IMMUNE-MEDIATED REACTIONS TO ALTHESIN (ALPHAXALONE)

J. Watkins, A. Clark, T. N. Appleyard and A. Padfield

SUMMARY

Measurements of complement conversion and white cell variations were made on sequential blood samples obtained from a single volunteer following repeated administration of Althesin. The results suggest a mechanism by which a clinically significant hypersensitivity reaction to the drug might be mediated. Studies of patients receiving routine anaesthesia revealed a very high incidence of subclinical "hypersensitivity" reaction, some of which appear to be immune-mediated. These reactions occurred irrespective of whether the patients were induced with Althesin, methohexitone or propanidid.

Althesin (Glaxo Laboratories Ltd, Greenford, Middlesex) comprises the potent pregnane derivative anaesthetic drug, 3α hydroxy-5α pregnane-11,20 dione (alphaxalone), a surfactant (cremophor) and a co-solubilizing related steroid (alphadolone acetate).

Although Althesin is a satisfactory induction agent, a number of adverse reactions which resemble immediate hypersensitivity responses have been reported (Sutton, Garrett and McArdle, 1974; Clarke et al., 1975). Reactions similar in character to those associated with Althesin have been reported for propanidid (Clarke, 1974), and, to a lesser degree, for thiopentone (Dundee and Wyant, 1974) and methohexitone (Driggs and O'Day, 1972; Wyatt and Watkins, 1975). Although histamine release appears to be a common factor in most of the reactions, the actual mechanisms remain obscure since any tests on the patients have usually been performed at a considerable time after the reaction. Of 30 patients who had exhibited a hypersensitivity type of reaction to i.v. anaesthesia and who were referred to us for investigation, only nine could be characterized as showing immune-mediated responses, and only one a pharmacological response (Watkins et al., 1976). It seemed that an understanding of the mechanisms of the adverse reaction was more likely to arise from detailed experiments with Althesin on a single healthy volunteer than from a random study of patients examined retrospectively. As a result of a preliminary investigation the experiments were extended to patients receiving a variety of anaesthetic induction agents.

METHODS

Experimental protocol

Outline. The first two experiments, 1 week apart, were designed to sensitize the volunteer to Althesin. Radio-labelled Althesin was used in these experiments to determine the pattern of elimination of the drug from the body. One month later the same volunteer was exposed to the surfactant alone. After a further month he was treated with sodium cromoglycate, an immune receptor blocker (Pepys et al., 1968), before a third exposure to Althesin.

Detailed procedure. The subject (J. W.: male; age 39 yr; wt 76 kg), was positioned supine and two i.v. cannulae were inserted: one in the left antecubital fossa, the other in the dorsum of the right hand. The latter was used as a route for the induction of anaesthesia and the former as a route for obtaining blood samples. On the first two occasions the anaesthetic was used as a route for the induction of anaesthesia and the former as a route for obtaining blood samples. On the first two occasions the anaesthetic was 1.5 ml of 14C-labelled Althesin mixed with 1.5 ml of plain Althesin. The total dose (3 ml) was injected over 20 s. Anaesthesia was produced on both occasions.

Blood samples were taken into heparinized tubes for lymphocyte activity studies and into EDTA tubes for plasma and for white cell counts. Samples were taken immediately before anaesthesia and then at intervals of 2, 5, 10, 20, 30 and 60 min after the drug injection. Exposure to cremophor EL (3 ml) alone followed the same procedure as that detailed above.

In the final experiment the volunteer inhaled the contents of four 20-mg “Spincap” cartridges (active
ingredient sodium cromoglycate, "Intal": Fisons Ltd, Pharmaceutical Division, Loughborough) before induction of anaesthesia with Althesin 3 ml.

Patient studies
The patients were attending the urology clinic, Hallamshire Hospital, Sheffield, for cystoscopy under general anaesthesia. Anaesthesia was induced with Althesin, methohexitone or propanidid and maintained with halothane and nitrous oxide in oxygen. The induction technique and blood sampling procedure followed the schedule described for the volunteer.

Althesin preparations. Standard formulations of Althesin were used. The radioactive preparation had a radiolabel on the 21 carbon atom of the alphaxalone molecule in a concentration of 5 μCi/ml.

Radioactive measurements of 14C-containing plasma samples. Plasma samples (1 ml) were solubilized with "Instagel" (Packard Instrument Co. Inc.) and counted. The residual radioactivity was plotted against the sampling time (fig. 1).

The total white blood cell and differential counts were measured in the Haematology Department of the Northern General Hospital, Sheffield, on EDTA blood samples.

Measurement of complement and complement conversion. Total complement C3 and C4 was assayed immunochemically. The degree of C3 conversion in samples was assessed by two-dimensional immuno-electrophoresis (Laurell, 1965; Watkins et al., 1976).

Investigation of unstimulated lymphocyte activity. Lymphocytes were separated from peripheral blood samples (5 ml) by centrifugation on a Ficoll–Triosil mixture (Harris and Ukaejiofo, 1970). Each preparation was divided into two equal aliquots to which was added either autologous plasma or inactivated foetal calf serum to a final concentration of 20%. Tripclicate cultures containing approximately 3 x 10^6 viable lymphocytes and 0.2 μCi of 3H-thymidine were incubated at 37 °C for 1 h and then harvested on glass fibre filters using an automatic harvesting system. Thereafter they were dried and counted. The results were expressed as counts per minute (c.p.m.).

RESULTS
The elimination pattern of 14C-Althesin in the volunteer (fig. 1) indicates (a) that the drug travels around the vascular system as a bolus for an appreciable period of time (2 or 3 min) and (b) that appreciable radioactivity persists in the plasma even after 24 h.

White blood cell patterns
Volunteer. The variations in total white cell counts following the administration of Althesin are illustrated in figure 2. There was no significant change caused by the administration of cremophor alone. The first exposure to Althesin caused a 20% increase in the total white cell count, peaking at 5 min after induction (compare with fig. 1). This effect disappeared entirely over the next 15 min. In contrast, the second Althesin induction caused a very significant reduction in the white cell count, over the same period. The third induction, following the administration of cromoglycate, resulted in an increase in the white cell count (fig. 2) similar to that of the first induction. The lymphocyte counts for the three Althesin inductions are illustrated (fig. 3); it can be seen that these cells show a similar apparent increase in numbers for all three experiments. The reduction in white cell count observed in the second experiment is the result of a temporary exclusion of polymorphs from the sampling point.

Patients. The data obtained from 21 patients are summarized in table I. A significant change in white cell types and numbers was observed in 10 out of 21 of these patients. The significance of variation in count was based on a measured deviation greater than 20% of the patient's white cell count before and after anaesthesia. A deviation of ±20% corresponded to twice the mean standard deviation for the total white cell counts in the same range measured routinely.
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Fig. 2. Variations in total white cell counts measured following the three inductions with Althesin. ○ = first induction; △ = second induction, 7 days later; • = third induction, following administration of cromoglycate. The error for counting white cells at this level as measured for similar total counts routinely approximates to a mean standard deviation of ±10%. This is less than the measured variations in these experiments. A similar error applies to the differential counts (fig. 3).

Table I. Changes in white blood cell counts in patients receiving general anaesthesia

<table>
<thead>
<tr>
<th>Induction agent</th>
<th>No. of patients</th>
<th>Total showing WBC response</th>
<th>Increasing WBC response (first exposure)</th>
<th>Decreasing WBC response (repeated exposure)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Althesin</td>
<td>7</td>
<td>3</td>
<td>3/6</td>
<td>0/1</td>
</tr>
<tr>
<td>Propanidid</td>
<td>7</td>
<td>4</td>
<td>3/4</td>
<td>1/2</td>
</tr>
<tr>
<td>Methohexitone</td>
<td>7</td>
<td>3</td>
<td>1/3</td>
<td>2/4</td>
</tr>
<tr>
<td>Totals</td>
<td>21</td>
<td>10</td>
<td>7/13</td>
<td>3/7</td>
</tr>
</tbody>
</table>

The white cell behaviour patterns were identical to those observed in the volunteer and there was no evidence to suggest modification by the halothane, nitrous oxide and oxygen mixture. Therefore the observed effects were attributed to the induction agents.

Increasing white cell counts occurred in seven of 13 patients receiving general anaesthesia for the first time. Decreasing polymorph counts were observed only in three out of seven patients who had received a previous exposure to the same anaesthetic, irrespective of the induction agent.

Lymphocyte activity studies

Plasma separated from the blood samples used for harvesting lymphocytes was retained to provide autologous plasma samples which conceivably would contain either Althesin or its metabolites. Lymphocytes were incubated with as many of these plasma samples as was practical and gaps in table II represent evaluations which were not possible technically. Foetal calf serum provided a control in all experiments.

Lymphocyte preparations obtained from the first Althesin induction showed no significant difference
in thymidine uptake either between samples obtained at the various times or between those samples incubated with autologous plasma and those with foetal calf serum (table II, columns A1). Lymphocytes harvested during the second experiment showed an uptake, on average, two- or three-fold greater than cells in the first experiment. Cells harvested at 1 min following induction (fig. 1) showed even greater thymidine uptake, irrespective of the incubation plasma (table II, columns A2). The uptake by lymphocytes after cromoglycate desensitization before the third induction was similar to that of the original experiment (table II, columns A1).

**Changes in complement proteins**

The C3 and C4 complement concentrations of the volunteer remained constant during anaesthesia. However, a small amount (< 10%) of transient C3 conversion was observed during the first experiment, 30 min after induction (fig. 4A), but this disappeared rapidly. Its appearance correlated with the observed increase in the total number of white blood cells. The second induction caused a greater degree of conversion which persisted throughout the 60-min period (fig. 4B). These reactions caused no observable clinical responses in the individual.

**DISCUSSION**

Following induction of anaesthesia with Althesin, the lymphoid cells in the vascular system are exposed to high concentrations of the drug. The persistence of plasma radioactivity at 24 h after induction implies plasma protein binding of Althesin metabolites and the existence of potentially antigenic material throughout the body. Both these factors could lead to an immune sensitization to the drug, and this would...
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normally imply the production of IgE antibodies. Subsequently, these antibodies liberate histamine in immune-mediated immediate hypersensitivity reactions without the intervention of complement. However, complement conversion is a feature of the observed anaphylactic responses to Althesin (Watkins et al., 1976). This may be explained by the production of non-IgE anaphylactic antibodies which utilize complement (Parish, 1970), or by a secondary mechanism involving complement directly. Pharmacological effects attributed to Althesin, involving limited histamine release into the plasma from basophil degranulation, have been reported previously (Doenicke et al., 1973). These effects are direct and not do involve complement. The apparent increase in white blood cell numbers in our volunteer on first administration is explicable by white cell chemotaxis being induced by C3 activation (fig. 4A). This C3 activation can occur by means of the "alternate pathway" mechanism without involving immune recognition, and again leads to vasoamine release. In contrast, the apparent decrease in polymorphs on the second induction, while also explicable in terms of complement activation, implies additionally a degree of immune recognition, since the complement activation pathway involving components C5, C6 and C7 (the products of which are specifically chemotactic for polymorphs) requires antigen–antibody interaction for initiation. High-level activation of these complement components results in temporary margination of polymorphs on the walls of capillaries such that at the sampling point in the lumen of the vein there appears to be a significant decrease in the number of these cells. The observation that cromoglycate apparently caused a reversion to the original increasing white cell pattern suggests that these particular patterns are not immune-mediated, but arise from activation of the C3 alternate pathway. Our studies in patients would suggest that the various complement activation mechanisms are not restricted to Althesin.

Thymidine uptake by lymphocytes is usually considered to correlate with DNA synthesis and to provide an indication of stimulation by a specific antigen. This may be a dangerous assumption, since the studies only measure thymidine uptake into the cell, a process which may be modified by chemical (anaesthetic drug) pre-treatment. However, thymidine uptake by lymphocytes at the second induction was consistently greater than in the first induction.

Two alternative conclusions can be drawn from this observation: either the lymphocytes were sensitized immunologically to Althesin, or the lymphocytes were activated as a result of the uptake of chemical substances liberated by Althesin from other cells. Whatever the mechanism it is unlikely to be a direct pharmacological effect, otherwise the responses in the volunteer to the first and the second Althesin exposures would have been identical. Obviously some type of drug recognition was induced. Pretreatment with sodium cromoglycate before the third Althesin exposure reduced thymidine uptake by lymphocytes to that of the initial exposure and this is consistent with the existence and blocking of immune receptor (recognition) sites.

We conclude that adverse reactions to i.v. induction agents may occur by three distinct mechanisms: (a) by direct pharmacological effects causing histamine release without involving immune mechanisms, (b) by immune-mediated hypersensitivity reactions and (c) by chemical activation of complement C3 leading to histamine release. The latter could occur by temporary blocking of inhibitor enzymes which normally prevent spontaneous C3 conversion. Whereas immune-mediated reactions will always require a previous exposure to the immunogen, the mechanisms (a) and (c) can occur at the first exposure. Our continuing studies in patients indicate that some degree of C3 activation is a frequent feature of induction with Althesin, methohexitone or propanidid, although the response is apparently restricted to half the sample populations, indicating that certain individuals may be genetically predisposed to exhibit this response. The steroid structure may be particularly effective in C3 activation; the apparently higher incidence of "Althesin responses" reflects this rather than a higher degree of immune sensitization. Nevertheless, the similarity of the frequency of adverse responses between Althesin and propanidid has brought suspicion upon the common constituent cremophor. While cremophor alone appears to have little significance, its surfactant properties may enhance the immunogenicity of both propanidid and Althesin. If this is so, chemical blocking of immune receptors in all patients likely to receive multiple exposures to either Althesin or to propanidid should certainly be considered. We hope to publish details of the effects of blocking procedures in volunteers in due course.

ACKNOWLEDGEMENT

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REFERENCES


REATIONS IMMUNOLOGIQUES INTERPOSEES A L’ALTHESINE (ALPHAXALONE)

RESUME

On a procedé à des mesures de conversion du complément et des variations dans les globules blancs sur des échantillons de sang séquentiel prélevés sur un seul volontaire après administration répétée d’Althesine. Les résultats semblent indiquer un mécanisme par lequel une réaction d’hypersensibilité clinique significative à ce médicament pourrait être interposée. Des études effectuées sur des patients anesthésiés d’une manière routinière ont révélé une très haute incidence de réactions d’hypersensibilité sous-cliniques, dont certaines semblent être immunologiques interposées. Ces réactions se sont produites lorsque les patients ont été traités à l’Althesine, au méthohexitone ou au propanidid.

IMMUN-AUSGEMITTELTE REAKTIONEN AUF ALTHESIN

ZUSAMMENFASSUNG


REACCIONES INMUNO-MEDIADAS AL ALTHESIN (ALFAXOLONA)

SUMARIO

Se efectuaron mediciones de conversión del complemento y variaciones leucocitarias en muestras seriadas hemáticas obtenidas de un voluntario tras la administración repetida de Althesin (“Alfathesin”). Los resultados sugieren un mecanismo según el cual una reacción de hipersensibilidad clínicamente significativa pudiera ser mediada. Estudios de pacientes sometidos a anestesia rutinaria revelaron una frecuencia muy elevada de reacción “de hipersensibilidad” subclínica, parte de la cual parece ser inmuno-mediada. Estas reacciones ocurrieron prescindiendo de si los pacientes eran inducidos con “Alfathesin”, metohexitone o propanidid.