \pm 0.3$ before and after IVIG. The time to reach maximal uptake in liver and spleen did not change after IVIG.

Sucrose density gradient ultracentrifugation experiments were performed in several patients before and after IVIG. Results of a representative experiment are shown in Figure 3. There is a rapid disappearance of the larger sized aggregates in the first 30 min after injection. No changes were seen after treatment with IVIG.
Figure 2. Clearance (A), Vss (B), first (C) and second (D) halflife in patients with hypogammaglobulinemia, before and after IVIG. Asterisk: p<0.05.

Figure 3. Sucrose density gradient ultracentrifugation profile of $^{123}$I-AIgG in plasma at different time points after injection in a patient with hypogammaglobulinemia after treatment with IVIG. The data are presented as percent of the radioactivity in plasma 3 min after injection $^{123}$I-AIgG.
probably explains the therapeutic efficacy of IVIG in immune thrombocytopenic purpura (13), where the immune complex is a particle, similarly to E-IgG. IVIG has also been administered therapeutically in patients with SLE, but results so far have been equivocal (14–16). The results presented here certainly offer no experimental evidence that IVIG has an effect on the clearance of soluble immune complexes by Fc receptor mediated mechanisms in vivo. Theoretically, if the manifestations of SLE are caused by deposition of soluble immune complexes in vessel walls and glomeruli, as a consequence of impaired Fc receptor function (1), IVIG induced Fc receptor blockade should not be expected to exert a beneficial effect in this disease. However, IVIG may have other immune modulatory effects, that may be of therapeutic use in patients with SLE. These include the proposed anti-idiotypic antibodies contained in the preparation (17), or its effects on autoantibody production (18, 19).

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REFERENCES
