Center for Biologics Evaluation and Research. 2007. Guidance for industry, clinical data needed to support the licensure of seasonal inactivated influenza vaccines. Rockville, MD: Food and Drug Administration, U.S. Department of Health and Human Services. pp 1–15.

Englund JA. 2007. The influence of maternal immunization on infant immune responses. J Comp Pathol 137:S16–S19. Review.

Fiore AE, Shay DK, Broder K, Iskander JK, Uyeki TM, Mootrey G, Bresee JS, Cox NS, Centers for Disease Control and Prevention (CDC), Advisory Committee on Immunization Practices (ACIP). 2008. Prevention and control of influenza: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 57:1–60.

Francis T, Pearson HE, Salk JE, Brown PN. 1944. Immunity in human subjects artificially infected with influenza virus, type B. Am J Public Health 34:317–334.

Freeman DW, Barno A. 1959. Deaths from Asian influenza associated with pregnancy. Am J Obstet Gynecol 78:1172–1175.

Greenberg M, Jacobziner H, Pakter J, Weisl BA. 1958. Maternal mortality in the epidemic of Asian influenza, New York City, 1957. Am J Obstet Gynecol 76:857–902.

Harper SA, Fukud K, Uyeki TM, Cox NJ, Bridges CB, Centers for Disease Control and Prevention (CDC) Advisory Committee on Immunization Practices (ACIP). 2004. Prevention and control of influenza: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 53:1–40.

Harris JW. 1919. Influenza occurring in pregnant women: A statistical study of thirteen hundred and fifty cases. JAMA 72:978–980.

Hartert TV, Neuzil KM, Shintani AK, Mitchel EF, Jr., Snowden MS, Wood LB, Dittus RS, Griffin MR. 2003. Maternal morbidity and perinatal outcomes among pregnant women with respiratory hospitalizations during influenza season. Am J Obstet Gynecol 189:1705–1712.

Hist GK. 1942. The quantitative determination of influenza virus and antibodies by means of red cell agglutination. J Exp Med 75:49–64.

Irving WL, James DK, Stephenson T, Laing P, Jameson C, Oxford JS, Chakraverty P, Brown DW, Boon AC, Zambon MC. 2000. Influenza virus infection in the second and third trimesters of pregnancy: A clinical and seroepidemiological study. BJOG 107: 1282–1289.

Longman RE, Johnson TR. 2007. Viral respiratory disease in pregnancy. Curr Opin Obstet Gynecol 19:120–122.

Mak TK, Mangtani P, Leese J, Watson JM, Pfeifer D. 2008. Influenza vaccination in pregnancy: Current evidence and selected national policies. Lancet Infect Dis 8:44–52. Review.

Mullooly JP, Barker WH, Nolan TF. 1986. Risk of acute respiratory disease among pregnant women during influenza A epidemics. Public Health Rep 101:205–211.

Munoz FM, Greisinger AJ, Wehmanen OA, Mouzoon ME, Hoyle JC, Smith FA, Glezen WP. 2005. Safety of influenza vaccination during pregnancy. Am J Obstet Gynecol 192:1098–1106.

Neuzil KM, Reed GW, Mitchel EF, Simonsen L, Griffin MR. 1998. Impact of influenza on acute cardiopulmonary hospitalizations in pregnant women. Am J Epidemiol 148:1094–1102.

Rothberg MB, Haessler SD, Brown RB. 2008. Complications of viral influenza. Am J Med 121:258–264. Review.

Saito S. 2000. Cytokine network at the feto-maternal interface. J Reprod Immunol 47:87–103. Review.

Stephenson I, Wood JM, Nicholson KG, Zambon MC. 2003. Sialic acid receptor specificity on erythrocytes affects detection of antibody to avian influenza haemagglutinin. J Med Virol 70:391–398.

Tsuda H, Michimata T, Hayakawa S, Tanebe K, Sakai M, Fujimura M, Matsushima K, Saito S. 2002. A Th2 chemokine, TARC, produced by trophoblasts and endometrial gland cells, regulates the infiltration of CCR4+ T lymphocytes into human decidua at early pregnancy. Am J Reprod Immunol 48:1–8.

Widelock D, Csizmas L, Klein S. 1963. Influenza, pregnancy and fetal outcome. Public Health Rep 78:1–11.

P Vermeersch,[1] K Op De Beeck,[1] B R Lauwerys,[2] K Van den Bergh,[1] M Develter,[1] G Mariën,[1] F A Houssiau,[2] X Bossuyt[1]

[1] Department of Laboratory Medicine, Immunology, University Hospitals Leuven, Catholic University of Leuven, Belgium; [2] Department of Rheumatology, Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, Brussels, Belgium

Correspondence to: Dr X Bossuyt, Laboratory Medicine, Immunology, University Hospitals Leuven, Herestraat 49, B-3000 Leuven, Belgium; Xavier.bossuyt@uz.kuleuven.be

Competing interests: None.

Ethics approval: Ethics approval was granted by the Institutional Review Board.

Accepted 23 January 2009

Ann Rheum Dis 2009;68:1791–1793. doi:10.1136/ard.2008.104190

REFERENCES

1. **Bøyne-Rauzy O,** Thabault S, Adoue D, *et al.* Anti-PCNA antibodies: prevalence and predictive value. *Joint Bone Spine* 2005;**72**:432–5.
2. **Fritzler MJ,** McCarty GA, Ryan JP, *et al.* Clinical features of patients with antibodies directed against proliferating cell nuclear antigen. *Arthritis Rheum* 1983;**26**:140–5.
3. **Tzang BS,** Chen TY, Hsu TC, *et al.* Presentation of autoantibody to proliferating cell nuclear antigen in patients with chronic hepatitis B and C virus infection *Ann Rheum Dis* 1999;**58**:630–4.

Etanercept during pregnancy and lactation in a patient with rheumatoid arthritis: drug levels in maternal serum, cord blood, breast milk and the infant's serum

Women with rheumatoid arthritis (RA) appear to be at a high risk of preterm delivery, preeclampsia and low birth weight infants.[1] Reduction of inflammatory activity is of particular importance in those desirous of having children.[2 3] The use of disease-modifying antirheumatic drugs, including biological preparations, has been shown to be essential for suppression of the activity of RA. Tumour necrosis factor inhibitors have been introduced for the treatment of RA, with the expectation of obtaining immediate and certain effect. Although no teratogenicity of these drugs has been recognised in animal experiments,[4 5] the safety of their use in pregnancy has not yet been adequately established. In this report, we discuss the clinical usefulness of etanercept with reference to the drug concentration in maternal and neonatal blood and breast milk.

A 40-year-old active RA patient (51.5 kg body weight; 153 cm height; body mass index 22), with a past history of two miscarriages at 7 weeks of pregnancy, was started on treatment with etanercept at 25 mg sq twice a week. She became pregnant soon after the drug produced a dramatic improvement in the clinical symptoms of RA, and strongly desired to continue the treatment with etanercept and prednisolone (9 mg/day) even during the pregnancy. Based on previous reports,[6–8] we decided to continue the treatment after obtaining informed consent from the patient. She delivered a female infant weighing 1906 g by caesarean section at 36 weeks and 2 days of gestation. The infant Apgar scores at one minute and 5 minutes were 8 and 9, respectively, and no abnormalities were observed.

To determine the alterations in the blood concentrations of etanercept during pregnancy and to determine the transfer ratio of the drug to the fetus during pregnancy, the etanercept concentrations in the maternal blood in each trimester of pregnancy and the cord blood immediately after delivery were measured.[9] The concentrations in the maternal blood were stable during pregnancy and the concentration in the cord blood, which was considered to represent the level in the blood of the infant, was approximately 1/30th of the concentration in the maternal blood. After childbirth, to confirm the degree of the transfer of etanercept to breast milk and to determine the possibility of the drug being detected in the blood of the infant during breast feeding, the etanercept concentrations in breast milk and serum of the baby were measured (see table 1). While the infant continued to be completely breast fed, the serum concentration of the drug in the infant decreased rapidly. By 12 weeks after delivery, etanercept could no longer be detected in the infant's serum, despite its detection in the breast milk,[10] and the infant (3885 g body weight; 51.9 cm height) had at least 800 ml/day of breast milk. The decrease in concentration with time in the infant's serum despite continuation of breast feeding indicates placental transfer, but not transfer via the breast milk. These findings suggest that etanercept may be relatively safe during lactation.

In conclusion, unchanging levels in the maternal serum during pregnancy, low levels in the cord blood and progressively decreasing concentrations in the infant's serum despite breast feeding were observed in this study.

A Murashima,[1,2] N Watanabe,[1,2] N Ozawa,[1] H Saito,[1] K Yamaguchi[1,2]

[1] Department of Perinatology, National Center for Child Health and Development, Tokyo, Japan; [2] Japan Drug Information Institute in Pregnancy, Tokyo, Japan

Correspondence to: Dr A Murashima, Division of Maternal Medicine, Department of Perinatology, National Center for Child Health and Development, Okura 2-10-1, Setagaya-ku, Tokyo 157-8535, Japan; murasima-a@nccho.go.jp

Table 1 Etanercept concentration in serum and breast milk

	Trimester				Post-delivery (week)		
	1st	2nd	3rd	Delivery	1	3	12
Maternal (ng/ml)							
Serum	3849	3589	3401	2239	2306	3512	2872
Breast milk							3.5
Neonatal (ng/ml)							
Serum				81*	21	2	ND

*Serum from umbilical cord blood.
ND, not detected.

Competing interests: None.

Provenance and peer review: Not commissioned; externally peer reviewed.

Accepted 5 February 2009

Ann Rheum Dis 2009;**68**:1793–1794. doi:10.1136/ard.2008.105924

REFERENCES

1. **Reed SD,** Vollan TA, Svec MA. Pregnancy outcomes in women with rheumatoid arthritis in Washington state. *Matern Child Health J* 2006;**10**:361–6.
2. **Wallace DJ.** The use of etanercept and other tumor necrosis factor-alpha blockers in infertility: it's time to get serious. *J Rheumatol* 2003;**30**:1897–9.
3. **Winger EE,** Reed JL. Treatment with tumor necrosis factor inhibitors and intravenous immunoglobulin improves live birth rates in women with recurrent spontaneous abortion. *Am J Reprod Immunol* 2008;**60**:8–16.
4. **Treacy G.** Using analogous monoclonal antibody to evaluate the reproductive and chronic toxicity potential for a humanized anti-TNFα monoclonal antibody. *Hum Exp Toxicol* 2000;**19**:226–8.
5. **Product information.** Enbrel. Thousand Oaks, CA: Immunex, 2001.
6. **Mahadevan U,** Kane S, Sandborn WJ, *et al.* Intentional infliximab use during pregnancy for induction or maintenance of remission in Crohn's disease. *Aliment Pharmacol Ther* 2005;**21**:733–8.
7. **Chambers CD,** Tutuncu ZN, Johnson D, *et al.* Human pregnancy safety for agents used to treat rheumatoid arthritis: adequacy of available information and strategies for developing post-marketing data. *Arthritis Res Ther* 2008;**8**:215.
8. **Cush JJ.** Biological drug use: US perspectives on indications and monitoring. *Ann Rheum Dis* 2005;**64**(Suppl 4):iv18–23.
9. **Korth-Bradley JM,** Rubin AS, Hanna RK, *et al.* The pharmacokinetics of etanercept in healthy volunteers. *Ann Pharmacother* 2000;**34**:161–4.
10. **Ostensen M,** Eigenmann GO. Etanercept in breast milk. *J Rheumatol* 2004;**31**:1017–18.

A patient's wish: anakinra in pregnancy

Patients with adult-onset Still's disease (AOSD) are at increased risk for adverse outcomes of pregnancy. An increased rate of preterm birth, intrauterine growth retardation and a possible influence of disease activity on pregnancy outcome have been reported.[1] Interleukin 1 (IL1) is thought to have a key role in the pathogenesis of AOSD. Interestingly, it has also been related to an increased risk of preterm birth in animal models.[2 3] An increasing number of reports on treatment of refractory AOSD with IL1 receptor antagonist (anakinra) emphasise its dramatic therapeutic effect and the rarity of its adverse events.[4 5] However, its effects in pregnancy are not well known. Here we report successful continuous treatment of AOSD during pregnancy and breastfeeding.

A 33-year-old woman with AOSD currently treated with recombinant IL1 receptor antagonist (IL1ra, anakinra) expressed the wish to become pregnant. Her disease course was refractory to treatment with prednisone alone or in combination with azathioprine or etanercept (fig 1). In July 2006 during a severe flare while receiving prednisone, anakinra was initiated at 100 mg/day. The patient became rapidly asymptomatic and prednisone was tapered and subsequently stopped in December 2006.

Despite extensive warning about the lack of knowledge of the effects of IL1ra on fetal development, she decided to continue anakinra during pregnancy. Two months after discontinuation of contraception, pregnancy was diagnosed. The adjusted risk for trisomy-21 based on age, β-HCG, PAPP-A and length of the nuchal fold was 1:8100. α-Fetoprotein was normal. Ultrasound repeatedly demonstrated normal growth and no evidence of malformation. Doppler analysis of the uterine artery was always normal. She remained free of signs of AOSD with the exception of one short flare after having missed a single dose of anakinra (fig 1). In April 2008, she gave spontaneous birth to a girl at 40+5 weeks of gestation: weight: 2700 g (5th centile), length 46 cm, APGAR Score 7/8/9, umbilical cord arterial pH 7.17. Birth was complicated by retention of the placenta requiring manual abruption. There were no pathological findings on thorough paediatric examination at discharge.

Postpartum, the mother decided to breastfeed despite continuation of treatment with anakinra. The child showed steady growth (10th to 25th centile) and inconspicuous psychomotor development during follow-up. The patient remained free of signs of AOSD until 4 months after delivery when a flare occurred during continuous monotherapy with anakinra.

To our knowledge, this is the first report of treatment of AOSD with anakinra during pregnancy and breastfeeding. Treatment with anakinra has not been recommended during pregnancy.[6] No teratogenic effects were demonstrated in animal studies of anakinra,[6] but nidation was thought to be compromised.[7] Of note, no additional risk for retaining the placenta has been described. Here, anakinra was highly effective in suppressing disease activity of AOSD, was very well tolerated and affected neither the conception nor the development of the child in utero or post partum. However, according to existing guidelines patients should continue to receive firm advice about the potential risks of inhibiting IL1.

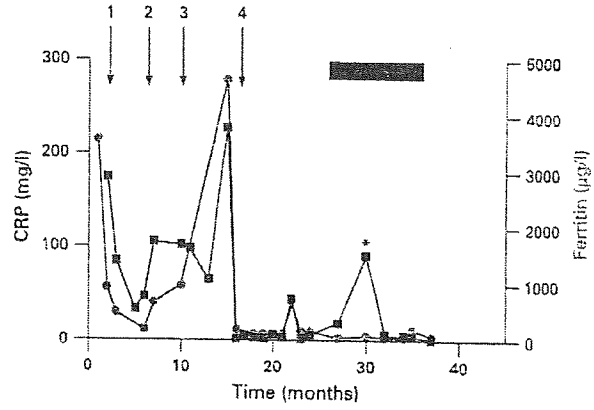


Figure 1 C-reactive protein (squares) and ferritin (circles) serum concentrations were monitored at the indicated time points. The black bar shows the duration of pregnancy. *Highlights the increase in C-reactive protein after a single missed injection of anakinra. The respective start of different therapeutic regimens is depicted by arrows as follows: (1) prednisone (initial dose 1 mg/kg/day); (2) prednisone + azathioprine (2 mg/kg/day); (3) prednisone + etanercept (2×25 mg subcutaneously/week); (4) anakinra subcutaneously 100 mg/day (first 6 months combined with tapered prednisone).

CMAJ PRACTICE

REVIEW

Safety of neuraminidase inhibitors against novel influenza A (H1N1) in pregnant and breastfeeding women

Toshihiro Tanaka MD, Ken Nakajima RPh, Atsuko Murashima MD PhD, Facundo Garcia-Bournissen MD, Gideon Koren MD, Shinya Ito MD

Published at www.cmaj.ca on June 15, 2009.

A new strain of influenza A virus (novel influenza A H1N1) that originated in swine has rapidly spread from the initial outbreak in Mexico and the southern United States to Canada and many countries in Europe and Asia. Consequently, the World Health Organization raised the level of alert for an influenza pandemic to 5 on Apr. 29, 2009.[1] Because many infected people are young,[2] the care of pregnant and lactating women is a concern.[3-6]

According to the US Centers for Disease Control and Prevention, the novel H1N1 influenza virus is susceptible to oseltamivir and zanamivir, neuraminidase-inhibitor antiviral medications, which target the early phase of the infection. However, this strain is resistant to adamantanes, such as amantadine and rimantadine.[7] The Centers for Disease Control and Prevention currently recommend antiviral treatment and chemoprophylaxis with either oseltamivir or zanamivir against novel H1N1 influenza for people at high risk of complications, including pregnant women.[3,4,8]

In this report, we summarize information about the safety of neuraminidase inhibitors for treatment of novel H1N1 influenza in pregnant and breastfeeding women. Although the information about drug safety in this report is also applicable to seasonal influenza and future pandemics, the management strategy presented in this article is specific to novel H1N1 influenza.

Evidence

We performed a literature search to identify reports of the use of oseltamivir or zanamivir during pregnancy, lactation and breastfeeding using MEDLINE (1950 to week 2 of May 2009) and EMBASE (1980 to week 19 of 2009) databases through the OVID system. The search terms were pregnancy, breastfeeding, human milk, lactation, influenza, oseltamivir, and zanamivir, or their various combinations. Relevant information was also gathered through the network of teratogen information services in Japan, where the use of oseltamivir and zanamivir for patients with confirmed influenza was relatively common even before the current pandemic.[9]

DOI:10.1503/cmaj.090866

Key points

- Pregnant women and infants are at high risk of influenza-related complications.
- Limited data suggest that oseltamivir is not a major human teratogen.
- Because of more data about its safety in pregnancy, the use of oseltamaivir is preferred over zanamavir during pregnancy.
- Oseltamivir and zanamivir are considered to be compatible with breastfeeding.

Influenza-related complications

Pregnancy

Little is known about whether influenza viruses are transmitted to the fetus through the placenta, although this class of viruses is not considered to be teratogenic in humans. Ács and colleagues[10] suggested indirect teratogenic effects of maternal influenza during pregnancy, possibly because of high fever, based on 1 case-control study and the known effects of hyperthermia, which is associated with an increased incidence of neural tube defects.[11]

The risk of morbidity from seasonal influenza is higher among pregnant women,[12,13] especially in the third trimester, than among nonpregnant and postpartum women.[12] This is consistent with increased mortality among pregnant women during past influenza pandemics.[14,15] Although the novel H1N1 influenza virus may not be as virulent as anticipated, the increased risk of complications during pregnancy should be taken into account when caring for affected patients. According to the Centers for Disease Control and Prevention, 20 recent infections of novel H1N1 influenza in the United States (15 confirmed and 5 probable) were in pregnant

From the Motherisk Program, Division of Clinical Pharmacology and Toxicology, Department of Paediatrics (Tanaka, Garcia-Bournissen, Koren, Ito), The Hospital for Sick Children, University of Toronto, Toronto, Ont.; and the Japan Drug Information Institute in Pregnancy, National Center for Child Health and Development (Nakajima, Murashima), Tokyo, Japan

Table 1: Outcomes of pregnancies in Japan after therapeutic exposure to oseltamivir in the first trimester

Characteristic	Toranomon Hospital[21] *n = 65*	Japan Drug Information Institute in Pregnancy *n = 25*
Time of exposure, gestational wk, range	1–12	2–10
No. of spontaneous abortions	1	2
No. of therapeutic abortions	0	1
Gestational age at birth, wk, range	35–41*	35–42
No. of preterm births	2*	2
Birth weight, g, range	2090–3810*	2418–3480
No. of infants with a low birth weight	3*	4
No. of infants with a major malformation	1†	0

*$n = 42$ (women exposed between gestational week 4 and 7 who had a live birth).
†Ventricular septal defect.

women. Of the 13 women for whom sufficient data were available, 3 were admitted to hospital; 1 of these patients died of respiratory complications. This patient was started on oseltamivir therapy 1 week after acute respiratory distress developed.[6] At present, the groups at high risk of influenza-related complications from the novel H1N1 influenza are the same as those for seasonal influenza. These groups include, but are not limited to, pregnant women and children aged 5 years or less.[8]

Lactation

Whether influenza viruses are passed into human milk is not known; however, respiratory droplets are likely to be the main mode of viral transmission. Because of the anti-infective benefits of human milk for infants, continuation of breastfeeding is recommended even if the mother is receiving treatment for novel H1N1 influenza infection.[3–5]

Pharmacotherapy

The Centers for Disease Control and Prevention recommendation[8] during the current pandemic is that drug treatment and chemoprophylaxis be considered, along with other public health measures, for patients at high risk of complications, including pregnant women and infants. Recent meta-analyses have suggested that oseltamivir and zanamivir may be modestly effective in alleviating symptoms of seasonal influenza in otherwise healthy adults[16] and children.[17] Routine use of these drugs is discouraged for patients at low-risk of complications from seasonal influenza, although these neuraminidase inhibitors are capable of reducing within-household spread of the disease, nasal viral load and lower respiratory tract complications.[16] Data about the effectiveness of these drugs in high-risk populations, specifically during the current pandemic, are limited.

Oseltamivir

Oseltamivir is a prodrug that is hydrolyzed by the liver to its active metabolite, oseltamivir carboxylate, with an elimination half-life of about 6–10 hours.[18] The therapeutic oral dosage for influenza, including novel H1N1 influenza, for adults is 75 mg taken twice daily for 5 days, starting within 48 hours of the initial symptoms to capture the early phase of viral replication. For chemoprophylaxis, the recommended dosage is 75 mg taken once daily for 10 days after exposure.[8] Therapeutic and prophylactic dosing schedules for children are similar (about 2 mg/kg twice a day for 5 days for treatment, and 2 mg/kg once a day for 10 days for prophylaxis).[8]

Pregnancy

A study using an ex vivo human placenta model showed that oseltamivir was extensively metabolized by the placenta.[19] Transplacental transfer of the metabolite was incomplete with minimal accumulation on the fetal side.[19] In postmarketing surveillance, 61 pregnant women who were exposed to oseltamivir with unknown timing were reported by the manufacturer.[20] Among these pregnancies, there were 10 abortions, including 6 therapeutic terminations, and 1 case each of trisomy 21 and anencephaly.[20] These findings are consistent with data from 2 Japanese teratogen information services (Toranomon Hospital,[21] and Japan Drug Information Institute in Pregnancy, National Center for Child Health and Development, Tokyo, Japan), which prospectively followed 90 pregnant women who took therapeutic doses of oseltamivir (75 mg twice a day for up to 5 days) during the first trimester (Table 1). In these 90 cases, there was 1 malformation (1.1%), which is within the incidence of major malformations in general population (1%–3%).

Lactation

Wentges-van Holthe and colleagues[22] reported the case of a lactating woman who received oseltamivir (75 mg twice daily for 5 days). The maximum milk concentrations of oseltamivir and its active metabolite were 38.2 ng/mL and 39.5 ng/mL (equivalent to 43.4 ng/mL of oseltamivir), respectively. The authors estimated that the infant would have been exposed to milk containing a maximum of 81.6 ng/mL oseltamivir–

equivalents, which corresponds to 0.012 mg/kg per day.[22] This is much smaller than the pediatric doses (2–4 mg/kg per day).

Zanamivir

Zanamivir is administered by inhalation with a dry powder inhaler. The bioavailability of the drug is 10%–20% by inhalation, compared with 2% by oral administration. About 90% of the absorbed dose is excreted unchanged in the urine. The elimination half-life in serum of zanamivir is between 2.5 and 5.1 hours.[23] The therapeutic dose is 10 mg inhaled twice daily for 5 days starting within 48 hours of the initial symptoms. For chemoprophylaxis, the dose is once daily for 10 days after exposure.[7,8] The recommended doses for children are the same.[8] Because zanamivir therapy requires the patient to voluntarily inhale through the device, oseltamivir may be preferred over zanamivir for young children.

Pregnancy

Three pregnant women were accidentally exposed to zanamivir during clinical trials.[24] Among these women, 1 pregnancy was spontaneously miscarried, 1 pregnancy was terminated, and 1 woman delivered a healthy baby.[24] The Japan Drug Information Institute in Pregnancy has information about 1 woman who took zanamivir at 4 weeks of gestation and delivered a healthy baby at term.

Lactation

A peak concentration of zanamivir in the serum after a 10 mg oral-inhalation dose ranges from 34 to 96 ng/mL.[23] Assuming a maternal serum concentration of 100 ng/mL, a milk-to-plasma ratio of 1.0 and an intake of milk of 150 mL/kg per day, the maximum amount of zanamivir that a 5 kg infant would ingest would be about 0.075 mg/day, which is much lower than the recommended prophylactic dosage for children of 10 mg/day inhalation.

Vaccine

The seasonal influenza vaccine does not appear to provide protection against novel H1N1 influenza.[25] Currently no vaccine for novel H1N1 influenza exists. However, vaccination for seasonal influenza should continue because of higher morbidity among pregnant women and possible concurrent epidemics with novel H1N1 influenza.[26] Once developed, it is unlikely that an inactivated vaccine against novel H1N1 influenza would be contraindicated for pregnant and lactating women, similar to regular influenza vaccines.[27,28]

Discussion

Pregnant women, especially those in the late stages of pregnancy, are at high risk of complications from influenza, including novel H1N1 influenza. Although the data are limited, this should be considered during the current novel H1N1 influenza pandemic.

If treatment or chemoprophylaxis is required for pregnant women during the current pandemic, oseltamivir appears to be the drug of choice because there are more data on its safety in pregnancy. The data suggest that oseltamivir is not a major teratogen for humans. Zanamivir may also be used, but there are less data available about its safety for pregnant women.

Both oseltamivir and zanamivir are considered to be compatible with breastfeeding. Continuation of breastfeeding by a woman taking these medications is unlikely to lead to substantial drug exposure by the infant. Adjustment of dose because of breastfeeding is not necessary. If mother–infant contact is clinically allowed, breastfeeding during oseltamivir or zanamivir treatment is acceptable. If an infant being breastfed by the mother receiving oseltamivir or zanamivir needs direct treatment or chemoprophylaxis, the recommended dose of oseltamivir or zanamivir for infants should be given. Therapy should start within 48 hours of the initial symptoms.

Prospective data collection with robust follow-up should continue for both oseltmivir and zanamivir.

This article has been peer reviewed.

Competing interests: None declared.

Contributors: Toshihiro Tanaka conceived and initiated this project, searched the literature, collected information, and drafted and revised the manuscript. Ken Nakajima searched Japanese literature, analyzed and interpreted the follow-up data collected from the Japan Drug Information Institute in Pregnancy, and drafted the paper. Atsuko Murashima critically interpreted the follow-up data collected from Japan Drug Information Institute in Pregnancy and drafted the paper. Facundo Garcia-Bournissen conceived the project, searched the Spanish literature, provided critical interpretation of the collected information and critically revised the draft. Gideon Koren provided critical interpretation of the data and revised the manuscript for key content. Shinya Ito searched literature, provided critical interpretation of the data, drafted the paper and revised it critically. All of the authors approved the final version submitted for publication.

Acknowledgements: Dr. Tanaka is supported by Nobel Pharma Scholarship, TFB Scholarship, Scholarship for Researchers through the Japanese Society of Clinical Pharmacology and Therapeutics, and The Research and Training Competition (RESTRACOMP) through the Hospital for Sick Children Research Institute.

Dr. Garcia-Bournissen has received funding from the Clinician Scientist Training Program. This program is funded by the Ontario Student Opportunity Trust Fund and the Hospital for Sick Children Foundation Student Scholarship Program.

Dr. Koren is the Ivey Chair in Molecular Toxicology, Department of Medicine, University of Western Ontario, and he holds the Research Leadership for Better Pharmacotherapy during Pregnancy and Lactation at The Hospital for Sick Children, Toronto, Ont.

Funding: The Japan Drug Information Institute in Pregnancy is supported by the Japanese Ministry of Health, Labor and Welfare. This report is supported by the National Center for Child Health and Development–Motherisk Collaborative Research Fund.

REFERENCES

1. Chan M. Influenza A (H1N1). Geneva (Switzerland): World Health Organization; 2009. Available: www.who.int/mediacentre/news/statements/2009/h1n1_20090429/en/index.html (accessed 2009 May 27).
2. Centers for Disease Control and Prevention. Update: novel influenza A (H1N1) virus infections — worldwide, May 6, 2009. *MMWR Morb Mortal Wkly Rep* 2009;58:453-8.
3. Centers for Disease Control and Prevention. Pregnant women and novel influenza A (H1N1) considerations for clinicians. Atlanta (GA): The Centers; 2009. Available: www.cdc.gov/h1n1flu/clinician_pregnant.htm (accessed 2009 May 27).
4. Centers for Disease Control and Prevention. What pregnant women should know about H1N1 (formerly called swine flu) virus. Atlanta (GA): The Centers; 2009. Available: www.cdc.gov/h1n1flu/guidance/pregnant.htm (accessed 2009 May 27).
5. Centers for Disease Control and Prevention. Novel H1N1 flu (swine flu) and feeding your baby: what parents should know. Atlanta (GA): The Centers; 2009. Available: www.cdc.gov/h1n1flu/breastfeeding.htm (accessed 2009 May 27).

6. Centers for Disease Control and Prevention. Novel influenza A (H1N1) virus infections in three pregnant women — United States, April–May 2009. *MMWR Morb Mortal Wkly Rep* 2009;58:497-500.
7. Centers for Disease Control and Prevention. Update: drug susceptibility of swine-origin influenza A (H1N1) viruses, April 2009. *MMWR Morb Mortal Wkly Rep* 2009;58:433-5.
8. Centers for Disease Control and Prevention. Interim guidance on antiviral recommendations for patients with novel influenza A (H1N1) virus infection and their close contacts. Atlanta (GA): The Centers; 2009. Available: www.cdc.gov/h1n1flu/recommendations.htm (accessed 2009 May 17).
9. Hata K, Koseki K, Yamaguchi K, et al. Limited inhibitory effects of oseltamivir and zanamivir on human sialidases. *Antimicrob Agents Chemother* 2008;52:3484-91.
10. Ács N, Banhidy F, Puho E, et al. Maternal influenza during pregnancy and risk of congenital abnormalities in offspring. *Birth Defects Res A Clin Mol Teratol* 2005; 73:989-96.
11. Moretti ME, Bar-Oz B, Fried S, et al. Maternal hyperthermia and the risk for neural tube defects in offspring: systematic review and meta-analysis. *Epidemiology* 2005;16:216-9.
12. Neuzil KM, Reed GW, Mitchel EF, et al. Impact of influenza on acute cardiopulmonary hospitalizations in pregnant women. *Am J Epidemiol* 1998;148:1094-102.
13. Dodds L, McNeil SA, Fell DB, et al. Impact of influenza exposure on rates of hospital admissions and physician visits because of respiratory illness among pregnant women. *CMAJ* 2007;176:463-8.
14. Harris JW. Influenza occurring in pregnant women: a statistical study of thirteen hundred and fifty cases. *JAMA* 1919;72:978-80.
15. Freeman DW, Barno A. Deaths from Asian influenza associated with pregnancy. *Am J Obstet Gynecol* 1959;78:1172-5.
16. Jefferson TO, Demicheli V, Di Pietrantonj C, et al. Neuraminidase inhibitors for preventing and treating influenza in healthy adults. *Cochrane Database Syst Rev* 2006;(3):CD001265.
17. Matheson NJ, Harnden A, Perera R, et al. Neuraminidase inhibitors for preventing and treating influenza in children. *Cochrane Database Syst Rev* 2007;(1): CD002744.
18. He G, Massarella J, Ward P. Clinical pharmacokinetics of the prodrug oseltamivir and its active metabolite Ro 64-0802. *Clin Pharmacokinet* 1999;37:471-84.
19. Worley KC, Roberts SW, Bawdon RE. The metabolism and transplacental transfer of oseltamivir in the ex vivo human model. *Infect Dis Obstet Gynecol* 2008;2008: 927574.
20. Ward P, Small I, Smith J, et al. Oseltamivir (Tamiflu) and its potential for use in the event of an influenza pandemic. *J Antimicrob Chemother* 2005;55(Suppl 1):i5-21.
21. Hayashi M, Yamane R, Tanaka M, et al. Pregnancy outcome after maternal exposure to oseltamivir phosphate during the first trimester: a case series survey [Japanese]. *Nihon Byoin Yakuzaishi Gakkai Zasshi* 2009;45:547-50.
22. Wentges-van Holthe N, van Eijkeren M, van der Laan JW. Oseltamivir and breastfeeding. *Int J Infect Dis* 2008;12:451.
23. Cass LM, Efthymiopoulos C, Bye A. Pharmacokinetics of zanamivir after intravenous, oral, inhaled or intranasal administration to healthy volunteers. *Clin Pharmacokinet* 1999;36(Suppl 1):1-11.
24. Freund B, Gravenstein S, Elliott M, et al. Zanamivir: a review of clinical safety. *Drug Saf* 1999;21:267-81.
25. Centers for Disease Control and Prevention. Serum cross-reactive antibody response to a novel influenza A (H1N1) virus after vaccination with seasonal influenza vaccine. *MMWR Morb Mortal Wkly Rep* 2009;58:521-4.
26. Pan American Health Organization. EOC situation report #7: influenza A/H1N1 in the Americas (Mexico, the United States, Canada). Geneva (Switzerland): The Organization; 2009. Available: new.paho.org/hq/index.php?option=com_content&task=view&id=1290&Itemid=569 (accessed 2009 May 27).
27. Fiore AE, Shay DK, Broder K, et al. Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2008. *MMWR Morb Mortal Wkly Rep* 2008;57(RR-7):1-60.
28. ACOG Committee on Obstetric Practice. ACOG committee opinion number 305, November 2004. Influenza vaccination and treatment during pregnancy. *Obstet Gynecol* 2004;104(5 Pt 1):1125-6.

Correspondence to: *Dr. Shinya Ito, Division of Clinical Pharmacology and Toxicology, Department of Paediatrics, The Hospital for Sick Children, 555 University Ave., Toronto ON M5G 1X8; fax 416 813-7562; shinya.ito@sickkids.ca*

Pediatr Cardiol (2009) 30:35–40
DOI 10.1007/s00246-008-9273-5

ORIGINAL ARTICLE

Outcome of Prenatally Diagnosed Isolated Congenital Complete Atrioventricular Block Treated with Transplacental Betamethasone or Ritodrine Therapy

Taiyu Hayashi · Masahide Kaneko · Ki-Sung Kim · Yoshihiko Eryu · Takahiro Shindo · Takayoshi Isoda · Atsuko Murashima · Yushi Ito · Haruhiko Sago

Received: 26 March 2008 / Accepted: 6 July 2008 / Published online: 26 July 2008

Abstract The effectiveness of transplacental drug therapy for prenatally diagnosed isolated congenital complete atrioventricular block (CCAVB) is controversial. Nine cases of prenatal isolated CCAVB were treated from 2002 to 2007. Ritodrine was administered transplacentally to all fetuses and betamethasone to those whose mothers tested positive for maternal anti-SSA/Ro antibodies. Six of the nine patients had an anti-SSA/Ro-positive mother and received transplacental betamethasone 4 mg/day at a median gestational age of 28 weeks (range, 24–31 weeks). No patients exhibited an improvement in the degrees of complete heart block, and one patient died in utero. No serious adverse events occurred. After the mean follow-up period of 1.7 ± 1.3 years, all five patients treated with transplacental betamethasone experienced a good cardiac function, whereas one of the three patients not treated with transplacental betamethasone experienced cardiomyopathy and died at the age of 4 months. Pacemaker implantation was required for seven of the eight live-born infants. Transplacental betamethasone therapy for the patients with isolated CCAVB neither improved the degree of atrioventricular block nor decreased the rate of patients requiring pacemaker implantation, but it probably reduced the risk for the development of myocardial disease.

Keywords Anti-SSA/Ro antibody · Atrioventricular block · Betamethasone · Congenial · Fetal hydrops

T. Hayashi (✉) · M. Kaneko · K.-S. Kim · Y. Eryu · T. Shindo · T. Isoda
Division of Pediatric Cardiology, National Center for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan
e-mail: hayashi-t@ncchd.go.jp

A. Murashima
Division of Maternal Internal Medicine, National Center for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan

Y. Ito
Division of Neonatology, National Center for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan

H. Sago
Division of Fetal Medicine, National Center for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan

Congenital complete atrioventricular block (CCAVB) is a relatively rare disease among children with normal heart structures, with an estimated incidence of 1 in 14,000–20,000 live births [18]. Isolated CCAVB often is associated with maternal anti-SSA/Ro or anti-SSB/La antibodies. Among mothers with positive test results for anti-SSA/Ro or anti-SSB/La antibodies, the rate of bearing an infant with isolated CCAVB is 1–2% [2, 7, 10], and 16% mothers with a previous affected offspring had a second affected child [4].

A previous study found that patients with positive maternal antibodies had a worse prognosis [20]. The deposition of the antibodies along the conduction system and the myocardium of the fetus may trigger immune-mediated inflammation and result in fibrosis of the atrioventricular node and endocardium [12]. Regardless of the maternal antibody status, pacemaker implantation was required for 63–95% of patients, and death due to dilated cardiomyopathy was not uncommon [4, 13, 20].

Transplacental steroid therapy is reported to be ineffective in terms of reversing the severity of atrioventricular block, but it probably could reduce fetal and neonatal

morbidity and mortality [14, 17]. However, reports on the clinical outcome of fetuses with isolated CCAVB who have received transplacental therapy are limited.

This study aimed to review the clinical course of infants with a prenatal diagnosis of isolated CCAVB who were treated with transplacental ritodrine, betamethasone therapy, or both.

Materials and Methods

We studied all cases of isolated CCAVB diagnosed prenatally at our institution from 2002 to 2007. Complete atrioventricular block was diagnosed when no mechanical relation existed between atrial and ventricular contraction on fetal M-mode echocardiography. Patients with complex cardiac anomalies such as heterotaxy syndrome and congenitally corrected transposition of the great arteries were not included in the study. Nine cases eventually were identified and included in the study.

The medical records of the affected patients were reviewed. The collected data included status at presentation, maternal symptoms of collagen disease, type and duration of transplacental therapy, changes in fetal heart rates, and outcome of pregnancy. Postnatal electrocardiograms were reviewed for the degree of atrioventricular block, heart rates, QRS widths, and QTc intervals. Data regarding postnatal treatment, presence or absence of cardiomyopathy, and status at the most recent follow-up assessment also were collected.

All mothers were tested for the presence or absence of anti-SSA/Ro and anti-SSB/La antibodies using the Ouchterlony double immunodiffusion test.

Statistical Analysis

The data are expressed as frequencies, mean ± standard deviation, and median and range, as appropriate. Comparisons between groups were performed using Fisher's exact test for categorical variables and the Mann–Whitney U test for continuous variables. All p values less than 0.05 were considered statistically significant.

Results

Clinical Features

Table 1 summarizes the clinical features of all nine cases included in the study. Five mothers had been pregnant previously, but none of their offspring had a history of complete atrioventricular block. The median gestational age at diagnosis was 27 weeks (range, 23–30 weeks), and all mothers were referred to our institution after fetal bradycardia was detected using a routine fetal ultrasound examination. Fetal hydrops was diagnosed in three fetuses at the time of diagnosis, and no other fetus had hydrops thereafter.

Eight mothers (89%) delivered live offspring, whereas one mother had an intrauterine fetal death. Two neonates (cases 3 and 7) had structural cardiac anomalies: small

Table 1 Characteristics of the nine cases with a prenatal diagnosis of isolated congenital complete atrioventricular block (CCAVB)

Case	Sex	Maternal antibodies	GA at diagnosis (weeks)	Prenatal treatment		Hydrops	GA at birth (weeks)	Birth weight (g)	CHD	Postnatal treatment	Pacemaker age (days)	Mode	Outcome (follow-up years)
				Ritodrine	Betamethasone								
1	M	SS-A	23	+	+	–	32	2,338	–	ISP	24	VVI	Alive (3.9)
2	F	–	30	+	–	+	30	1,624	–	ISP, DOA	4	VVI	Alive (2.9)
3	M	–	27	+	–	+	28	1,332	VSD	ISP, DOA	4	VVI	Alive (2.8)
4	F	SS-A	24	+	+	–	35	1,634[a]	–	ISP, DOA			Alive (1.6)
5	M	SS-A	29	+	+	–	36	2,300[a]	–	ISP	40	VVI	Alive (1.0)
6	F	SS-A	27	+	+	–	37	2,460	–	ISP	297	VVI	Alive (0.8)
7	F	–	28	+	–	–	37	2,816	PS	ISP	11	VVI	DCM, died at 4 months
8	F	SS-A	29	+	+	–	36	1,884[a]	–	ISP	3	VVI	Alive (0.4)
9	–	SS-A	23	+	+	+	–	–	–	–	–	–	IUFD at 30 weeks

GA, gestational age; CHD, congenital heart disease; SS-A, anti SS-A/Ro antibody; ISP, isoprenaline; VVI; DOA, dopamine; IUFD, intrauterine fetal death

[a] Small for gestational age

muscular ventricular septal defect in the former and pulmonary valve stenosis in the latter. Postnatal electrocardiography confirmed the diagnosis of CCAVB in all live-born infants.

Six mothers tested positive for anti-SSA/Ro antibodies, whereas none tested positive for anti-SSB/La antibodies. Among the six mothers with positive anti-SSA/Ro antibodies, four experienced some symptoms of collagen disease. However, only one mother had a previous diagnosis of collagen disease. None of the mothers had been treated with steroids at the time of referral to our institution.

Transplacental Therapy and Outcome of Pregnancy

Beta-mimetic agent (ritodrine) was administered to all nine mothers as soon as CCAVB was diagnosed. Five fetuses (56%) showed a persistent increase in their heart rate by more than 5 bpm for at least 2 weeks after initiation of the beta-mimetic agent (Fig. 1).

Betamethasone was additionally administered if the mothers tested positive for anti-SSA/Ro antibodies. All six mothers with anti-SSA/Ro antibodies received betamethasone therapy at the median gestational age of 28 weeks (range, 24–31 weeks). Betamethasone therapy was initiated at a dose of 4 mg/day in all cases. In two cases, the same dose of betamethasone was maintained for 10 weeks (case 4) or 6 weeks (case 9) until the end of pregnancy. In four cases, the dose was tapered after 2–4 weeks.

The betamethasone therapy did not improve the degree of atrioventricular block in any patients treated. Three mothers exhibited adverse effects attributable to the transplacental betamethasone therapy, which included mood disorder, insomnia, and increased appetite. However, betamethasone therapy was not discontinued for any of these mothers because serious adverse effects did not occur.

Of the three fetuses with hydrops, one (case 9) died in utero at the gestational age of 30 weeks. In this case, the fetal heart rate was 42 bpm at the time of diagnosis—the lowest among all the cases. It even decreased to 35 bpm, indicating absence of response to transplacental therapy with ritodrine and betamethasone. In the other two fetuses, the initial fetal heart rate was 55 bpm (case 3) or less (case 2) at the time of referral to our institution. Because the maternal anti-SSA/Ro antibodies were negative, both of these fetuses were treated with transplacental ritodrine alone. However, increases in the fetal heart rates were subtle, and these two fetuses showed no improvement in fetal hydrops. Their mothers underwent preterm cesarean section at the gestational age of 30 weeks (case 2) and 28 weeks (case 3).

Of the six fetuses without hydrops, all but one infant were born after the gestational age of 35 weeks. In case 1, the fetus was delivered by cesarean section at 32 weeks gestation because of placenta previa and vaginal bleeding.

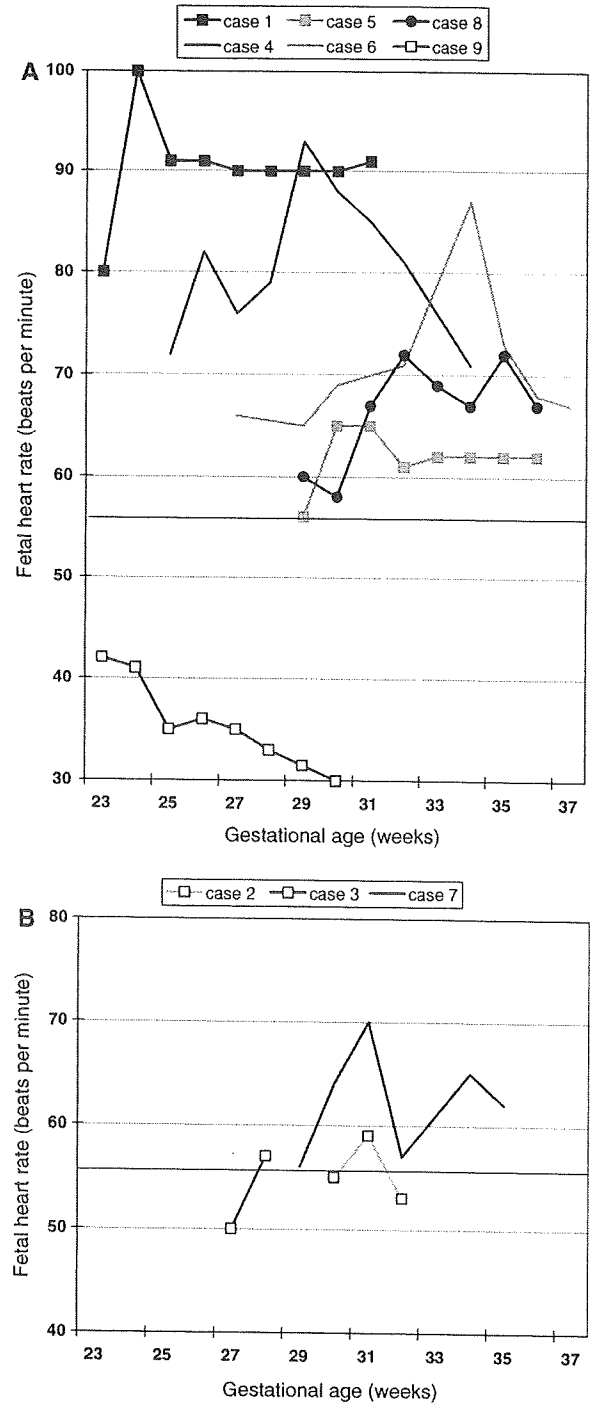


Fig. 1 Fetal heart rate of patients treated transplacentally with betamethasone and ritodrine (**a**) and with ritodrine alone (**b**). Five (56%) of the fetuses had a persisting increase in heart rate of more than 5 bpm for at least 2 weeks after initiation of the beta-mimetic agent. No cases showed improvement in the degree of complete heart block. In the cases with fetal hydrops (open square), the fetal heart rates were lower than 55 bpm at least once. In the cases without fetal hydrops, the fetal heart rates never fell below 55 bpm throughout pregnancy

In none of the six cases did the fetal heart rate fall below 55 bpm throughout pregnancy (Fig. 1). The combined rate of intrauterine fetal death and premature delivery (before 35 weeks gestation) was significantly higher in the fetuses with hydrops than in those without hydrops (100% vs. 17%; $p = 0.047$).

The mean birth weight was 2,049 ± 507 g (range, 1332–2816 g). Three infants were small for gestational age, and all these infants tested positive for maternal anti-SSA/Ro antibodies.

Postnatal Treatment

Eight pregnancies resulted in live births, and isoprenaline infusion was initiated during the neonatal period for all eight infants. Isoprenaline therapy was successfully tapered for two infants (cases 4 and 6), both of whom tested positive for maternal anti-SSA/Ro antibodies. The remaining six infants needed pacemaker implantation at a median age of 7.5 days (range, 3–40 days). The indications for pacemaker implantation were bradycardia with ventricular rates less than 55 bpm in five infants and ventricular dysfunction with a wide QRS escape rhythm in one infant (case 7).

Three patients with birth weights less than 2,000 g were treated initially with temporary epicardial pacing. Two of these three patients (cases 2 and 3) were born prematurely due to fetal hydrops. We had adopted a staged approach for hydropic fetuses. Before placing temporary epicardial leads, we stabilized their respiratory and circulatory condition by isoprenaline and dopamine infusion, supplementation of pulmonary surfactant, and if necessary, surgical drainage of pulmonary effusion. Although pericardial effusion developed in one patient, requiring surgical drainage, temporary pacing could be successfully switched to permanent pacemaker implantation in all the patients when their body weights increased to 2,500 g.

For the patients discharged without pacemaker implantation, the QRS widths and QTc intervals tended to be shorter than for those discharged after pacemaker implantation (QRS widths, 64 ± 5.7 vs. 97 ± 28 ms; $p = 0.05$; QTc intervals, 374 ± 67.9 vs. 454 ± 379 ms; $p = 0.08$). However, we found no statistically significant differences.

Outcome of Affected Infants

After the mean follow-up period of 1.7 ± 1.3 years (range, 0.4–3.9 years), seven patients were alive and one patient (case 7) had died of dilated cardiomyopathy at the age of 4 months. In this case, fetal echocardiography performed at the 28th gestational week had shown myocardial hypertrophy. Because the maternal anti-SSA/Ro antibody was negative, the patient had not received transplacental steroid therapy. At birth, echocardiography showed pulmonary valve stenosis and severe heart failure, with a left ventricular ejection fraction of 50% and a left ventricular end-diastolic diameter of 22 mm. Although the baby was treated postnatally with pacemaker implantation and methylprednisolone pulse therapy in addition to pharmacologic therapy with diuretics, vasodilators, and inotropic agents, the cardiac function continued to deteriorate, and the patient died at the age of 4 months.

The remaining seven patients experienced a good cardiac function without the development of any significant valve insufficiency. Of the two patients discharged initially without pacemaker implantation, one (case 6) experienced an abrupt pause in ventricular rhythm that exceeded 3.7 s on Holter monitoring at the age of 9 months and therefore received pacemaker implantation. Finally, at the mean follow-up period of 1.7 ± 1.3 years, one of the eight patients (13%) had experienced dilated cardiomyopathy, and the remaining seven (88%) had required a permanent pacemaker.

Discussion

With fetal echocardiography coming into wide use, cases of isolated CCAVB are increasingly being identified prenatally, mostly before the gestational age of 30 weeks [13]. Because prenatally diagnosed CCAVB cases are reported to have higher mortality and require pacemaker implantation more frequently [13], various types of transplacental therapy have been attempted [1, 5]. Although transplacental therapy with steroids, beta-mimetics, or both is becoming commonplace, reports on the outcome of these therapies are limited, and there is no widely accepted regimen of transplacental therapy to date.

Maternally administered steroids have been used for the treatment of isolated CCAVB. Although several studies [9, 19] have reported that heart block of less advanced degrees reverted to normal sinus rhythm after transplacental steroid therapy, reports documenting the reversal of complete atrioventricular block are rare [12]. However, it has been suggested that transplacental steroid therapy may reduce the incidence of more severe myocardial disease. Jaeggi et al. [14] reported that the rate of immune-mediated postnatal complications such as hepatitis, myocarditis, and endocardial fibroelastosis was significantly lower for cases treated with transplacental dexamethasone administration. These authors recommended transplacental dexamethasone therapy for all isolated CCAVB cases regardless of the maternal antibody status.

Our study also suggests the efficacy of transplacental steroid therapy in preventing myocardial disease. In our series, all the six mothers testing positive for anti-SSA/Ro antibodies had received transplacental betamethasone

therapy. One fetus with hydrops died in utero. Although the transplacental betamethasone therapy neither improved the degree of complete atrioventricular block nor prevented postnatal pacemaker implantation, none of the five surviving infants experienced dilated cardiomyopathy, and all experienced a good cardiac condition with normal left ventricular ejection fraction. The only patient who died of dilated cardiomyopathy in our series had not received betamethasone prenatally.

It is reported that transplacental steroid therapy may cause oligohydramnios, which sometimes prompts premature delivery or fetal death [14]. No patients in our series experienced such serious adverse effects. Prenatal exposure to dexamethasone may lead to adverse obstetric events such as spontaneous abortion, stillbirth, and neonatal adrenal insufficiency [6]. In addition, there is concern about the adverse neurodevelopmental effects of prenatal steroid exposure. Brucato et al. [3] studied 14 children with isolated CCAVB who had been exposed prenatally to a high dosage of dexamethasone and found that all these patients had normal intelligence at the mean age of 5 years.

One recent large study of extremely low-birth-weight infants by Lee et al. [16] showed that prenatal betamethasone exposure was associated with increased likelihood of unimpaired neurodevelopmental status at corrected ages of 18 to 22 months compared with prenatal dexamethasone exposure or no prenatal steroid exposure. Although the dose and the duration of steroids administered in transplacental therapy for CCAVB differ from those for extremely low-birth-weight infants, prenatal dexamethasone exposure may be more harmful than prenatal betamethasone exposure in terms of neurodevelopmental status. Therefore, transplacental therapy with betamethasone currently seems to be better than therapy with dexamethasone. The efficacy and safety of transplacental betamethasone therapy for CCAVB should be evaluated in a prospective randomized trial.

Transplacental therapy with beta-mimetics is reported to increase the fetal ventricular heart rate in some CCAVB cases, but this approach does not seem to be universally effective [14, 17]. Whereas Jaeggi et al. [14] used beta-mimetics only for cases with a fetal ventricular rate less than 55 bpm, we administered beta-mimetics to all the mothers regardless of the fetal ventricular rate because we consider that all affected fetuses will benefit from an increase in their ventricular rate. In our series, five (56%) of nine fetuses showed a persistent increase in their heart rate by more than 5 bpm for at least 2 weeks after the initiation of the beta-mimetic agent. Unfortunately, the transplacental ritodrine therapy was not effective for three fetuses with hydrops in our series, suggesting the limitations of the therapy in severely affected cases.

Former reports have shown that risk factors for poor prognosis for isolated CCAVB patients include fetal hydrops, positive maternal anti-SSA/Ro antibodies, and endocardial fibroelastosis [13, 20]. In our series, the combined rate of intrauterine fetal death and premature delivery (before 35 weeks gestation) was significantly higher for fetuses with hydrops than for fetuses without hydrops. We did not find any differences in prognosis between fetuses born to mothers with and those without anti-SSA/Ro antibodies, partly because of too few cases. However, it is noteworthy that one fetus experienced dilated cardiomyopathy and two experienced hydrops despite negative maternal anti-SSA/Ro antibodies. It seems that patients without maternal anti-SSA/Ro antibodies do not always have a better prognosis than those with maternal anti-SSA/Ro antibodies.

No patients in our series experienced endocardial fibroelastosis. The heart rates of the three fetuses with hydrops were 55 bpm or less at the time of referral to our institution, and the fetus with the lowest heart rate eventually died in utero. Thus, a low fetal heart rate could possibly predict poor prognosis. Grove et al. [11] reported that a fetal heart rate lower than 55 bpm before 28 weeks gestation was associated with greater likelihood of a poor outcome. Jaeggi et al. [13] reported that the majority of fetuses with a heart rate lower than 55 bpm did not survive the perinatal period, but this observation did not reach statistical significance.

A large proportion of patients with isolated CCAVB require pacemaker implantation. Previous reports have shown that pacemaker implantation was required for 63% to 95% of patients [4, 13, 20]. In our series, pacemaker implantation was necessary for seven patients (88% of the live-born infants) after the mean follow-up period of 1.7 ± 1.3 years. Of the five live-born infants who had received the transplacental betamethasone therapy, four (80%) required pacemaker implantation. Five patients underwent pacemaker implantation during the neonatal period, one at 40 days of age and one more patient at the age of 9 months.

For the premature hydropic fetuses, we adopted the staged pacing approach, as formerly reported [8, 21]. Before placing the temporary epicardial leads, we stabilized the respiratory and circulatory condition of the patients by isoprenaline and dopamine infusion, supplementation of pulmonary surfactant, and if necessary, surgical drainage of pulmonary effusion. This approach successfully bridged the interval until a permanent pacemaker could be implanted. A previous report described temporary epicardial pacing for a neonate weighing as little as 930 g [21]. Although the decision of performing premature delivery must be carefully considered, the initial staged pacing approach with the use of temporary

epicardial pacing can improve the outcome of premature hydropic fetuses with isolated CCAVB.

Although all our patients, except for one who experienced dilated cardiomyopathy and died at 4 months of age, had a good cardiac condition, a close long-term follow-up is essential. Kurosaki et al. [15] reported that the total mortality rate for patients with isolated CCAVB who underwent pacemaker implantation during the neonatal period was 30% after a median follow-up period of 5.6 years, and that the cumulative probability of freedom from dilated cardiomyopathy at 10 years was 59%.

The limitations of our study need to be addressed. Our series included only a small number of patients, and the follow-up periods were relatively short. The gestational ages at which the transplacental drug therapy was initiated may have been too late in some cases. In addition, the ways of tapering betamethasone varied across cases, although the initial dose was 4 mg/day in all cases.

In conclusion, transplacental betamethasone therapy for the patients with isolated CCAVB neither improved the degree of atrioventricular block nor decreased the rate of patients requiring pacemaker implantation, but it probably decreased the risk for the development of myocardial disease. Fetal hydrops in patients with isolated CCAVB relates to intrauterine fetal death and premature delivery. A large proportion of patients with isolated CCAVB require pacemaker implantation. The initial staged pacing approach with temporary epicardial pacing can improve the outcome for premature hydropic fetuses with isolated CCAVB.

References

1. Barclay CS, French MAH, Ross LD, Sokol RJ (1987) Successful pregnancy following steroid therapy and plasma exchange in a woman with anti-Ro (SS-A) antibodies: case report. Br J Obstet Gynaecol 94:369–371
2. Brucato A, Frassi M, Franceschini F et al (2001) Risk of congenital complete heart block in newborns of mothers with anti-Ro/SSA antibodies detected by counterimmunoelectrophoresis: a prospective study of 100 women. Arthritis Rheum 44:1832–1835
3. Brucato A, Astori MG, Cimaz R et al (2006) Normal neuropsychological development in children with congenital complete heart block who may or may not be exposed to high-dose dexamethasone in utero. Ann Rheum Dis 65:1422–1426
4. Buyon JP, Hiebert R, Copel J et al (1998) Autoimmune-associated congenital heart block: demographics, mortality, morbidity, and recurrence rates obtained from a national neonatal lupus registry. J Am Coll Cardiol 31:1658–1666
5. Carpenter RJ, Strasburger JF, Garson A et al (1986) Fetal ventricular pacing for hydrops secondary to complete atrioventricular block. J Am Coll Cardiol 8:1434–1436
6. Costedoat-Chalumeau N, Amoura Z, Hong DLT et al (2003) Questions about dexamethasone use for the prevention of anti-SSA related congenital heart block. Ann Rheum Dis 62:1010–1012
7. Costedoat-Chalumeau N, Amoura Z, Lupoglazoff JM et al (2004) Outcome of pregnancies in patients with anti-SSA/Ro antibodies: a study of 165 pregnancies, with special focus on electrocardiographic variations in the children and comparison with a control group. Arthritis Rheum 50:3187–3194
8. Deloof E, Devlieger H, Van Hoestenberghe R, Vandenberghe K, Daenen W, Gewllig M (1996) Management with a staged approach of the premature hydropic fetus due to complete congenital heart block. Eur J Pediatr 156:521–523
9. Friedman DM, Kim MY, Copel JA et al (2008) Utility of cardiac monitoring in fetuses at risk for congenital heart block: the PR interval and dexamethasone evaluation (PRIDE) prospective study. Circulation 117:485–493
10. Gladman G, Silverman ED, Yuk-Law et al (2002) Fetal echocardiographic screening of pregnancies of mothers with anti-Ro and/or anti-La antibodies. Am J Perinatol 19:73–80
11. Grove AMM, Allan LD, Rosenthal E (1996) Outcome of isolated congenital complete heart block diagnosed in utero. Heart 75:190–194
12. Hornberger LK, Sahn DJ (2007) Rhythm abnormalities of the fetus. Heart 93:1294–1300
13. Jaeggi ET, Hamilton RM, Silverman ED et al (2002) Outcome of children with fetal, neonatal, or childhood diagnosis of isolated congenital atrioventricular block. J Am Coll Cardiol 39:130–137
14. Jaeggi ET, Fouron JC, Silverman ED et al (2004) Transplacental fetal treatment improves the outcome of prenatally diagnosed complete atrioventricular block without structural heart disease. Circulation 110:1542–1548
15. Kurosaki K, Miyazaki A, Watanabe K, Echigo S (2008) Long-term outcome of isolated congenital complete atrioventricular block pacing since neonatal period: experience at a single Japanese institution. Circ J 72:81–87
16. Lee BH, Stoll BJ, McDonald SA, Higgins RD (2008) Neurodevelopmental outcomes of extremely low-birth-weight infants exposed prenatally to dexamethasone versus betamethasone. Pediatrics 121:289–296
17. Maeno Y, Himeno W, Saito A et al (2005) Clinical course of fetal congenital atrioventricular block in the Japanese population: a multicentre experience. Heart 91:1075–1079
18. Michaelsson M, Engle MA (1972) Congenital complete heart block: an international study of the natural history story. Clin Cardiol 4:86–101
19. Saleeb S, Copel J, Friedman D, Buyon JP (1999) Comparison of treatment with fluorinated glucocorticoids to the natural history of autoantibody-associated congenital heart block: retrospective review of the research registry for neonatal lupus. Arthritis Rheum 42:2335–2345
20. Villain E, Coastedoat-Chalumeau N, Marijon E, Boudjemline Y, Piette JC, Bonnet D (2006) Presentation and prognosis of complete atrioventricular block in childhood, according to maternal antibody status. J Am Coll Cardiol 48:1682–1687
21. Weindling SN, Saul JP, Triedman JK et al (1994) Staged pacing therapy for congenital complete heart block in premature infants. Am J Cardiol 74:412–413

Practice of Pharmaceutical Care ファーマシューティカル・ケアの実践 65

「妊娠と薬外来」における薬剤相談の取り組み

筑波大学附属病院 薬剤部　幸田 幸直　香取 久美
同大学附属病院 産科婦人科　濱田 洋実

はじめに

このほど日本病院薬剤師会に妊婦・授乳婦専門薬剤師制度が立ち上がった。妊婦や授乳婦を対象にした専門薬剤師・認定薬剤師が、その専門性を活かして、病棟で、外来で活躍する日も近いと感じている。その活躍の場の大きな部分を占めるものとして「妊娠と薬外来」があると考えている。「妊娠と薬外来」は、1980年代の終わりごろ、虎の門病院に「妊娠と薬相談外来」が開設されたのが最初で[1]、その後、2003年末に国立成育医療センターに「妊娠・授乳と薬相談外来」が開設されるなど、いくつかの医療機関に妊娠と薬の外来が開設されるようになった[2]。しかしながら、これらの医療機関だけでは膨大な相談要請に応じることができないことから、2005年10月に国立成育医療センター内に「妊娠と薬情報センター」が設立され、国家事業として「妊娠と薬情報センター事業」が動き出した[2]。

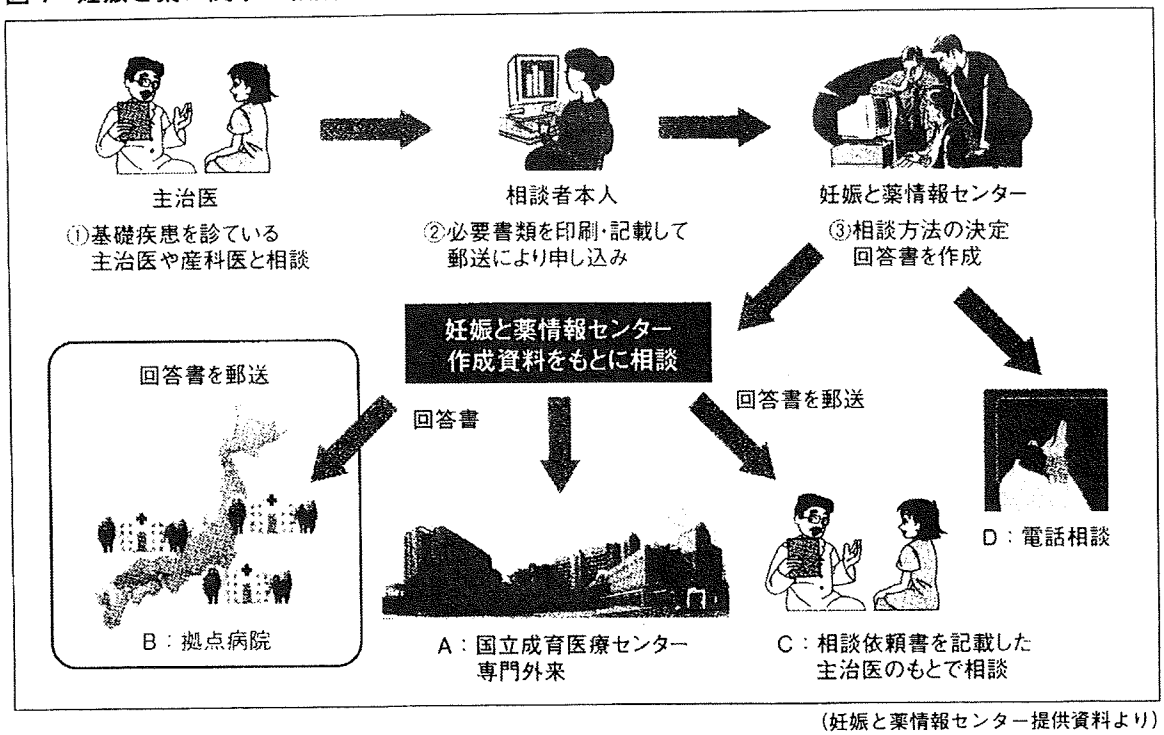
「妊娠と薬外来」の開設と運営

筑波大学附属病院（以下、当院）は、2007年に妊娠と薬情報センター事業に拠点病院として参画し、自費診療の相談外来として「妊娠と薬外来」を開設した。当院では、それまで産科外来の一部として産科医師による胎児外来が運営され、そこで妊娠と薬の相談を受けていたが、妊娠と薬情報センター事業への参画を機に薬剤師も相談業務を担当するようになった。

妊娠と薬情報センター事業は、図1に示されるように、妊娠と薬情報センターによって作成された資料をもとに相談を実施している。具体的には、Aのように国立成育医療センターの専門外来での相談、Bのように各地の拠点病院外来での相談、Cのように主治医のもとでの相談、Dのように妊娠と薬情報センターとの直接の電話相談の4通りの方法がある。当院の妊娠と薬外来は、妊娠と薬情報センター事業に拠点病院として参画してからは、Bの拠点病院という位置づけで業務を遂行している。

相談の手順は、図1に示されるように、相談者本人が妊娠と薬情報センターに郵送で申し込み、その際、当院での相談を希望すると、妊娠と薬情報センターから相談日程の指示が出され、それに基づいて当院の

図1　妊娠と薬に関する相談の方法



（妊娠と薬情報センター提供資料より）

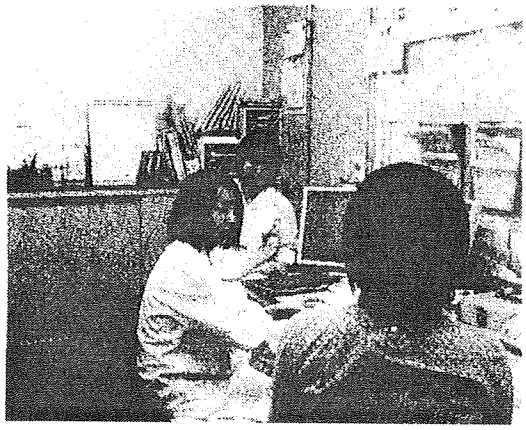
表1　問診票と回答書に記載される内容

問診票（相談者→妊娠と薬情報センター）	回答書（妊娠と薬情報センター→拠点病院）
・記入日 ・相談者の氏名、住所、生年月日、連絡先電話番号 ・過去の妊娠と薬情報センター利用の有無 ・相談の内容 ・相談のきっかけ ・妊娠の有無（現在、妊娠している場合は、その状況） ・妊娠暦 ・相談対象の薬剤 ・嗜好品 ・職業環境 ・既往歴、治療中の疾患名	・全般的な注意点 ・相談薬剤に関する情報 （回答書の転載・複製・翻訳・譲渡権は、妊娠と薬情報センターが保有）

予約センターで妊娠と薬外来の予約をとることになる。その後、外来予約日の数日前までに、相談者が作成した「問診票」と妊娠と薬情報センターが作成した相談内容に対する「回答書」が、妊娠と薬情報センターから当院薬剤部に郵送されてくる。相談当日は、その問診票と回答書に基づいて相談が行われる。

表1に問診票と回答書に記載される内容を示す。また**写真1**に相談(説明)状況を示す。

写真１　筑波大学附属病院「妊娠と薬外来」における相談（説明）状況



薬剤師（左）、医師（中央）、患者（右）

このように、当院では2007年５月から新装となった「妊娠と薬外来」で相談を開始した。相談は、毎週木曜日の14時30分から１人30分の枠を設けて実施しており、現在（2009年３月末）までの相談件数と相談を受けた薬剤数は延べ54件で、284剤となった。**図２**に月次の推移を示す。

「妊娠と薬外来」の相談実態[3]

これまで実施してきた薬剤相談について、その取り組みを見直すべく、2000年２月から2008年４月の間に相談を受けた内容について解析した。

８年間余にわたる相談者は延べ100名（すべて女性、年齢は平均29.5±5.4歳）であり、そのうち91名は妊婦であった。相談件数は延べ100件であり、そのうち「妊娠と薬外来」となってからの相談件数は26件であった。相談を受けた薬剤は延べ390剤であり、そのうち「妊娠と薬外来」となってからの薬剤数は144剤であった。

薬剤の服用理由は、神経症（パニック障害、社会不安障害）が15件と最も多く、次いで精神病（うつ病、統合失調症）が13件、感冒が10件と続き、そのほか多種多様の疾患の治療となっていた。服用薬剤数は、１剤が最も多く29％、次いで２剤と４剤が15％、３

図２　「妊娠と薬外来」における相談の件数と薬剤数

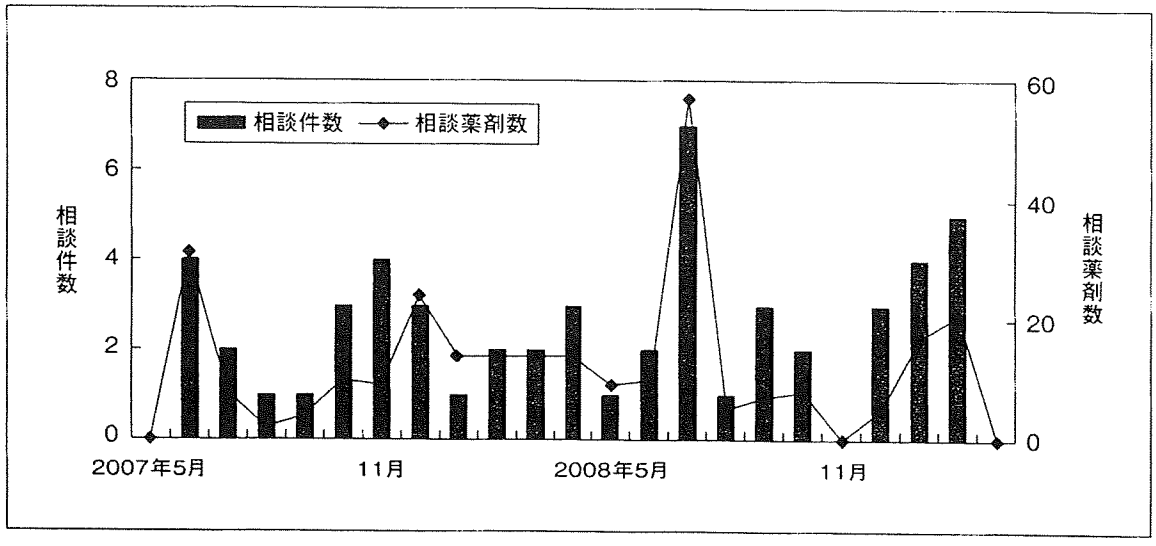


表2 相談を受けた薬剤の種類と延べ件数(2000年2月～2008年4月)[3]

	種類	延べ件数	全相談薬剤数に占める割合(%)	累積割合(%)
催眠鎮静薬・抗不安薬	13	52	13.3	13.3
解熱消炎鎮痛薬	14	33	8.5	21.8
精神神経用薬	13	31	7.9	29.7
消化性潰傷治療薬	12	29	7.4	37.2
総合感冒薬	12	17	4.4	41.5
健胃消化薬	3	12	3.1	44.6
気管支拡張薬	6	12	3.1	47.7
ビタミン剤	7	12	3.1	50.8
鎮咳薬	9	12	3.1	53.8
抗ヒスタミン薬	5	11	2.8	56.7
その他	—	169	—	—

剤が12%、6剤が10%、5剤が5%と続いた。相談を受けた薬剤の種類と延べ件数は、薬剤の種類では解熱消炎鎮痛薬が最も多く14種類、次いで催眠鎮静薬・抗不安薬と精神神経用薬がそれぞれ13種類、消化性潰瘍治療薬と総合感冒薬がそれぞれ12種類と続いた。薬剤の件数では催眠鎮静薬・抗不安薬が最も多く延べ52件(13.3%)、次いで解熱消炎鎮痛薬が延べ33件(8.5%)、精神神経用薬が延べ31件(7.9%)、消化性潰瘍治療薬が延べ29件(7.4%)、総合感冒薬が延べ17件(4.4%)と続いた。**表2**に延べ件数の上位10種類の薬剤を示す。

薬剤の服用時期と相談時期は、服用時期では絶対過敏期(妊娠4～7週)に服用した薬剤の相談が最も多く44名(44.0%)で、その服用時期は平均5.2±1.1週であった。次いで無影響期(妊娠0～4週)に服用した薬剤の相談が16名(16.0%)で、その服用時期は平均2.4±1.1週であった。また妊娠期間を通じて服用を継続した薬剤の相談は、24名(24.0%)であった。相談の時期については、絶対過敏期に服用した薬剤についての相談は10.4±4.2週、無影響期に服用した薬剤についての相談は8.5±2.3週であり、服用時期にかかわらず服用の3～5週後に相談を受ける場合が多かった。相対過敏期(妊娠7～12週)、比較過敏期(妊娠12～16週)、潜在過敏期(妊娠16週～出産)に服用した薬剤についての相談の時期は、20.0±8.0週と妊娠第2三半期にかけて多く、妊娠期間を通じて服用を続けていた薬剤の相談の時期は7.8±2.6週と妊娠第1三半期に集中していた。

相談を受けた薬剤390件(配合剤が含まれていることから薬効成分としては358成分になる)について、胎児危険度分類基準であるFDA分類およびオーストラリア分類にあてはめたところ、FDA分類およびオーストラリア分類に収載されていた薬剤は144成分で40.2%、いずれか一方の分類に収載されていた薬剤は65成分で18.2%、いずれの分類にも収載されていなかった薬剤は149成分で

表3　相談を受けた薬剤(成分)の危険度カテゴリー[件(%)][3)]

	A分類	B分類			C分類	D分類	X分類	未収載
		B1分類	B2分類	B3分類				
FDA分類	2(0.6)	54(15.1)			85(23.7)	41(11.5)	3(0.8)	173(48.3)
オーストラリア分類	46(12.8)	11(3.1)	23(6.4)	15(4.2)	64(17.9)	9(2.5)	0(0.0)	190(53.1)

41.6％となった。それらをFDA分類およびオーストラリア分類の危険度カテゴリーで分類した結果を**表3**に示す。また、いずれの分類にも収載されていなかった薬剤としてロキソプロフェンナトリウム水和物、エチゾラム、クロチアゼパム、ブロチゾラム、ロフラゼプ酸エチルなどがあったが、これらはFDA分類やオーストラリア分類で危険性が高いとされているベンゾジアゼピン系薬や非ステロイド系抗炎症薬に属する薬剤であったことから、今後これら薬剤個々の情報収集が必要と考えられる。

おわりに

妊娠中の薬剤の服用を考えたとき、まず頭に浮かぶのは催奇形性であり、「妊娠中に薬剤を服用しなければ奇形は発現しない」、「奇形が発現する(した)のは妊娠中に服用した薬剤によるものである」などと考えている人は多い。しかし、出生児における奇形の自然発生率は2～3％であり、服用した薬剤に全く催奇形性がなかったとしても、出生児100人に2人程度には奇形がみられることになる。この自然発生率に対する理解、すなわち服用薬剤の影響が自然発生率にどのくらい加算されるかを理解してもらうことが重要であり、薬剤を必要以上に恐れることなく、また侮ることなく、冷静に確率論的に受け入れてもらうことこそ、「妊娠と薬外来」に課せられた重要な使命ではないかと考えるが、いかがだろうか。

参考文献

1）林　昌洋：外来カウンセリングの実際．虎の門病院「妊娠と薬相談外来」．月刊薬事　48(2)：217-225，2006
2）中島　研：妊娠・授乳と薬相談外来と妊娠と薬情報センター．臨床薬理　37(6)：337-341，2006
3）香取久美ほか：妊娠と薬外来における服用薬剤の相談実態．医療薬学フォーラム2008，東京，2008年7月

A High Dose of Intravenous Immunoglobulin Increases CD94 Expression on Natural Killer Cells in Women with Recurrent Spontaneous Abortion

Shigeki Shimada[1,2], Masamitsu Takeda[1], Jun Nishihira[3], Masanori Kaneuchi[1,2], Noriaki Sakuragi[1,2], Hisanori Minakami[1], Hideto Yamada[4]

[1]Department of Obstetrics and Gynecology, Hokkaido University Graduate School of Medicine, Sapporo, Japan;
[2]Women's Health Educational System, Hokkaido University Graduate School of Medicine, Sapporo, Japan;
[3]Department of Medical Management and Informatics, Hokkaido Information University, Ebetsu, Japan;
[4]Department of Obstetrics and Gynecology, Kobe University Graduate School of Medicine, Kobe, Japan

Keywords
CD94, immunoglobulin, natural killer cell

Correspondence
Hideto Yamada, Department of Obstetrics and Gynecology, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho Chuo-ku, Kobe 650-0017, Japan.
E-mail: yhideto@med.kobe-u.ac.jp

Submitted July 6, 2009;
accepted August 11, 2009.

Citation
Shimada S, Takeda M, Nishihira J, Kaneuchi M, Sakuragi N, Minakami H, Yamada H. A high dose of intravenous immunoglobulin increases CD94 expression on natural killer cells in women with recurrent spontaneous abortion. Am J Reprod Immunol 2009; 62: 301–307

doi:10.1111/j.1600-0897.2009.00739.x

Problem
A high dose of intravenous immunoglobulin (HIVIg) therapy is effective in various diseases such as autoimmune diseases, and also is expected to have efficacy in recurrent spontaneous abortion (RSA). The aim of this study was to understand immunological mechanisms of this therapy.

Method of study
By flowcytometric analyses, we examined phenotypic changes of a variety of immunological cells including natural killer (NK) cells, cytotoxic T cells, regulatory T cells and macrophages in peripheral blood of RSA women with HIVIg therapy (n = 8).

Results
Expression percentages of inhibitory CD94 on NK cells significantly (P = 0.01) increased after the therapy (58.8 ± 21.4% versus 71.0 ± 17.6%).

Conclusion
Mechanisms of possible efficacy of HIVIg therapy for RSA may include enhancement of CD94 expression and subsequent suppression of NK cell cytotoxicity.

Introduction

A high dose of intravenous immunoglobulin (HIVIg) therapy is effective and widely used in various diseases including idiopathic thrombocytopenic purpura, Guillain–Barré syndrome, Kawasaki's disease, and myasthenia gravis.[1–4] To assess the efficacy of this therapeutic option in women with recurrent spontaneous abortion (RSA) of unexplained etiology, randomized, double-blind, and placebo-controlled trials of a medium dose of intravenous immunoglobulin (IVIg) therapy, in which 20–40 g of immunoglobulin is infused weekly or every 2–4 weeks during early pregnancy, have been performed.[5–10] Conclusions drawn from these IVIg trials are controversial. However, recent reports of meta-analysis[11] and systematic review[12] concerning efficacy of IVIg therapy suggest that a medium dose of IVIg are effective among women with secondary RSA.

On the other hand, our group tried HIVIg therapy for RSA women, in which 100 g of immunoglobulin was infused intravenously over the course of 5 days during early pregnancy. A high live birth rate was

observed among these women who had a history of four or more spontaneous abortions and underwent HIVIg therapy.[13,14] Additionally, using a mouse model of immunological reproductive failure, a recent study demonstrated that intraperitonial injection of a high dose of immunoglobulin restored the fecundity.[15]

We previously reported that HIVIg therapy reduced natural killer (NK) cell activity[14] and Th1/Th2 balance[16] in the blood of RSA women. However, immunological mechanisms of possible efficacy of HIVIg therapy for RSA have not been fully elucidated. In this study, to understand these immunological mechanisms, using flowcytometric analyses we examined phenotypic changes of a variety of immunological cells including NK cells, cytotoxic T cells (CTLs), regulatory T cells (Tregs) and macrophages in peripheral blood of RSA women with HIVIg therapy.

Subjects and methods

Patient Characteristics

Study subjects consisted of eight consecutively seen Japanese patients (30–41 years old), who had a history of four or more (mean 4.9, range 4–7) consecutive abortions, in the Hokkaido University Hospital. The previous abortions of all the eight patients occurred <10 weeks of gestation. Only one patient experienced a full-term normal delivery followed by five consecutive abortions. All the patients had received therapies of luteal hormone, herbal medicine, steroid hormone, low dose aspirin and/or heparin during their previous pregnancies. They underwent examinations of ultrasound, hysterosalpingography, endometrial biopsy and conventional blood analyses for RSA screening, and were diagnosed as having RSA of unexplained etiology. The conventional blood analyses included chromosome karyotypes of couple; measurements of progesterone in mid-luteal phase, prolactin, thyroid, liver, kidney functions, haemostatic coagulation factors such as d-dimer, factor XII, protein C, protein S; and autoimmune factors such as antinuclear antibody, complements, anticardiolipin, beta 2-glycoprotein I-dependent anticardiolipin antibodies and lupus anticoagulant.

They underwent HIVIg therapy (intact type immunoglobulin 20 g daily in the course of 5 days; a total 100 g) with written informed consent immediately after a gestational sac was detected in a uterus by ultrasound. The peripheral blood samples were obtained prior to commencement of HIVIg (4–5 weeks of gestation) and 1–3 days after completion of HIVIg (5–6 weeks of gestation). The gestational age of all pregnancies was determined from basal body temperature.

Two of the eight pregnancies ended in spontaneous abortion. Of the two abortions, one was with chromosome 10 trisomy, and the other with 18 trisomy. The other six pregnancies ended in full-term normal deliveries.

Flow Cytometric Analysis

The peripheral blood samples were suspended in phosphate buffered saline (PBS) containing 0.2% bovine serum albumin (BSA) and 0.1% sodium azide. A lysing solution containing NH4Cl and EDTA was added for 10 min at room temperature to lyse the erythrocytes. Peripheral blood cells were washed twice with PBS and resuspended with 1 mL of PBS before flow cytometric analyses using antibodies as follows. For the NK cell analyses, the cells were stained with Peridinin–Chlorophyll–Protein Complex (PerCP)-conjugated anti-CD3 (SK7) mAb (Becton Dickinson, San Jose, CA, USA), fluorescein isothiocyanate (FITC) conjugated anti-CD3 (HIT3a) mAb (Becton Dickinson), allophycocyanin (APC) -conjugated anti-CD56 (NKH-1) (Beckman Coulter, Inc., Fullerton, CA, USA), anti-CD158a (EB6)- R-phycoerythrin (PE) (Immunotech, Marseille, France) and anti-CD94 (HP-3B1)-PE (Immunotech). Cytotoxic T cells were analyzed with mAb as follows: PerCP-conjugated anti-CD3 (SK7) mAb (Becton Dickinson), anti-CD8 (B9.11)-APC (Immunotech) and anti-CD28 (CD28.2)-PE (Immunotech). For the staining of intracellular perforin, the cells were washed and fixed with fixation buffer (CALTAG Laboratories, Burlingame, CA, USA), and then washed with permeabilization buffer (CALTAG). These fixed and permeabilized cells were stained with mouse anti-human perforin (dG9) (Becton Dickinson) with RPE-Cy5-conjugated F(ab)2 fragment of rabbit anti-mouse immunoglobulins (DAKO Cytomation, Glostrup, Denmark), and then stained with anti-CD3 (SK7)-FITC (Becton Dickinson), anti-CD56 (NKH-1)-PE (Beckman Coulter) and anti-CD8 (B9.11)-APC (Immunotech). Tregs were analyzed with mAb as follows: PerCP-conjugated anti-CD4 (SK3) mAb, anti-CD25 (2A3)-APC (Becton Dickinson), anti-FOXP3 (PCH101)-FITC (eBioscience, San Diego, CA, USA), anti-CD28 (CD28.2)-FITC (Im-

American Journal of Reproductive Immunology 62 (2009) 301–307