Clinical experiment with immunosuppressors in the treatment of Rh - isoimmunization

by
A. SAIZ MARTI

In the Rh-immunized patients intrauterine transfusion offers the possibility of reducing perinatal mortality (1); but unless it is used with due care and caution, it can have the effect of increasing rather than reducing this mortality rate. It is a « desperate » and « aggressive » therapy for foetus, which should only be used in seriously effected and immature foetuses (2).

It is clear that the prophylaxis based on the introduction of anti-D immunoglobulin would in future be able to dispose of this problem of Rh-isoimmunization. But how long would this take? (3).

We should note the important necessity of discovering a therapeutic method that is more efficient for serious cases. A whole series of tests were carried out with the aim of inhibiting formation of serum antibodies, or to eliminate or neutralize those already formed, with the eventual aim of inhibiting or reducing maternal sensitivity or to exert a favourable influence on the course of foetal illnesses.

We have used immunosuppressors as therapeutic agents and observed their effectiveness in preventing rejection during organic grafts and in the treatment of auto-immune disorders. In view of the similarity between hemolitic anaemia in the adult and foetal erythroblastosis, we investigated this clinically.

At the same time, based on the innocuity of the use of cytostatics during gestation (4, 5, 6, 7, 8, 9, 10, 11), and at the same time bearing in mind the possible teratogenic risk, we restricted its use to the beginning of the 5th month of pregnancy.

With the aim of producing tolerance and desensitivity in patients that are very highly sensitized to the Rh antigen, a technique was adopted which utilises the immunosuppression of the immunoanamnestic response by chemical means (10, 11).

MATERIALS AND METHODS

Six patients were treated (five of whom were pregnant) and all were considerably immunized with antibodies, comprising between 1/16 and 1/256. All the patients had histories of erythroblastosis foetalis, foetal mortality and homozygote husbands (see Table I).

The immunosuppressor drugs used in these tests were: 6-mercaptopurine, azathioprine, arabinoside cytosine, cyclophosphamide and prednisone.

The first three cases were treated with 6-mercaptopurine at a dosage of 150 mg/day, orally, and prednisone 30 mg/day, orally. In the second case, two weeks before labour was induced, the doses of 6-mercaptopurine and prednisone were doubled due to the patient sustaining an accidental fall, which did not, however, lead to any external haemorrhage.

Obstetrics Department. The Municipal Hospital of Jativa (Spain).

Table I. *Obstetric history of patients with Rh isoimmunization treated with immunosuppressor drugs*

	Case 1 20.4.70 Gr/V-P/I	Case 2 22.1.71 Gr/III-P/1	Case 3 8.8.71 Gr/X-P/II	Case 4 25.2.72 Gr/IV-P/III	Case 5 10.1.72 Gr/IV-P/1	Case 6 25.9.72 not pregnant
Blood group Antibodies Husband	A Rh neg. 1/64 A Rh pos. CDe/CDe	B Rh neg. 1/32 O Rh pos. CDE/cDE	A Rh neg. 1/256 A Rh pos. CDe/CDe	A. Rh neg. 1/16 A Rh pos. cDe/CDe	A Rh neg. 1/16 A Rh pos. CDe/CDe	A Rh neg. 1/64 A Rh pos. CDe/cDE
History:	*1st p.:* R.N. alive. Caesarean section Transfusion blood incomp. *2nd p.:* Aborted at 20 weeks *3rd. p.:* R.N. died with jaundice on the 4th day *4th p.:* Twins died 1st and 2nd day with jaundice	*1st p.:* R.N. alive *2nd p.:* Aborted at 24 weeks Mixed abortion at 44 weeks	*1st p.:* R.N. alive *2nd p.:* Aborted at 12 weeks *3rd p.:* R.N. alive *4th p.:* Aborted at 8 weeks transfusion incompatible *5th p.:* R.N. died, 24 weeks *6th p.:* Aborted at 8 weeks *7th p.:* R.N. died at 28 weeks *8th p.:* R.N. died at 26 weeks *9th p.:* R.N. died at 24 weeks	*1st p.:* R.N. alive *2nd p.:* R.N. alive transfusion incompatible *3rd p.:* R.N. alive, jaundice	*1st p.:* R.N. alive, Caesarean *2nd p.:* R.N. dead, Caesarean *3rd p.:* R.N. dead, Caesarean	*1st p.:* R.N. dead *2nd p.:* 24 weeks Mixed abortion *3rd p.:* R.N. dead, 28 weeks *4th p.:* R.N. dead, 26 weeks *5th p.:* R.N. dead, 24 weeks *6th p.:* (7.8.70) R.N. dead, 23 weeks

In the fourth case, the treatment was changed to azathioprine at a dosage of 150 mg/day, orally, and prednisone at a dosage of 30 mg/day. This change was made as a result of a complication in the form of a hepatic cholostasis suffered by the third pregnant patient with the 6-mercaptopurine.

The fifth case was treated with arabinoside cytosine, due to its low degree of toxicity, at a dosage of 100 mg/day and prednisone 30 mg/day, both taken orally.

In these five cases, treatment started in the 7th, 7th, 6th, 7th and 6th month of gestation respectively (Table II).

The sixth case, the non-pregnant patient,, was treated with various chemical agents, each with a different action mechanism on the basis of the polychemical therapy used in the treatment of leukaemias (Mathe, 1971; Dubois, 1972). In this way, we made use of an alkyl cytostatic, cyclophosphamide, pyrimidine antimetabolite, arabinoside cytoxine and a corticoid: prednisone. The pattern of treatment was as follows.

The cyclophosphamide at a dosage of 15 mg/kg, reached 1000 mg/day, introduced intravenously in a single dose in a 500 cc glucose saline drip (25th September 1972), together with prednisone at a dosage of 0.5 mg/day, or 30 mg/day, orally, in a single dose, for 10 days. On the 10th November 1972, the dosages were doubled: the cyclophosphamide dose was increased to 2000 mg/day, and the prednisone was increased to 30 mg/day in accordance with the previous pattern. On the 26th December 1972, 2000 mg cyclophosphamide and 30 mg prednisone were administered a day for 10 days, together with arabinoside cytosine at a dosage rate of 5 mg/kg/day, orally, representing 300 mg/day in a single dose for a period of 10 days. On the 20th February 1973, 2000 mg (30 mg/kg) cyclophosphamide was administered intravenously in a single dose. Prednisone at a dosage of 200 mg/day (3.5 mg/kg/day) orally, in a single dose, for a period of 8 days, reducing by 10 mg daily for a period of 30 days until a final level of 40 mg/day is reached, then reducing to 30 mg/day for a further week and concluding with a dosage of 5 mg/day in the subsequent week. The arabinoside cytosine is administered at a dosage of 500 mg/day (8.3 mg/kg/day) in five doses a day, every three days for a month.

For antiemetic purposes, the patient was given metoclopramide parenterally at the beginning of the four cycles with cyclophosphamide. As hepatic protection, the patient was given hepatic extracts and vitamin B Complex parenterally. She was given as a prophylactic against infection doxycycline (100 mg/day orally) during the first three cycles, while in the fourth cylce, she was also given penicillin-streptomycin (400,000 i.u. - 1gr) parenterally. The treatment was supervised in the patient's home, with a 10 day period of confinement during the first three cycles and one month in the last. As might be expected, the patient was also receiving anovulatory contraceptive treatment.

In the six cases that were studied, the haematic constitution was checked every week, with the exception of the sixth patient, where it was checked every three days. In this latter case also, an indirect Coombs test was carried out every 30 days with titration of antibodies. Further, a protein study, a proteinogram and a titration of immunoglobulin were carried out. In the third, fourth and fifth cases, a spectrophotometric curve was prepared for the amniotic fluid.

Lastly, a study was made in all the patients of their blood groups, the Rh factor, bilirubin, haemoglobin in the blood of the umbilical cord and a direct Coombs test on the umbilical cord. Similarly, all the pregnant patients the labor was

Table II. *Prenatal immunosuppression treatment for Rh isoimmunization*

Case No.	Dose 6-Mercaptopurine Maximum dose	Dose Prednisone Maximum dose	Dose Azathioprine maximum dose	Dose Arabinoside maximum dose	Duration of the treatment	Adverse Reactions
1.	150 mg/day=1.7 mg/kg/day, orally	30 mg/day=0.3 mg/kg/day, orally			7th-9th month	
2.	a) 150 mg/day=2 mg/kg/day b) 300 mg/day=4 mg/kg/day	30 mg/day=0.4 mg/kg/day 90 mg/day=1.2 mg/kg/day			7th-9th month	
3.	150 mg/day=1.9 mg/kg/day	30 mg/day=0.3 mg/kg/day			6th-8th month	Hepatic Cholostasis
4.		30 mg/day=0.5 mg/kg/day	150 mg/day=2.5 mg/kg/day, orally		7th-9th month	
5.		30 mg/day=0.4 mg/kg/day		100 mg/day=1.3 mg/kg/day	5th-8th month	

	Treatment	Indirect Coombs Test before treatment	Indirect Coombs Test after treatment	Umbilical cord blood test Direct Coombs test	Umbilical cord blood test Hb g%ml	Umbilical cord blood test Bilirubin mg%ml	Treatment of E.F.
1. R.M.C.	6-Mercaptopurine Prednisone	1/64	1/16	Positive	11.6	6	Exchange transfusion
2. F.B.C.	6-Mercaptopurine Prednisone	1/32	1/32		16.2	2	none
3. T.F.C.	6-Mercaptopurine Prednisone	1/256	1/32	—	—	—	none, due to death by hydrops foetalis
4. C.S.B.	Azathioprine Prednisone	1/16	1/4	Positive	18.4	3	none
5. J.P.T.	Arabinoside cytosine Prednisone	1/16	1/1 o 24	Positive	11.2	6	Exchange transfusion
6. E.V.F.	Cyclophosphamide Arabinoside cytosine Prednisone	1/64	Negative	—	—	—	none, due to non-pregnancy

Clinical Exper. Obst. & Gynecology
Jan. 1974 Vol. I, pp. 55-61

induced between the 36th and 38th weeks of their pregnancies, with the exception of the third and fifth cases, whose labor commenced spontaneously after an intrauterine transfusion had been carried out.

RESULTS AND CONCLUSIONS

The first fact that deserves to be pointed out is the stability and the progressive reduction of the amount of agglutinins in the serum (see Table II); in the first case, the reduction was from 1/64 to 1/16, in the second case from 1/32 to 1/32, in the third from 1/256 to 1/32, in the fourth from 1/16 to 1/4 and in the fifth from 1/16 to 1/1024. The sixth case, the non-pregnant patient, is the most extraordinary of all, since from a titration level of 1/64 at the commencement of the immunosuppressor treatment we subsequently obtained a *negative result in the Coombs test.* The second case also merits some comment, as she commenced treatment at a level of 1/32, dropped to 1/16 and then to 1/8; the patient then suffered a traumatic fall and when the Coombs test was carried out, the result was found to be 1/32 again. This could well have been caused by a placentary micro-transfusion. With regard to the fifth case, it is necessary to emphasize the complications that arose when an amniocentesis was performed and a placentary foetal vessel was punctured, as a result of which the degree of sensitivity increased a titration of 1/1024.

In all six cases (including the non-pregnant patient) there were no signs of significant toxicity with the dosages of chemical immunosuppressors, such as signs of leukopenia, loss of weight or mortality. We should only point out the complication of a hepatic cholostasis with the 6-mercaptopurine in the third case and nausea from the cyclophosphamide in the sixth patient.

In the subjects with prenatal therapy, all the foetuses were born living and were Rh positive, with a positive direct Coombs in the umbilical cord. One foetus died (third case) but the rest are surviving and do not have any apparent signs of damage as a result of the immunosuppressor treatment. This third infant had hydrops foetalis and we would stress the obstetric history of six foetuses that died from serious erythroblastosis after sensitization with a transfusion of Rh positive accidentally, with the additional occurrence of minor haemorrhages in the fifth month of pregnancy. In this case, it could well be that the immunosuppressor treatment should have been verified before the sixth month and at a higher dosage, in view of the serious history of Rh isoimmunization.

The total amount of bilirubin that we observed in the blood of the cords of the foetuses, whose mothers we had been treating was 6 mg% (1st case); 2 mg% (2nd case); not ascertained (3rd case); 3 mg% (4th case); and 6 mg% (5th case). The quantity of haemoglobin was 11, 16, 18 and 11 g% respectively.

In two of the newly born babies of the patients treated with 6-mercaptopurine and azathioprine (2nd and 4th cases), it was possible to avoid having to perform a total blood transfusion, by maintaining the bilirubin levels within a safe margin (20 mg%) (See Table II).

In the first and fifth patients, it was decided to perform a total blood transfusion and in this connection it should be mentioned that these had histories of high neonatal mortality.

In the non-pregnant case (6th case) with immunosuppressive therapy, the response was observed that this highly sensitized patient had shown previously in her pregnancies, with her history of 6 pregnancies and 6 lost babies as a

Clinical Exper. Obst. & Gynecology
Jan. 1974 Vol. I, pp. 55-61

result of serious neonatal haemolytic Rh affliction. The commencement of immunosuppressive treatment was two years from the date on which she had lost her last baby (7th August 1970). It can be seen that the level of agglutinins fell from 1/64 to 1/32 with the first cycle of cyclophosphamide at a dosage of 16.6 mg/kg/day and prednisone at a dosage of 0.5 mg/kg/day, which induced us to repeat the treatment with a second cycle, increasing the dose of cyclophosphamide to 33.3 mg/kg/day (twice the previous dose). After this we obtained a spectacular reduction of serum antibodies to 1/2. Encouraged by these results, we proceeded with a third cycle at the same doses and drug as in the second cycle and also introduced arabinoside cytosine at a dosage of 5 mg/kg/day. However, this time the level of agglutinines remained static (1/2) and the leukocytes, erythrocytes and platelets decreased, but did not reach a critical level. When the blood levels dropped, we decided on a fourth cycle of treatment with the same doses of cyclophosphamide as used previously (33.3 mg/kg/day), but at the same time, we increased the dosage of arabinoside cytosine to 8.3 mg/kg/day, every three days, and prednisone to 3.3 mg/kg/day. We persevered with this treatment as the blood elements remained stable, and in the month during which the fourth cycle was carried out, we conducted a Coombs test on the 20th March 1973 which was *negative.* This test is still being continued together with other practical tests (in saline, albuminose, bromeline, papain medium) and continues to be *negative.*

With regard to the toxicity, this was not very significant for the doses of both drugs that were used, with the exception of a slight leucocyte reduction in the third cycle and the insignificant sense of nausea, which lasted for one day only when cyclophosphamide was used in the four cycles.

As a preliminary clinical experiment, we have demonstrated that by chemotherapeutic means « an immunological desensitization » can ben produced in a woman who is not pregnant (6th case), but who is very sensitive and with a history of severe neonatal haemolitic disease.

At the same time, we have shown that prenatal immunosuppressive treatment in highly sensitive women with serious histories of Rh isoimmunization can produce a state of « immunological tolerance » or immunitary paralysis.

The preliminary data, despite the fact that the case-sheet is still insufficient for a final judgement to be made, indicates:

1) The possibility of producing tolerance and desensitization in a sensitive patient with chemotherapeutic agents at non-toxic dosages.

2) The apparent suitability of these immunosuppressive agents in the prevention of the reduction of foetal losses, in serious cases of Rh isoimmunization, particularly where there are serious anamnesic antecedents; this treatment is preferable to intrauterine transfusions and may well preclude the necessity of postnatal total blood transfusions ([11]).

3) The final hope for super-sensitized female patients with histories of hydrops foetalis and foetuses dying before the 25th week of pregnancy, or who have had to have an intrauterine transfusion unsuccessfully in previous pregnancies...

The total desensitization obtained with chemotherapeutic immunoppressors outside pregnancy can offer, as a result of our contribution, a hopeful solution to all future pregnancies of this type which would otherwise be seriously threatened.

SUMMARY

A 6 patients (five pregnant women and one not pregnant) already highly sensitized with obstetrics history of erythroblastosis are presented. Demonstrating the use therapeutic of chemical agents (6-Mercaptopurine, Azathioprine, Prednisone, Cyclophosphamide, Cytosine arabinoside) as a means of immunosuppression of anamnestic Rh-immune response. Suppression of this anamnestic response have been beneficial in all cases but one, by this method immunoparalysis. In the case not pregnant, is reported a desensitization down to make the titer of the indirect Coombs test negative, then hope for future pregnancies in these highly sensitized women might be theoretically offered. Methods for producing a state of immunologic tolerance in the Rh problems.

Translated by Samil-Pabyrn Foundation

BIBLIOGRAPHY

1. Queenan J. T.: *Conferencia extraordinaria en el Xth Hispano-Portugese Congress on Obstetrics and Gynaecology,* P. de Mallorca, 1970. - 2. De La Fuente P. et al.: *Paper presented at the Xth Hispano-Portugese Congress on Obstetrics and gynaecology,* P. de Mallorca, 1970. - 3. Caballero Gordo A., De Augustin P.: Arch. Fac. Med. 2, 173-200, 1970. - 4. Hager E. B.: The New Physician 15, 162-185, 1966. - 5. Gillibrand P. N.: Proc. R. Soc. Med. 59, 834, 1966. - 6. Van Ypersele De Strihou Ch.: Brux. Med. 338, 1967. - 7. Kaufman J. J.: J. Am. med. Ass. 200, 162, 1967. - 8. Board J. A., Lee H. M., Draper D. A., Hume D. M.: Obstet. Gynec. 29, 318, 1967. - 9. Hume D. M.: *Human Transplantation, ed Rapaport and Dansset, Grune and Stratton,* New York and London, 1968. - 10. Vecchietti G., Onnis A.: *Gli antimetaboliti nel trattamento delle neoplasie genitali femminili.* Cedam Ed. - Padova, 1967. - 11. Onnis A., Vecchietti G.: Min. Gin.: 22, 9, 459-462, 1970.

The effect on Rh$^+$ new borns of immunosuppressive drugs administered to their Rh$^-$ mothers during pregnancy. A pediatrician's viewpoint

by

F. F. RUBALTELLI

In recent years, clinical research on the effects of immunosuppressive drugs administered during the course of pregnancy has been carried out by Onnis and co-workers. The drugs were administered on a continuous basis during the last 20 weeks of gestation to women with unoperable neoplasms, and the newborns of these mothers showed no apparent effects as a result of this treatment ([1]).

Later, using the same substances, Onnis treated pregnant women who had a

From the Paediatric Clinic the University of Padua.