Intramuscular versus intravenous anti-D for preventing Rhesus alloimmunization during pregnancy (Review)

Okwundu CI, Afolabi BB

This is a reprint of a Cochrane review, prepared and maintained by The Cochrane Collaboration and published in *The Cochrane Library* 2013, Issue 1

http://www.thecochranelibrary.com

TABLE OF CONTENTS

[Intervention Review]

Intramuscular versus intravenous anti-D for preventing Rhesus alloimmunization during pregnancy

Charles I Okwundu[1,2], Bosede B Afolabi[3]

[1]Centre for Evidence-Based Health Care, Faculty of Health Sciences, Stellenbosch University, Tygerberg, South Africa. [2]South African Cochrane Centre, South African Medical Research Council, Tygerberg, South Africa. [3]Department of Obstetrics and Gynaecology, University of Lagos, Lagos, Nigeria

Contact address: Charles I Okwundu, ciokwundu@sun.ac.za.

Editorial group: Cochrane Pregnancy and Childbirth Group.
Publication status and date: New, published in Issue 1, 2013.
Review content assessed as up-to-date: 15 November 2012.

Citation: Okwundu CI, Afolabi BB. Intramuscular versus intravenous anti-D for preventing Rhesus alloimmunization during pregnancy. *Cochrane Database of Systematic Reviews* 2013, Issue 1. Art. No.: CD007885. DOI: 10.1002/14651858.CD007885.pub2.

ABSTRACT

Background

Antibodies to the red cell Rhesus D (RhD) antigen can be produced during pregnancy in a RhD-negative mother carrying a RhD-positive fetus, in particular following feto-maternal haemorrhage at birth or following any procedure that may cause feto-maternal haemorrhage. While the first baby is usually not harmed, these antibodies may cause haemolytic disease of the fetus/newborn (HDFN) in subsequent RhD-positive babies. RhD incompatibility is a major cause of HDFN.

To reduce the risk of HDFN, anti-D is given to RhD-negative mothers at 28 or 30 weeks of pregnancy and within 72 hours of potential maternal exposure to fetal red cells. Anit-D is currently available in both intramuscular (IM) and intravenous (IV) preparations.

Objectives

To compare the efficacy and effectiveness of IM versus IV anti-D IgG in preventing RhD alloimmunization in RhD-negative pregnant women.

Search methods

We searched the Cochrane Pregnancy and Childbirth Group's Trials Register (30 September 2012).

Selection criteria

Randomized controlled trials, quasi-randomized trials and cluster-randomized trials comparing IM and IV anti-D for preventing RhD alloimmunization in RhD-negative pregnant women.

Data collection and analysis

Two review authors independently assessed trials for inclusion and assessed trial quality. Two review authors extracted data. Data were checked for consistency by both authors.

Main results

Two studies involving 447 (with sample sizes 14 and 432) RhD negative women were included. The studies compared IM and IV administration of anti-D prophylaxis. In both studies the women received a 1500 IU (300 microgram) dose of Rhophylac during week 28 of gestation. There was no incidence of RhD alloimmunization in either of the studies, as the sample size was insufficient for meaningful comparison of this uncommon outcome. One of the studies found that the mean anti-D IgG concentrations after IV and IM administration differed up to seven days (36.1 (2.6) ng/mL IV; 19.8 (8.7) ng/mL IM on day seven). However, from two to three weeks post-administration, the concentrations were similar for both routes of administration. None of the women involved in the studies developed antibodies against the RhD antigen.

Authors' conclusions

It appears that IM and IV administration of anti-D are equally effective. The number of included studies and the number of participants are not enough to assess whether there are any differences. Anti-D can be administered by IM or IV injection. The choice of IM or IV route of administration will depend on the available preparations, the dose to be administered and also on the patients' preferences. This review found insufficient information upon which to guide practice due to the limited number of included studies, small sample sizes and methodological limitations.

PLAIN LANGUAGE SUMMARY

Intramuscular versus intravenous anti-D for preventing Rhesus alloimmunization during pregnancy

Antibodies to the red cell Rhesus D (RhD) antigen may be produced by a RhD-negative mother if she is carrying a RhD-positive baby. Antibodies form with the entry of fetal blood into the maternal circulation (feto-maternal haemorrhage) at birth or following a procedure (such as amniocentesis). Spontaneous sensitisation occurs antenatally at about 28 to 30 weeks gestation. The maternal condition that results from this is known as RhD alloimmunization or sensitization. The antibodies can cause haemolytic disease in the baby resulting in anaemia, oedema and possible death. While the first baby is usually not harmed, the antibodies (directed against antigens inherited from the father) may cause haemolytic disease in subsequent RhD-positive babies.

Anti-D (anti-D immunoglobulin G) is obtained from human plasma and contains high levels of antibody to the fetal RhD antigens. Following administration to the mother, a positive antibody screen is found as the anti-D crosses the placenta and binds to the fetal red blood cells. Since andi-D is derived from pooled donor plasma, there is a risk of transmission of blood-borne diseases.

Anti-D immunoprophylaxis is recommended for RhD-negative mothers at 28 or 30 weeks of pregnancy and within 72 hours of potential maternal exposure to fetal red cells to prevent the mother developing antibodies during the pregnancy. RhD negative mothers also receive postpartum anti-D after a RhD-positive baby to reduce the risk of sensitization during the next pregnancy. Present routes of administration for this product include intramuscular of intravenous routes. This review aimed to compare the efficacy and effectiveness of intramuscular versus intravenous anti-D in preventing rhesus alloimmunization in RhD-negative pregnant women.

We identified two completed randomized controlled studies, involving 447 RhD-negative women. The findings suggest that intramuscular and intravenous anti-D in the 28th week of pregnancy are equally effective in preventing RhD antibody formation (alloimmunization) during the pregnancy. None of the women developed antibodies against the RhD antigen. The small number of studies, low number of participants and methodological limitations mean that we do not have sufficient information to guide practice. The choice of intramuscular or intravenous route of administration will depend on available preparations, the dose to be administered and the woman's preference.

BACKGROUND

One of the greatest successes of modern obstetrics has been the dramatic reduction in the prevalence of rhesus D (RhD) alloimmunization and deaths from haemolytic disease of the fetus/newborn (HDFN) with the introduction of anti-D immunoglobulin in the 1970s (Parker 2008).

Antibodies to the red cell RhD antigen are produced during preg-

nancy in a RhD-negative mother carrying a RhD-positive fetus. These antibodies are produced following feto-maternal haemorrhage at birth (Chilcott 2002) or following any procedure that may cause feto-maternal haemorrhage.The maternal condition that results from this is known as RhD alloimmunization or sensitization (Moise 2005).

Frequency of RhD negativity is higher in Caucasian/European (15%) than in African (5%) or South American (8%) populations. It occurs much more rarely in indigenous (i.e. Eskimo, Native American), East Asian (i.e. Japanese, Chinese) populations (Sameer 2008). While the first baby is usually not harmed, these antibodies (directed against antigens inherited from the father) may cause haemolytic disease in subsequent RhD-positive babies. The perinatal effects of maternal RhD alloimmunization are referred to as haemolytic disease of the fetus/newborn (HDFN), and fetal manifestations of the disease are more appreciated with newer technologies such as cordocentesis and fetal ultrasonography (Sameer 2008). Fetal anaemia and hydrops fetalis can result if the maternal antibody reaches a significant level (Moise 2005). A major cause of HDFN is an incompatibility of the RhD blood group between the mother and fetus. Most commonly, haemolytic disease is triggered by the D antigen, although other Rh antigens, such as c, C, E, e, Fy, Kell and Jk systems can also cause problems (Sameer 2008). HDFN due to anti-D was a significant cause of morbidity and mortality prior to the introduction of immunoprophylaxis with anti-D immunoglobulin. However, anti-D immunoprophylaxis has made HDFN a preventable disease (Urbaniak 1998).There has been a reduction in mortality from 1.2 per 1000 births to 0.02 per 1000 births (Tovey 1992). However HDFN still remains a problem for RhD-negative mothers and their babies.

It is recommended that intramuscular (IM) or intravenous (IV) anti-D be given to RhD-negative mothers at 28 or 30 weeks of pregnancy and within 72 hours of potential maternal exposure to fetal red cells (Fung 2003). Potential sensitization events in RhD-negative mothers include in-utero procedures (such as amniocentesis and chorionic villus sampling), antepartum haemorrhage, external cephalic version or other invasive procedures. Routine anti-D is given at 28 or 30 weeks of pregnancy because spontaneous sensitization occurs most commonly from around that time. Following administration of anti-D, a positive antibody screen will be found in the woman (Hartwell 1998). Anti-D crosses the placenta and binds to fetal red blood cells, without causing haemolysis, anaemia or jaundice. If RhD-negative mothers do not receive postpartum anti-D IgG prophylaxis after a RhD-positive baby, the incidence of sensitization during the next pregnancy is 12% to 16%, compared to 1.6% to 1.9% in mothers receiving postpartum prophylaxis (Fung 2003).

Anti-D immunoglobulin G is a blood product containing a high titre of antibody to RhD antigens of red blood cells. It is obtained from human plasma and is effective in the prevention of RhD alloimmunization. Since anti-D is derived from pooled donor plasma, there is a risk of transmission of blood-borne diseases (National Blood 2003).

Present routes of administration for this product include IM or IV. Until recently the only anti-D immunoglobulin approved by the US Food and Drug Administration (FDA) for this indication required IM injection, an inconvenient and painful route for the relatively large volume that may be required (Anderson 1999). Both IM and IV preparations of anti-D are now available in the US, Canada and the UK (Fung 2003; Moise 2005; Parker 2008). Some are intended for IM use only while others may be given by either the IM or IV route (Parker 2008). There are no data to suggest that any route of administration is superior to any other in terms of efficacy, or that one is likely to cause more harm than the other.

Optimally, the most effective and convenient and least painful approach should be the treatment of choice for preventing RhD alloimmunization. We review here the effects of IM compared with IV anti-D for the prevention of RhD alloimmunization in pregnancy.

OBJECTIVES

To compare the efficacy and effectiveness of IM versus IV anti-D IgG in preventing RhD alloimmunization in RhD-negative pregnant women.

METHODS

Criteria for considering studies for this review

Types of studies

Randomized controlled trials (RCTs), quasi-randomized trials and cluster-randomized trials comparing IM and IV anti-D in preventing RhD alloimmunization in RhD-negative pregnant women.

Types of participants

RhD negative pregnant women with negative Kleihauer test (a test that detects fetal cells in the maternal blood) at 28 weeks' gestation.

Types of interventions

IM versus IV anti-D (administered routinely at 28 or 30 weeks, or following a potential sensitization event).

Types of outcome measures

Primary outcomes

- Incidence of RhD alloimmunization

Secondary outcomes

- Maternal serum anti-D concentration (measured at any time after administration)
- Neonatal morbidity (including anaemia, jaundice, hydrops fetalis) in the indexed and subsequent pregnancies if reported by the studies
- Patient's preferred route of administration
- Adverse events (as reported by the primary studies)

Search methods for identification of studies

Electronic searches

We contacted the Trials Search Co-ordinator to search the Cochrane Pregnancy and Childbirth Group's Trials Register (30 September 2012).

The Cochrane Pregnancy and Childbirth Group's Trials Register is maintained by the Trials Search Co-ordinator and contains trials identified from:

1. monthly searches of the Cochrane Central Register of Controlled Trials (CENTRAL);
2. weekly searches of MEDLINE;
3. weekly searches of EMBASE;
4. handsearches of 30 journals and the proceedings of major conferences;
5. weekly current awareness alerts for a further 44 journals plus monthly BioMed Central email alerts.

Details of the search strategies for CENTRAL, MEDLINE and EMBASE, the list of handsearched journals and conference proceedings, and the list of journals reviewed via the current awareness service can be found in the 'Specialized Register' section within the editorial information about the Cochrane Pregnancy and Childbirth Group.

Trials identified through the searching activities described above are each assigned to a review topic (or topics). The Trials Search Co-ordinator searches the register for each review using the topic list rather than keywords.

We did not apply any language restrictions.

Data collection and analysis

Two review authors (C Okwundu and B Afolabi) independently assessed identified studies for inclusion.

Selection of studies

Two studies were identified and selected for inclusion in the review.

Data extraction and management

We designed a form to extract data. For eligible studies, both review authors extracted the data using the agreed form. We resolved discrepancies through discussion. Data were entered into Review Manager software (RevMan 2011) and checked for accuracy.

Extracted information included the following.

Study details: citation, study design and setting, time period.

Participant details: study population demographics, sample size, and attrition rate.

Intervention details: type of drug, dose, and route of administration.

Outcome details: serum anti-D concentration, incidence of RhD alloimmunization, adverse events, neonatal morbidity and preferred route of administration.

When information regarding any of the above was unclear, we attempted to contact authors of the original reports to provide further details.

Assessment of risk of bias in included studies

Both review authors independently assessed risk of bias for each study using the criteria outlined in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). We resolved any disagreement by discussion.

(1) Random sequence generation (checking for possible selection bias)

We described for each included study the method used to generate the allocation sequence in sufficient detail to allow an assessment of whether it should produce comparable groups.

We assessed the method as:

- low risk of bias (any truly random process, e.g. random number table; computer random number generator);
- high risk of bias (any non-random process, e.g. odd or even date of birth; hospital or clinic record number);
- unclear risk of bias.

(2) Allocation concealment (checking for possible selection bias)

We described for each included study the method used to conceal the allocation sequence and determine whether intervention allocation could have been foreseen in advance of, or during recruitment, or changed after assignment.

We assessed the methods as:

- low risk of bias (e.g. telephone or central randomization; consecutively numbered sealed opaque envelopes);

- high risk of bias (open random allocation; unsealed or non-opaque envelopes, alternation; date of birth);
- unclear risk of bias.

(3) Blinding of participants, personnel and outcome assessors (checking for possible performance bias)

We described for each included study the methods used, if any, to blind study participants and personnel from knowledge of which intervention a participant received. We considered studies to be at low risk of bias if they were blinded, or if we judged that the lack of blinding would be unlikely to affect results. We assessed blinding separately for different outcomes or classes of outcomes. We assessed the methods as:

- low, high or unclear risk of bias for participants;
- low, high or unclear risk of bias for personnel;
- low, high or unclear risk of bias for outcome assessment.

(4) Incomplete outcome data (checking for possible attrition bias due to the amount, nature and handling of incomplete outcome data)

We described for each included study, and for each outcome or class of outcomes, the completeness of data including attrition and exclusions from the analysis. We stated whether attrition and exclusions were reported, the numbers included in the analysis at each stage (compared with the total randomized participants), reasons for attrition or exclusion where reported, and whether missing data were balanced across groups or were related to outcomes. We assessed the methods as:

- low risk of bias (e.g. 20% or less missing data; missing outcome data balanced across groups);
- high risk of bias (e.g. numbers or reasons for missing data imbalanced across groups; 'as treated' analysis done with substantial departure of intervention received from that assigned at randomization);
- unclear risk of bias.

(5) Selective reporting (checking for reporting bias)

We described for each included study how we investigated the possibility of selective outcome reporting bias and what we found. We assessed the methods as:

- low risk of bias (where it is clear that all of the study's pre-specified outcomes and all expected outcomes of interest to the review have been reported);
- high risk of bias (where not all the study's pre-specified outcomes have been reported; one or more reported primary outcomes were not pre-specified; outcomes of interest are reported incompletely and so cannot be used; study fails to include results of a key outcome that would have been expected to have been reported);
- unclear risk of bias.

(6) Other bias (checking for bias due to problems not covered by (1) to (5) above)

We assessed whether each study was free of other problems that could put it at risk of bias:

- low risk of other bias;
- high risk of other bias;
- unclear whether there is risk of other bias.

(7) Overall risk of bias

We made judgements about whether studies were at high risk of bias, according to the criteria given in the *Handbook* (Higgins 2011).

Dealing with missing data

For all outcomes, we carried out analyses, as far as possible, on an intention-to-treat basis, i.e. we attempted to include all participants randomized to each group in the analyses, and all participants were analysed in the group to which they were allocated, regardless of whether or not they received the allocated intervention.

Assessment of heterogeneity

We planned to assess statistical heterogeneity in each meta-analysis using the T^2, I^2 and Chi^2 statistics. However, we could not perform any statistical test of heterogeneity since we did not perform a meta-analysis. The two included studies were similar in terms of the study participants and the intervention administered.

Assessment of reporting biases

We planned to investigate for publication bias using funnel plots If we found 10 or more studies. However, there were not enough studies to assess for publication bias.

Data synthesis

Review authors CO and BA independently extracted data from the included studies. All of the extracted information were rechecked by both authors. We did not perform a meta-analysis because the two studies did not report the findings in a way that would allow for meta-analysis. Also, for the primary outcome (incidence of Rh-alloimmunization), there were no events.

We planned to carry out statistical analysis using the Review Manager software (RevMan 2011). We also planned to use a fixed-effect meta-analysis for combining data where it was reasonable to assume that studies were estimating the same underlying treatment effect: i.e. where trials examined the same intervention, and the trials' populations and methods were judged to be sufficiently similar. If we had found clinical heterogeneity sufficient to expect

that the underlying treatment effects differed between trials, or if substantial statistical heterogeneity was detected, we planned to use random-effects meta-analysis to produce an overall summary if an average treatment effect across trials was considered clinically meaningful.

Subgroup analysis and investigation of heterogeneity

We planned to investigate any significant statistical heterogeneity using subgroup analyses and sensitivity analyses. We planned to carry out the following subgroup analyses based on race, dose and frequency of anti-D, mode of sensitization (e.g. following amniocentesis, antepartum haemorrhage or external cephalic version). However, we could not perform a subgroup analysis because of limited number of included studies.

Sensitivity analysis

We planned to conduct the following sensitivity analyses: excluding studies at high risk of bias, such as quasi-randomized studies and studies with missing outcome data; repeating analyses using a random-effects model when substantial heterogeneity was found; excluding studies published as abstracts or non-peer reviewed publication.

RESULTS

Description of studies

See: Characteristics of included studies.
See Characteristics of included studies.

Results of the search

The search of the Cochrane Pregnancy and Childbirth Group's Trials Register retrieved two trial reports.

Included studies

Two studies that involved about 447 women met the inclusion criteria (Bichler 2003; MacKenzie 2004). *See* Characteristics of included studies for further details. Both the dose of anti-D used and the timing were similar in both studies. The two studies involved RhD-negative women and both were multi-centre studies; one was conducted in seven centres in Germany (Bichler 2003) while the other was conducted in 22 centres in the USA and the United Kingdom (MacKenzie 2004). The intervention tested in the studies was Rhophylac 300 microgram administered intravenously or intramuscularly at the 28th week of gestation and within 72 hours after delivery of a RhD-positive child. The outcomes reported in the two studies were serum anti-D concentration, adverse events and RhD alloimmunization.

Risk of bias in included studies

See 'Risk of bias' graph and 'Risk of bias' summary tables for the included studies Figure 1; Figure 2. In general, the overall methodological quality of the included studies was acceptable.

Figure 1. 'Risk of bias' graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

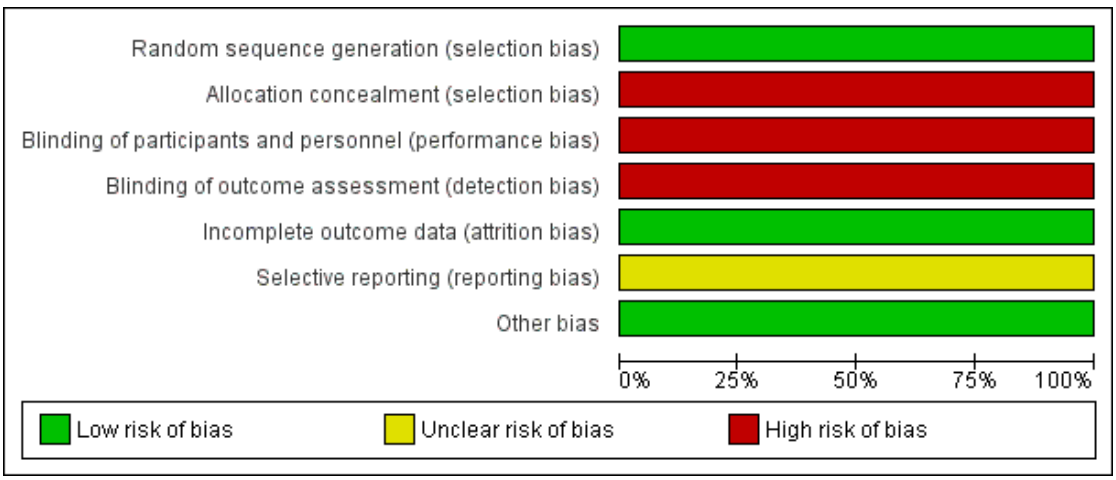

Figure 2. 'Risk of bias' summary: review authors' judgements about each risk of bias item for each included study.

Allocation

Bichler 2003 described the use of block randomization while MacKenzie 2004 described the use of computer-generated random numbers for generating the random sequence. However, there was no concealment of allocation in either study.

Blinding

Both studies (Bichler 2003; MacKenzie 2004) were open-label and there was no blinding.

Incomplete outcome data

The were similar rates of attrition in both the placebo and treatment arms in both studies (Bichler 2003; MacKenzie 2004).

Selective reporting

All the outcomes specified in the protocols were reported for both studies.

Other potential sources of bias

No other potential sources of bias were identified.

Effects of interventions

Primary outcomes

1) RhD alloimmunization

The two included studies reported on the RhD alloimmunization. However, there were was no incidence of RhD alloimmunization in either of the studies. In Bichler 2003, antibody tests performed six to eight months later were negative for all women, suggesting that no RhD alloimmunization occurred. Also, in MacKenzie 2004, none of the women involved developed antibodies against the RhD antigen.

Secondary outcomes

1) Maternal serum anti-D concentration (measured at any time after administration)

The mean anti-D immunoglobulin concentrations after IV and IM administration were different up to seven days [36.1 (2.6) ng/mL IV; 19.8 (8.7) ng/mL IM on day seven]; however, they were comparable from two to three weeks post-administration (Bichler 2003).

2) Neonatal morbidity (including anaemia, jaundice, hydrops fetalis) in the indexed and subsequent pregnancies

Neither of the studies reported on this outcome.

3) Patient's preferred route of administration

Neither of the studies reported on this outcome.

4) Adverse events

In Bichler 2003, there were a total of seven adverse events; three in the IV group, four in the IM group. All adverse events were considered not to be related to the study drug by the investigators. "One woman complained about oesophagitis. Influenza-like symptoms were reported for three women. One of them also suffered from neuritis". MacKenzie 2004 did not report any adverse events in either of the study arms.

DISCUSSION

Intramuscular (IM) and intravenous (IV) preparations of anti-D immunoglobulin are now available in many countries. Though the peak serum levels are achieved faster after IV than IM injection (Bichler 2003; MacKenzie 2004), findings from Bichler 2003 suggests that the serum concentration of anti-D administered intramuscularly or intravenously are similar two to three weeks after administration. However, because of the small number of studies, small sample sizes and methodological limitations, the available data are insufficient to assess whether there are any differences between administering anti-D intramuscularly or intravenously to prevent RhD alloimmunization during pregnancy. The choice of the route of administration should depend or other factors (including costs, patient choices, available preparations and dose of anti-D to be administered) rather than on efficacy.

Potential biases in the review process

We conducted a comprehensive search to ensure that all relevant completed or ongoing studies were identified. There was no language restriction. We also reduced potential bias in the conduct of this review by having both review authors independently scan through the search output, extract data, and assess the methodological quality of each study.

AUTHORS' CONCLUSIONS

Implications for practice

The findings from this review suggest that the serum concentration of anti-D administered intravenously or intramuscularly are similar two to three weeks after administration. Intramuscular and intravenous administration of anti-D immunoglobulin are equally effective in preventing RhD alloimmunization. However, because of the limited number of included studies, small sample sizes and methodological limitations we do not have sufficient information upon which to guide practice.

Implications for research

Any future studies comparing intramuscular versus intravenous anti-D for preventing RhD alloimmunization should also aim to provide information on the cost-effectiveness and patients' preferences.

ACKNOWLEDGEMENTS

As part of the pre-publication editorial process, this review has been commented on by three peers (an editor and two referees who are external to the editorial team), a member of the Pregnancy and Childbirth Group's international panel of consumers and the Group's Statistical Adviser.

REFERENCES

References to studies included in this review

Bichler 2003 ***{published data only}***
Bichler J, Schondorfer G, Pabst G, Andresen I. Pharmakokinetics of anti-D IgG in pregnant RhD-negative women. *BJOG: an international journal of obstetrics and gynaecology* 2003;**110**:39–45.

MacKenzie 2004 ***{published data only}***
MacKenzie IZ, Bichler J, Mason GC, Lunan CB, Stewart P, Al-Azzawi F, et al.Efficacy and safety of a new, chromatographically purified rhesus (D) immunoglobulin. *European Journal of Obstetrics & Gynecology and Reproductive Biology* 2004;**117**(2):154–61.

Additional references

Anderson 1999
Anderson B, Aziza TS, Gootenberg JE, Sandler SG. Successful prevention of post-transfusion Rh alloimmunization by intravenous Rho (D) immune globulin (WinRho SD). *American Journal of Hematology* 1999;**60**(3): 245–7.

Chilcott 2002
Chilcott J, Lloyd Jones M, Wight J, Forman K, Wray J, Beverley C. *A review of the clinical effectiveness and cost effectiveness of routine anti-D prophylaxis for pregnant women who are Rhesus (RhD) negative*. London: National Institute of Clinical Excellence, 2002.

Fung 2003
Fung K, Eason E. *SOGC Clinical Practice Guideline: Prevention of Rh Alloimmunization*. SOGC, September 2003.

Hartwell 1998
Hartwell E. Use of Rh Immune globulin. ASCP Practice Parameter. *American Journal of Clinical Pathology* 1998; **110**:281–302.

Higgins 2011
Higgins JPT, Green S, editors. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.

Moise 2005
Moise KJ. *Management of Rhesus Alloimmunization in pregnancy*. Business Briefing: Womens' Health Care, 2005.

National Blood 2003
National Blood Authority. *Guidelines on the prophylactic use of Rh D immunoglobulin (anti-D) in obstetrics*. Canberra: NHMRC, 2003.

Parker 2008
Parker J, Wray J, Gooch A, Robson S, Qureshi H. *Guidelines for the use of prophylactic anti-D immunoglobulin*. BCSH, June 2008.

RevMan 2011
The Nordic Cochrane Centre, The Cochrane Collaboration. Review Manager (RevMan). 5.1. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2011.

Sameer 2008
Sameer W, Prashant G. Haemolytic disease of the newborn. http://www.emedicine.com/ped/topic959.htm (accessed January 2008).

Tovey 1992
Tovey LA. Oliver memorial lecture. Towards the conquest of Rh haemolytic disease: Britain's contribution and the role of serendipity. *Transfusion Medicine* 1992;**2**:99–109.

Urbaniak 1998
Urbaniak S. The scientific basis of antenatal prophylaxis. *British Journal of Obstetrics and Gynaecology* 1998;**105 Suppl 18**:11–8.

* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Bichler 2003

Methods	Randomized control trial.
Participants	14 RhD-negative women at 28 weeks of gestation. There were 6 women in the intravenous anti-D arm and 8 in the intramuscular anti-D arm
Interventions	Single antenatal injection of 300 microgram of Rhophylac by intravenous or intramuscular route
Outcomes	• Mean anti-D IgG concentrations after intravenous and intramuscular administration. • Adverse events.
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	The allocation of the women to the treatment groups was determined by block randomization
Allocation concealment (selection bias)	High risk	There was no concealment of allocation.
Blinding of participants and personnel (performance bias) All outcomes	High risk	The study was open-label.
Blinding of outcome assessment (detection bias) All outcomes	High risk	The study was open-label.
Incomplete outcome data (attrition bias) All outcomes	Low risk	There was no loss to follow-up.
Selective reporting (reporting bias)	Unclear risk	This cannot be assessed since we did not find the study protocol
Other bias	Low risk	We did not identify any other potential source of bias.

MacKenzie 2004

Methods	Open-label randomized control trial.	
Participants	432 RhD-negative women. 216 participants were randomized into each study arm	
Interventions	Intravenously or intramuscularly Rhophylac 300 microgram at the 28th week of gestation and within 72 hours after delivery of an RhD-positive child	
Outcomes	Incidence of RhD immunization.	
Notes	22 centres in the US and United Kingdom.	
Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Use of computer-generated random numbers.
Allocation concealment (selection bias)	High risk	There was no concealment of allocation.
Blinding of participants and personnel (performance bias) All outcomes	High risk	The study was open-label.
Blinding of outcome assessment (detection bias) All outcomes	High risk	The study was open-label.
Incomplete outcome data (attrition bias) All outcomes	Low risk	There was no loss to follow-up.
Selective reporting (reporting bias)	Unclear risk	This cannot be assessed since we did not find the protocol of the study
Other bias	Low risk	We did not identify any other potential source of bias.

RhD: Rhesus D

DATA AND ANALYSES

Comparison 1. Intramuscular versus intravenous anti-D

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 RhD alloimmunization	2	446	Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
2 Serum anti-D concentration	0	0	Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]

Analysis 1.1. Comparison 1 Intramuscular versus intravenous anti-D, Outcome 1 RhD alloimmunization.

Review: Intramuscular versus intravenous anti-D for preventing Rhesus alloimmunization during pregnancy

Comparison: 1 Intramuscular versus intravenous anti-D

Outcome: 1 RhD alloimmunization

Study or subgroup	Intramuscular anti-D n/N	Intraveneous anti-D n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Bichler 2003	0/8	0/6		0.0 [0.0, 0.0]
MacKenzie 2004	0/216	0/216		0.0 [0.0, 0.0]
Total (95% CI)	**224**	**222**		**0.0 [0.0, 0.0]**

Total events: 0 (Intramuscular anti-D), 0 (Intraveneous anti-D)

Heterogeneity: $Chi^2 = 0.0$, $df = 0$ ($P<0.00001$); $I^2 = 0.0\%$

Test for overall effect: $Z = 0.0$ ($P < 0.00001$)

Test for subgroup differences: Not applicable

CONTRIBUTIONS OF AUTHORS

Both authors (Charles Okwundu (CO) and Bosede Afolabi (BA) were involved in data collection and assessment of methodological quality of the included studies. CO wrote the draft review.

DECLARATIONS OF INTEREST

None known.

SOURCES OF SUPPORT

Internal sources

- Centre for Evidence-Based Health Care, Stellenbosch University, South Africa.
- South African Cochrane Centre, Medical Research Council, Cape Town, South Africa.

External sources

- No sources of support supplied

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

The methods section has been updated to reflect the Pregnancy and Childbirth Group's updated methods text and the latest *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011).

The outcomes have been separated into 'Primary' and 'Secondary' outcomes.