IDENTIFICATION AND QUANTITATION OF HYPERSENSITIVITY REACTIONS TO INTRAVENOUS ANAESTHETIC AGENTS

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SUMMARY

It is not possible to distinguish between direct pharmacological effects and immune-mediated hypersensitivity reactions by clinical observation alone and errors may occur in the absence of laboratory tests. A convenient and simple test is the measurement of plasma complement C3 consumption and conversion in sequential blood samples taken at intervals over the 24 h following an adverse response.

Interest in adverse reactions to i.v. anaesthetic agents, particularly those appearing to be related to hypersensitivity, has increased in recent years (Clarke et al., 1975). Reports of reactions to propanidid (Epontol) attributed to hypersensitivity are frequent (Clarke, 1974). Reports of hypersensitivity to thiopentone (Dundee and Wyant, 1974) and methohexitone (Driggs and O'Day, 1972), although fewer than those associated with propanidid, are similar in character. The new steroid anaesthetic, Althesin, has caused a significant number of adverse reactions of the hypersensitivity type (Sutton, Garrett and McArdle, 1974). Unfortunately it is not possible to distinguish readily between direct pharmacological effects and immune-mediated hypersensitivity reactions, the latter implying previous sensitization of the patient either to the anaesthetic agent itself or to a cross-reacting antigen. It is important to distinguish the mechanism of the adverse response because (a) the patient may require further anaesthesia at some later date, (b) it affords a critical assessment of the advantages and disadvantages of any new anaesthetic drug and (c) there may be legal implications of death during anaesthesia.

Recent investigations have suggested that certain transient changes may occur in the blood of patients who suffer an adverse reaction following the administration of an i.v. anaesthetic agent. Alterations in the concentrations of certain circulating components of the complement system, of immunoglobulin IgE and of white cell numbers have been apparent from serial sampling of blood from such patients (Watkins, Appleyard and Ward, 1975). These changes may be quantitated by simple laboratory techniques. The measurement of consumption and conversion of complement component C3 in sequential blood samples taken over 24 h following an adverse response provides a convenient laboratory assay of the adverse reactions.

METHODS

Blood samples

Venous blood (5 ml) was collected into a heparinized tube as soon as possible after the onset of the reaction and the time, relative to onset, was noted. Three further heparinized blood samples (5 ml) were collected at convenient times over the next 24 h. A further sample was taken not sooner than 5 days after the event to provide a "base-line" profile of the patient. The plasma was separated from these samples by centrifugation and stored at —20 to —25 °C pending analysis.

C3 assay: quantitation

This may be achieved by any of the standard immunochemical methods employed for the quantitation of specific plasma proteins. Suitable methods are the single radial immunodiffusion (SRID) procedure (Mancini, Carbonara and Heremans, 1965), rocket immunoelectrophoresis (Laurell, 1967) and automated immunoprecipitin (AIP) analysis (Davis, West and Ho, 1972). A suitable antiserum is Behringwerke rabbit anti-human C3 (Behringwerke A. G., Marburg-Lahn, W. Germany*). Absolute quantitation in each method is made by reference to a calibration curve constructed using commercially available standard reference serum (for example

* Obtainable in the U.K. from Hoechst Pharmaceuticals, Hounslow, Middlesex.
C3 assay: degree of conversion

This was estimated by two-dimensional immunoelectrophoresis (Laurell, 1965; Axelsen, Krell and Weeke, 1973). Satisfactory conditions for the C3 assay are:

(a) First dimension. Glass plates (5x5 cm) were coated uniformly with 1% agarose 2.4 ml (L’Industrie Biologique Française) in 0.05 mol/litre Barbital buffer, pH 8.6, containing 0.00125 mol/litre calcium lactate, at 50-55 °C. The plates were then put aside to set.

A well was punched into each gel with a cylindrical cutter (1-mm diameter), 12 mm from one edge and 10 mm from the bottom of the plate. Using a microsyringe, a plasma sample (2 μletre) was transferred into the well. The plate was then electrophoresed in a conventional low-voltage electrophoresis apparatus, either with the use of a cooling plate or in a cold room at 4 °C, such that the proteins migrated parallel to the bottom edge of the plate. Tris-Barbital buffer, 0.06 ionic strength, pH 8.8 (High Resolution Buffer, Gelman Instrument Co., Ann Arbor, Michigan), is suitable for the electrophoresis (2½ h, 5-8 V/cm gel, 7-8 mA/plate).

Separate plates are required for each sample and ideally all the samples obtained, from a particular patient should be electrophoresed together.

(b) Second dimension. The gel was cut in a line 12 mm from, and parallel to, the bottom edge of the plate. The gel beyond this line was scraped away cleanly and discarded. The remaining strip, 50 x 12 mm, contained the first dimension electrophoresis. The discarded gel was replaced with a warm solution (2 ml) of 1% agarose containing 0.0075 ml Behringwerke C3 antiserum per millilitre agarose, which was allowed to set. Electrophoresis was then carried out at right-angles to the original direction of protein migration, such that the proteins were then forced into the antibody-containing gel (18 h, 2 V/cm, 2.5-3.5 mA/plate).

The C3 and C3 breakdown products appeared as joined twin peaks, outlined by the precipitated antigen–antibody complex, towards the end of this second electrophoresis. The outline may be intensified by drying down and staining the gel with amido black. Measurement of peak areas may be achieved by any convenient method, such as planimetry, and the degree of conversion may be calculated directly from the relative peak areas.

RESULTS

Table I compares the usefulness of various techniques in assessing an immediate hypersensitivity reaction observed in a patient in whom anaesthesia was induced with Althesin. In terms of C3 quantitation, the absolute values obtained by the various techniques vary because of inherent anomalies in the techniques themselves, but the relative C3 concentrations, measured on sequential plasma samples, are consistent regardless of the assay procedure. The method of choice for most non-routine laboratories would be SRID and for this purpose simple-to-use kits are available in the M-Partigen range from Hoechst Pharmaceuticals.

<table>
<thead>
<tr>
<th>Time following clinical reaction (h)</th>
<th>C3 concentration (mg/litre)</th>
<th>C3 conversion (%)</th>
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<tbody>
<tr>
<td></td>
<td>SRID</td>
<td>Rocket</td>
</tr>
<tr>
<td>2</td>
<td>612</td>
<td>570</td>
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<tr>
<td>6</td>
<td>376</td>
<td>460</td>
</tr>
<tr>
<td>12</td>
<td>272</td>
<td>430</td>
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<tr>
<td>24</td>
<td>548</td>
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The immediate impression from the quantitation of C3 was that this protein is consumed for up to 12 h after the hypersensitivity reaction, "normal" values returning after 24 h. However, the sequence was a projection of the initial rapid hypersensitivity response whereby about 90% (in this patient) of C3 was converted to the biologically inactive breakdown product C3c (via the "active" molecule C3b) with the liberation of the small, biologically extremely active polypeptide moiety C3a (anaphylactoxin). Both native C3 and C3c were estimated as total C3 by immunochemical assay. Two-dimensional immunoelectrophoresis revealed the preponderance of the electrophoretically faster C3c protein in the plasma of the patient immediately following the reaction. Conversion appeared more usually to be about 50-60% and revealed itself in a characteristic twin peak (fig. 1A), the continuity of the peak profiles indicating the close relationship of the two molecular species involved. Elimination of the C3 breakdown product from the body proceeded at a faster rate than repopulation by synthesis of native C3, so that initially the "apparent" C3 concentration (native C3 + C3c) appeared to decrease, although it is clear from table I that the relative proportion of the native
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C3 species was actually increasing progressively following the initial reaction. After 24 h only a small amount of C3c remained (fig. 1A). In patients showing no apparent adverse reaction to the drug there was no obvious complement conversion (fig. 1B) or consumption.

Some degree of in vitro conversion of C3 did occur, the extent varying with the pretreatment of the plasma samples. However, if sequential samples were taken and treated as described, the magnitude of the in vitro effect was insignificant relative to the in vivo conversion. Even if total conversion had occurred as a result of gross mishandling of specimens, the apparent C3 consumption artefact in sequential samples would still indicate the initial in vivo conversion.

DISCUSSION

Immune-mediated hypersensitivity reactions require a prior sensitization of the individual, involving IgE antibody production. These antibodies become bound to mast cells or to basophils. Subsequent challenge with the specific antigen results in immune recognition by the bound IgE receptors which then cause immediate histamine release from the cells, resulting in the manifestations of anaphylaxis, including smooth muscle contraction, dilatation of the vascular system and tissue oedema. To date, we have received plasma samples from patients considered to have shown such anaphylactic responses to Althesin (26), methohexitone (2), and thiopentone (2). Unfortunately, sequential blood samples were obtained from only eight patients receiving Althesin, one receiving methohexitone, and one receiving thiopentone. Complement C3 involvement in the reaction mechanism was observed in seven out of the eight who received Althesin and those receiving methohexitone and thiopentone. The patients had all been exposed previously to the respective anaesthetic agents. The absence of complement involvement following Althesin was unusual in that the adverse response became significant clinically only 10–15 min after induction, following a minor gynaecological
procedure and the return to the recovery room. We consider that Althesin was not involved directly in this particular reaction. No evidence of measurable complement involvement was seen in sequential samples obtained from 20 patients receiving Althesin, propanidid, methohexitone or thiopentone, without adverse response.

It would appear, therefore, that there is reasonable evidence to indicate C3 involvement in the adverse response and conversely the usefulness of the C3 assay, particularly the degree of conversion, for assessing hypersensitivity-type reactions. This is embarrassing since, classically, complement is not considered to be involved in immediate hypersensitivity reactions! The complement conversion pattern with Althesin response differs from that in patients responding to methohexitone or to thiopentone because (a) it appears to involve an exaggerated activation of C3 by the alternate pathway, with subsequent "immediate hypersensitivity" characteristics, (b) complement conversion appears to be immediate, repopulation of humoral native C3 and elimination of the C3 breakdown products proceeding smoothly over about 24 h and (c) rapid recovery of the patients, given some type of corticosteroid therapy, within a short time of the onset. We have suggested previously a mechanism for Althesin action to explain this phenomenon (Watkins, Appleyard and Ward, 1975) which resembles that observed in patients with hereditary angioneurotic oedema (HANE). Multiple experiments on a healthy volunteer have indicated immune recognition of Althesin by lymphocytes even in the absence of a full clinical response, and we are forced to conclude that we are indeed dealing with an immune phenomenon (Watkins, et al., 1976).

Reactions to thiopentone and methohexitone (Wyatt and Watkins, 1975) appear to be "classical" hypersensitivity reactions to barbiturate-type drugs, with marked IgE involvement. Complement conversion in these patients may be a secondary effect involving drug retention in the body. Both C3 and C4 elimination appears to be progressive over 24 h and normal concentrations are not restored for 2–3 days following the reaction. Mild but unpleasant symptoms probably associated with complement conversion, such as oedema, appear to persist in these patients for 2–3 days.

In conclusion, we would suggest that, irrespective of the actual mechanism involved, measurement of C3 consumption and conversion on sequential plasma samples provides a simple and convenient method for assessing hypersensitivity reactions mediated in some way through immune mechanisms.

ACKNOWLEDGEMENTS

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REFERENCES


IDENTIFICATION ET ESTIMATION QUANTITATIVE DES REACTIONS HYPERSENSIBLES AUX AGENTS ANESTHESIQUES INTRAVEINEUX

RESUME

Il n'est pas possible de distinguer par la seule observation clinique, les effets pharmacologiques directs des réactions hypersensibles immuno-médiatrices et il peut se produire des erreurs si on ne la confirme pas par des essais en
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laboratoires. Un test pratique et simple consiste à mesurer la consommation et la conversion du complément C3 du plasma dans des échantillons de sang séquentiel prélevés à intervalles pendant les 24 h qui suivent une réaction adverse.

IDENTIFIKATION UND ERMESSUNG DER UBEREMPFINDLICHKEITSREAKTION AUF INTRAVENÖSE NARKOSEMITTEL

ZUSAMMENFASSUNG

IDENTIFICACION Y CUANTIFICACION DE LAS REACCIONES DE HIPERSENSIBILIDAD A LOS AGENTES ANESTESICOS INTRAVENOSOS

SUMARIO
No es posible distinguir entre los efectos farmacológicos directos y las reacciones de hipersensibilidad mediadas por el inmunización, por la observación clínica solamente y pueden producirse errores a falta de pruebas de laboratorio. Una prueba sencilla y conveniente consiste en la medición del consumo y la conversión de complemento de plasma C3 en muestra de sangre secuencial tomada a intervalos de más de 24 horas después de una reacción desfavorable.