Production of human immunoglobulin anti-D (Rh₀) for intravenous administration, for a national Rh prophylaxis programme

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Before the introduction of immunsuppression by passively administered anti-D(Rh₀) immunoglobulin G, haemolytic disease of the newborn due to blood-group incompatibility was a serious cause of perinatal mortality, in spite of advances in prenatal assessment of disease severity and in pre- and post-natal treatment. Since 1966 it has been accepted that Rh isoinmunization following pregnancy can be prevented in most cases by administration of anti-D immunoglobulin G. World Health Organisation working groups (World Health Organization, 1967, 1971) made recommendations for the use of Immunoglobulin Anti-D for the Prevention of Rh Sensitization, by radioimmunoassay (Hughes-Jones, 1967). In accordance with British Pharmacopaeia (1975) and The Therapeutic Substances (Saorstat Eireann) Regulations (1934), donations of source plasma are acknowledged to provide comprehensive prophylactic treatment available and the requirements for self-sufficiency in the Republic of Ireland have been discussed (O'Riordan, 1968). It was estimated that the annual requirement to provide comprehensive prophylactic treatment would be 8000-10000 doses. After study of various aspects, it was decided that an intravenously administered product is most suitable.

Source plasma containing anti-D immunoglobulin is obtained by plasmapheresis of Rh-primed volunteers as described by O'Riordan (1972). Each donation of plasma is tested for hepatitis-B surface antigen. Annually, donors together contribute approximately 41 litres of plasma, containing an average of 41 μg of anti-D immunoglobulin/ml. From this source plasma, 9600 minimum 100 μg doses of human immunoglobulin anti-D (Rh₀) for intravenous administration are prepared annually.

The method of preparation is essentially that of Hoppe et al. (1967), involving the use of DEAE-Sephadex A-50 ion-exchange chromatography followed by ethanol precipitation, sterile filtration and freeze-drying. Aseptic precautions are observed. Fresh DEAE-Sephadex A-50 is used, equilibrated according to manufacturer's instructions. Swelling is performed at 80-100°C to minimize bacterial and pyrogen contamination.

Portions (100-260ml) of anti-D plasma (in acid/citrate/dextrose), stored below -30°C, thawed at 4°C and centrifuged to remove cryoprecipitate, from a minimum of ten donors are pooled for fractionation. Controls and assays are performed in accordance with British Pharmacopoeia (1973), British Pharmacopoeia Addendum (1975) and The Therapeutic Substances (Saorstat Eireann) Regulations (1934).

Initially quantitative assay of anti-D (factor) antibody was performed by the World Health Organisation Reference Centre for the Use of Immunoglobulin Anti-D for the Prevention of Rh Sensitization, by radioimmunoassay (Hughes-Jones, 1967). In 1972, quantitative determination by use of the Technicon Autoanalyser (Gunson et al., 1972) was introduced by The Blood Transfusion Service Board. Ampoules are submitted at random to the Blood Group Reference Laboratory, London, U.K., for reference assay.

Recovery of anti-D immunoglobulin after chromatography averages 83%. An insignificant loss occurs after ethanol precipitation (79% recovery of original activity). Filtration contributes substantially to loss (69% of recovery of original activity). Batches prepared from plasma with high lipid content sometimes have to be refiltered, contributing to loss. It is desirable to obtain plasma from fasting donors. Overall recovery, after the freeze-drying, is 64%.

Freeze-dried anti-D (factor) immunoglobulin, after slow re-constitution with 2ml of saline (0.9% NaCl), is ready soluble. Tests for sterility, pyrogens, toxicity and hepatitis-B surface antigen are always satisfactory. Immunoglobulin content varies from 10 to 30 mg per minimum 100 μg dose. Trace amounts of immunoglobulin A, transferrin, β-lipoprotein and chylomicrons are found in some batches. Immunoglobulin G antibodies other than anti-D immunoglobulin are also found. Gel filtration and ultracentrifugation reveal minor amounts of aggregate in some batches after the freeze-drying. Before being freeze-dried the product exhibits little anti-complement activity; an increase occurs in most batches after the freeze-drying. Experience indicates that the anti-complement activity in anti-D immunoglobulin prepared as described is of little clinical significance compared with the severe reactions reported to accompany intravenous administration of other immunoglobulin preparations (Barandum et al., 1962). Becker (1974) showed that preparations of immunoglobulin G eluted from DEAE-Sephadex A-50 with 25 mM-phosphate buffer are free from hepatitis-B surface antigen.

A sufficient quantity of Human Immunoglobulin Anti-D (Rh₀) was available to provide comprehensive Rh prophylaxis in Ireland from 1971. A marked fall in the incidence of haemolytic disease of the newborn and Rh isoinmunization has resulted.

Much knowledge has accumulated during the past decade on the effectiveness of Rh prophylaxis programmes in various countries (Clarke & Whitfield, 1979; Ecklund, 1978; McMaster Conference on Prevention of Rh Immunization, 1979). Although a complete analysis of data relating to the programme in Ireland has not been completed, it appears that failure to protect against Rh isoinmunization by postnatal administration of intravenous Human Immunoglobulin Anti-D (Rh₀) is small. Experience indicates that the product prepared by DEAE-Sephadex chromatography is efficacious and safe. Over 70000 standard minimum 100 μg doses have been administered intravenously, and only two allergic reactions were reported. Additionally, in four instances where inadvertent transfusion of Rh-incompatible blood took place during the 1971-1979 period, 5000-8000 μg of anti-D immunoglobulin were given intravenously in divided doses over 48-72h with no apparent side effects. The anti-D immunoglobulin for intravenous administration produced as described is a safe, effective and economical product for a national Rh prophylaxis programme.

Dr. J. P. O'Riordan, National Director of The Blood Transfusion Service Board, initiated and directed the Rh-factor prophylaxis programme discussed. Successful implementation is also due to Dr. T. Walsh, Mr. J. Cann, Dr. J. Kerrane and other staff of the Blood Transfusion Service Board. Many experts contributed to this programme by performing assays. Volunteer donors of source plasma are acknowledge.
The structural and functional identity of [11β-3H]prostacyclin

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Prostacyclin, an unstable compound that is formed from prostaglandin endoperoxides (Moncada et al., 1976), has been shown to be 30-40 times more potent than prostaglandin E1 as an inhibitor of platelet aggregation. It has been suggested (Moncada & Vane, 1979) that the functionally important prostanooid mediating platelet aggregation is prostacyclin. The inherent instability of the molecule, which possesses not only a highly strained enol ether group but also an intramolecular proton source (Chiang et al., 1979), makes quantification of this compound in biological fluids a challenging analytical problem. In order to develop g.l.c.-mass-spectrometric assays for the metabolites of prostacyclin and its stable hydrolysis product 6-oxo-prostaglandin F1α, 'H labels were required in these compounds.

NaBH₄ reduction of prostaglandin D₁ in ethanol at -20°C, followed by acidic work up, provided [11β-3H]prostaglandin F₁α, which was methylated with diazomethane and purified on Lipidex 5000, and eluted with heptane/chloroform (80:20, v/v). The methyl ester was cyclized to the iodoether (Nicolaou et al., 1977) with iodine in dichloromethane, and then purified on silica preparative layer plates (developed with methanol-ether (10:90, v/v)). Dehydrohalogenation with sodium ethoxide, followed by hydrolysis with NaOH, afforded [11β-3H]prostacyclin as its sodium salt ([259 MBq/mmol (7 mCi/mmol)]. A portion was acidified to pH 3, extracted into ethyl acetate and evaporated to give 6-oxo-[11β-3H]prostaglandin F₁α. Radiochromatography and t.l.c. (spraying with vanillin/H₂SO₄) indicated that a single compound had been formed.

The structural identity of this hydrolysis product was established by g.l.c.-mass spectrometry with a suitable derivative [methoxime, trimethylsilyl] ether, methyl ester. The analysis showed a single compound had been formed with a retention time of 9.2 min on a Finnigan 4000 automated g.l.c.-mass spectrometer, utilizing a 2 mm × 2 mm (internal diam.), OV-1 column at 255°C. The mass spectrum showed a molecular ion (M⁺) at m/e 629, the high-mass ions could be assigned as fragments from M⁺: m/e 614 M⁺-Me, m/e 598 M⁺-OMe, m/e 558 M⁺-C₅H₁₁, m/e 508 M⁺-(OMe + Me₂SiOH), m/e 468 M⁺-(Me₂SiOH + C₅H₁₁), m/e 449 M⁺-(Me₂SiOH + Me₂SiOH), m/e 418 M⁺-(Me₂SiOH + Me₂SiOH + OMe), m/e 378 M⁺-(C₅H₁₁ + Me₂SiOH + Me₂SiOH).

* Abbreviation: Me₂Si, trimethylsilyl.

The hydrolysis product derived from [11β-3H]prostacyclin ran as a single band on t.l.c. The band was eluted with methanol and derivatized (methoxyamine hydrochloride, diazomethane, and N0-bis(trimethylsilyl) trifluoroacetamide) for g.l.c.-mass-spectrometric analysis. The reconstructed total ion current indicated that a new compound with a shorter retention time (tR = 6.1 min) had been formed. Its mass spectrum was different from that of 6-oxo-[11β-3H]prostaglandin F₁α. The structure was assigned as the bis(trimethylsilyl) ether, methyl ester of 6-methoxy[11β-3H]prostaglandin I₁. The mass spectrum showed an ion at m/e 510, which is consistent with the loss of methanol from M⁺ at m/e 542. The ions at the upper mass range could readily be assigned: m/e 439 M⁺-(MeOH + C₅H₁₁), m/e 423 M⁺-(MeOH + CH₃CH₂CO₂Me), m/e 420 M⁺-(MeOH + Me₂SiOH), m/e 389 M⁺-(MeOH + OMe + Me₂SiOH), m/e 330 M⁺-(MeOH + Me₂SiOH + Me₂SiOH). A plausible mechanism for the formation of this ketal involves an acid-catalysed reaction of methanol at the ketone function of 6-oxo-prostaglandin F₁α. Subsequent intramolecular cyclization from the 6α-hydroxy group, with displacement of water, would provide the observed product. This facile process is of particular interest in assays for 6-oxo-prostaglandin F₁α, which rely on a t.l.c. step.

The chemical identity of [11β-3H]prostacyclin having been established, its biological activity was measured. The labelled compound was compared with authentic prostacyclin for activation of adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] of a neuronal/somatic-cell hybrid. The NCB-20 (mouse neuroblastoma cell) × (brain cell of foetal Chinese hamster) hybrid (Minna et al., 1975) was cultured, and cell homogenates prepared for assay of adenylate cyclase activity as described previously (MacDermot et al., 1979). The enzyme activity was determined by a modification (Sharma et al., 1975) of method C of Salomon et al. (1974). Incubations were performed at 30°C and pH 8.5 for 9 min in order to minimize the hydrolysis of prostacyclin. Protein was determined by a modification of the method of Lowry et al. (1951). The increase in enzyme activity as a function of prostanooid concentration was similar for the synthetic labelled prostacyclin and the authentic unlabelled prostacyclin. An Eadie–Hofstee plot showed the Kₘ = 28 μM for authentic prostacyclin, and Kₘ = 36 μM for [11β-3H]prostacyclin. (Kₘ is the concentration of prostacyclin producing half-maximal activation of adenylate cyclase.)

The utilization of both chemical and biochemical techniques has established the identity of synthetic [11β-3H]prostacyclin, which is now available for metabolic studies.