

Experimental Study Concerning Safety Dosage of OK-432 for Intrauterine Treatment

Keiko Ogita, Tomoaki Taguchi and Sachiyo Suita, Department of Pediatric Surgery, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

OBJECTIVE: Clinical intrauterine treatment for fetal cystic hygroma has so far been performed in a few patients; however, it is still difficult to evaluate the results. The aim of this study is to establish the safe dosage of OK-432 in the intrauterine treatment of fetal cystic hygroma.

METHODS: OK-432 was injected either subcutaneously behind the neck of the fetuses or into the amniotic cavity through the uterine wall of pregnant Japanese white rabbits at 27 days of gestation. Saline was administered to the controls. The dosage and the site of injection were as follows: group 1, OK-432, 0.01 KE (0.25 KE/kg) in 0.2 mL saline per fetus, subcutis; group 2, OK-432, 0.02 KE (0.5 KE/kg) in 0.2 mL saline per fetus, subcutis; group 3, OK-432, 0.04 KE (1 KE/kg) in 0.2 mL saline per fetus, subcutis; group 4, OK-432, 0.01 KE in 0.2 mL saline per fetus, amniotic cavity; group 5, OK-432, 0.04 KE in 0.2 mL saline per fetus, amniotic cavity; group 6, saline, 0.2 mL per fetus, subcutis; group 7, saline, 0.2 mL per fetus, amniotic cavity. All fetuses were delivered at 29 days of gestation.

RESULTS: The mother's rectal temperature was mostly in the normal range throughout the experiment. There was no significant difference between any of the seven groups in fetal body weight. The C reactive protein values of all fetuses were negative. The appearance of the skin of all the fetuses was normal. The histopathological findings of the skin in the OK-432 groups showed a moderate infiltration of monocytes and plasma cells. No pathological changes were observed in the heart, lung, liver or kidneys of any of the fetuses.

CONCLUSION: Based on this rabbit experiment, we determined that OK-432 may be safely used at a dose of up to 1 KE/1 kg of fetal body weight as an intrauterine treatment for fetal cystic hygroma. [*Asian J Surg* 2006;29(3):202-6]

Key Words: cystic hygroma, fetus, intrauterine treatment, OK-432, safety

Introduction

Fetal cystic hygroma is a congenital malformation of the lymphatic system, appearing most often around the neck.¹ Cases of cystic hygroma, with a huge neck mass, are at risk of developing airway obstruction, which can result in death, while cases associated with hydrops fetalis are at risk of suffering from intrauterine fetal death due to

circulatory failure.^{2,3} Intrauterine treatment for cystic hygroma using OK-432, which is a biological response modifier with an anti-tumour effect and is used in sclerotherapy to treat neonatal cystic hygroma, has recently begun to be used clinically in order to overcome this severe condition. However, there have so far been only a few reports on this treatment modality.³⁻⁸ The indications and safety have also not yet been established. Therefore, the aim of this study is

Address correspondence and reprint requests to Dr Keiko Ogita, Department of Pediatric Surgery, Graduate School of Medical Sciences, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812-8582, Japan.
E-mail: ogita@ped surg.med.kyushu-u.ac.jp • Date of acceptance: 28 February 2005

to establish the safe dosage levels of OK-432 for the intrauterine treatment of fetal cystic hygroma, referring to the practical dosages of human neonatal cases. This is the first report to evaluate the safe dosages of OK-432 using animal fetal models.

Materials and methods

Pregnant Japanese white rabbits (body weight, 3.8–4.0 kg) were obtained (Kyudo Co., Saga, Japan). At 27 days of gestation (full term, 31 days), the operation was performed under sodium pentobarbital intravenous anaesthesia (35 mg/kg) as follows: after carrying out a median laparotomy, the entire uterus was exposed from the abdominal cavity. OK-432 (Picibanil®, Chugai Pharmaceutical Co., Tokyo, Japan) was then administered to the fetus by a transuterine puncture using a 27-gauge needle. The sites administered OK-432 included the subcutaneous tissue behind the neck of the fetuses or the amniotic cavity. The experimental groups were classified into seven groups according to the site and dosage (Table). Regarding the dosage of OK-432, we administered 0.027 KE of OK-432 to a rabbit fetus weighing 40 g, based on the calculations in which a neonate weighing 3,000 g is administered 2 KE of OK-432 (0.67 KE/kg) as a maximal volume. Therefore, we administered OK-432 at dosages ranging from 0.01 KE (0.25 KE/kg) to 0.04 KE (1 KE/kg) for each fetus. The groups, in which OK-432 was injected into the amniotic fluid, were studied regarding any adverse effects caused by OK-432 in the case of leakage into the amniotic fluid. OK-432 was dissolved by saline and the administration volume was 0.2 mL for each group. The pregnancy was allowed to continue until 29 days of gestation, and all

fetuses were then delivered by caesarean section. The mother’s rectal temperature was measured before operation, immediately after operation, and at 1, 2 and 24 hours after operation, and just before the caesarean section. The fetuses were weighed and blood was aspirated to measure the C reactive protein (CRP) values. Next, the fetuses were sacrificed and a part of their lung, heart, liver, kidney and skin at the site of injection was fixed with 10% formaldehyde and examined with haematoxylin-eosin staining.

All results are expressed as mean ± SD. An unpaired *t* test was used for the statistical analysis. The level of significance was set at *p* < 0.05.

This experiment was reviewed by the Committee on the Ethics of Animal Experiments in the Graduate School of Medical Sciences, Kyushu University and it was carried out under the Guidelines for Animal Experiments in Kyushu University.

Results

The mother rabbits and fetuses were all alive throughout the experimental period. The rectal body temperature of the mothers was mostly in the normal range during the experiment in all groups except for the rectal temperature just after the operation, which was slightly below the normal range (Figure 1).

The mean body weight of the fetuses showed no significant difference among groups. Serum CRP levels of all fetuses were below 0.6 mg/dL. However, the mean CRP value of group 3, which was injected with 0.04 KE of OK-432 subcutaneously, was the highest. There was no statistically significant difference between any of the groups (Table).

Table. Dose and site of administration, and summary of results

Group	Medication	Injected part	Volume (per fetus)	Calculated dose (KE/kg fetus)	Mean body weight of fetus (g)	Serum C reactive protein (mg/dL)	Infiltration of inflammatory cells
1 (n=6)	OK-432	Subcutis	0.01 KE	0.25	38.5 ± 3.15	0.13 ± 0.05	+
2 (n=9)	OK-432	Subcutis	0.02 KE	0.5	37.0 ± 11.5	0.17 ± 0.13	+
3 (n=8)	OK-432	Subcutis	0.04 KE	1.0	40.1 ± 4.52	0.34 ± 0.32	+
4 (n=4)	OK-432	Amniotic cavity	0.01 KE	0.25	39.5 ± 4.20	0.15 ± 0.06	-
5 (n=8)	OK-432	Amniotic cavity	0.04 KE	1.0	41.5 ± 2.88	0.14 ± 0.05	-
6 (n=6)	Saline	Subcutis	0.2 mL	0	34.2 ± 5.85	0.13 ± 0.05	-
7 (n=4)	Saline	Amniotic cavity	0.2 mL	0	40.0 ± 4.08	0.10 ± 0.0	-

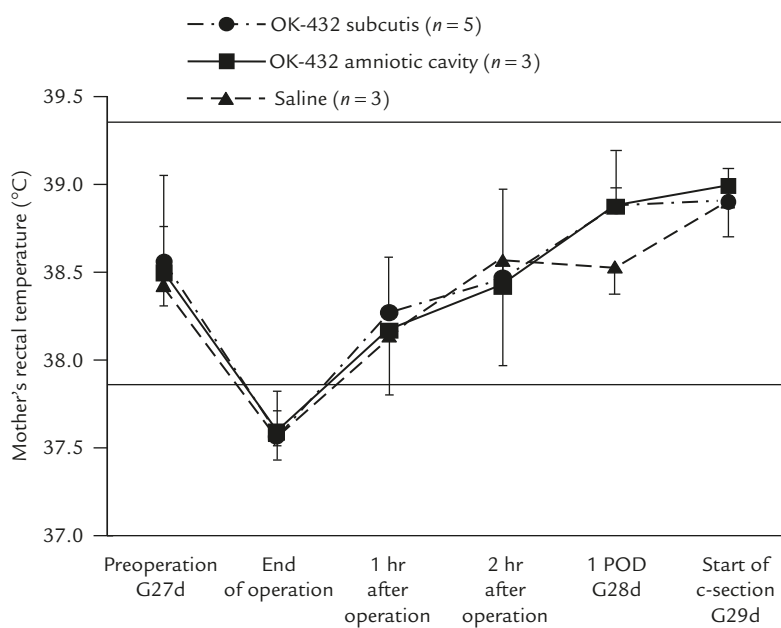


Figure 1. The changes in the mothers' rectal temperature. The temperatures were mostly in the normal range during the experiment in all groups, except for the rectal temperature just after the operation, which was slightly below the normal range. The normal temperature range was 38.65 ± 0.73°C.

The macroscopic findings of the nuchal skin injected with saline or OK-432 showed a normal appearance in all fetuses (Figures 2A and B). The systemic skin of all fetuses injected with OK-432 into the amniotic cavity also showed a normal appearance. The histopathological findings of the nuchal skin injected with saline were almost normal in appearance (Figure 2C). In contrast, the histopathological findings of the nuchal skin injected with OK-432 showed a moderate infiltration of monocytes and plasma cells (Figure 2D), and no significant difference was observed in the degree of infiltration of inflammatory cells dependent on the dosage. The histopathological findings of the nuchal skin injected with OK-432 into the amniotic cavity showed a normal appearance.

The macroscopic and histopathological findings of the heart, lung, liver and kidney of the fetuses showed a normal appearance in all groups.

Discussion

OK-432 has been reported to have several adverse effects, including fever and fatigue as general conditions, and tenderness, swelling, reddening and induration as local findings. An increased white blood cell count and increased CRP level have also been commonly observed.⁹ In this study, all mother rabbits and fetuses remained alive throughout the experimental period without any

spontaneous abortions. In addition, no fetuses showed any toxic responses considered to be an adverse effect related to OK-432.

Regarding the dosage, OK-432 has been reported to be administered at a range of 1–2 KE at a concentration of 1 KE/1 mL of saline in neonates.¹⁰ In this study, based on the calculations in which a neonate weighing 3,000 g is administered 2 KE of OK-432 (0.67 KE/kg) as a maximal volume, a rabbit fetus of 40 g is administered OK-432 of 0.027 KE. Therefore, we administered OK-432 at dosages ranging from 0.01 KE (0.25 KE/kg) to 0.04 KE (1 KE/kg) for each fetus. Moreover, the groups, in which OK-432 was injected into the amniotic fluid, were studied regarding any adverse effects caused by OK-432 in case of leakage into the amniotic fluid. The volume of injection was determined considering about the equality among each group and the concentration. Regarding the mothers in this study, the maximal dosage of OK-432 for the mothers was 0.08 KE/kg and no adverse effects were observed, including fever. This dosage equals that for a fetus of a pregnant woman, who weighs 55 kg in body weight and is administered OK-432 at a dose of 4.4 KE.

On the other hand, the mean CRP value of group 3 was the highest and the histopathological findings of group 3 showed a mild infiltration of inflammatory cells, which consisted of either plasma cells or monocytes. These findings might be consistent with those of inflammatory effect of OK-432. Moreover, there have been

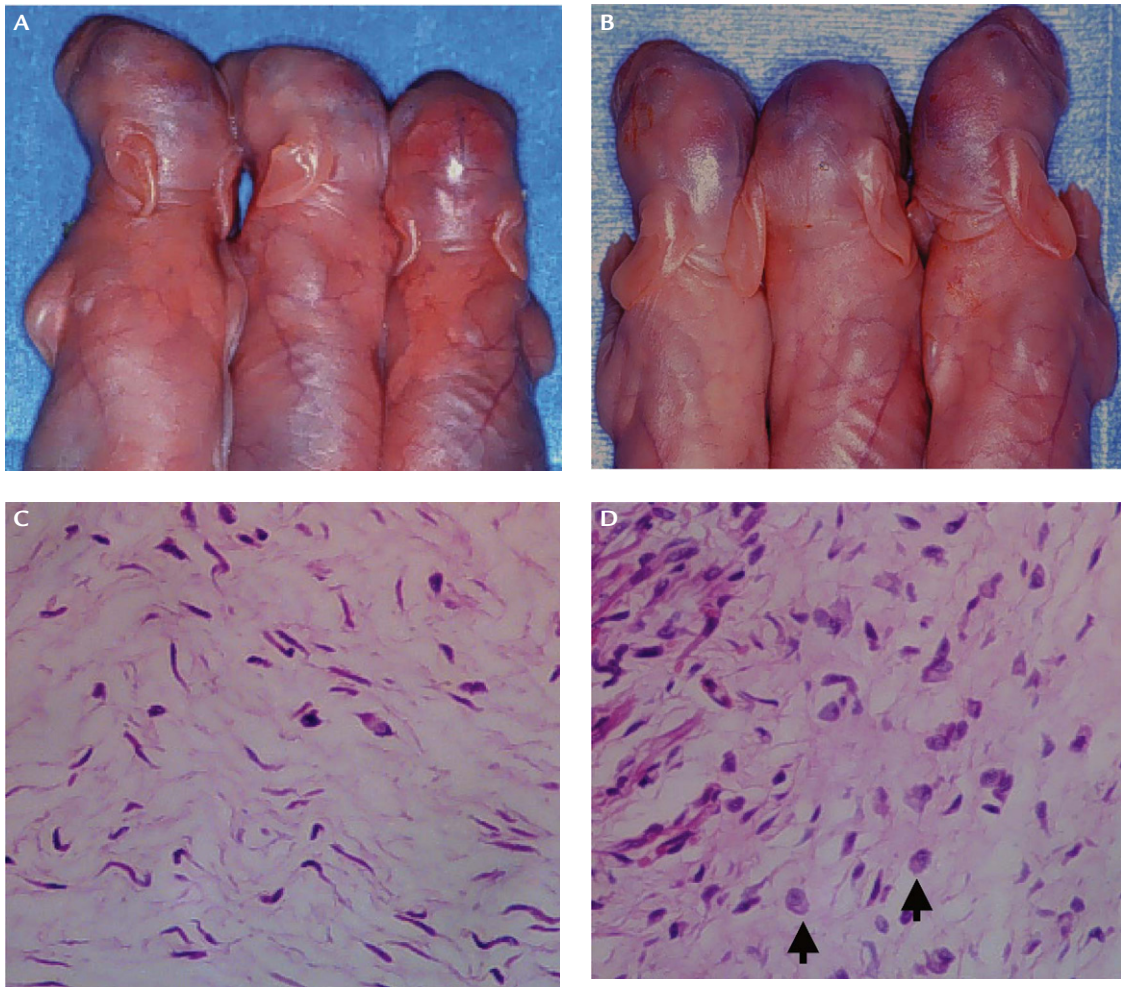


Figure 2. Macroscopic findings of nuchal skin in (A) group 6 and (B) group 3 show a mostly normal appearance. Histopathological findings of nuchal skin in (C) group 6 show a mostly normal appearance and those in (D) group 3 show moderate infiltration of inflammatory cells, such as plasma cells (arrows) and monocytes in the subcutaneous tissue (haematoxylin and eosin, 200 \times).

reported effective neonatal cases treated by the dosage of OK-432, which was 1 KE/kg.^{2,10} Therefore, we suggest that the dosage, 1 KE/kg, of OK-432 is appropriate to treat fetal cystic hygroma.

In conclusion, no adverse effects were observed in either the mothers or the fetuses when OK-432 was injected into the fetuses either subcutaneously or into the amniotic cavity at a dose of up to 1 KE/1 kg of the fetal rabbit's body weight. Our above findings therefore suggest that human intrauterine treatment of fetal cystic hygroma by the administration of OK-432 is a safe and effective therapeutic modality.

References

1. Chervenak FA, Glenn I, Blakemore KJ, et al. Fetal cystic hygroma: cause and natural history. *N Engl J Med* 1983;309:822-5.
2. Samuel M, McCarthy I, Boddy S. Efficacy and safety of OK-432 sclerotherapy for giant cystic hygroma in a newborn. *Fetal Diagn Ther* 2000;15:93-6.
3. Ogita K, Suita S, Taguchi T, et al. Outcome of fetal cystic hygroma and experience of intrauterine treatment. *Fetal Diagn Ther* 2001;16:105-10.
4. Kuwabara Y, Sawa R, Otsubo Y, et al. Intrauterine therapy for the acutely enlarging fetal cystic hygroma. *Fetal Diagn Ther* 2004;19:191-4.
5. Sasaki Y, Chiba Y. Successful intrauterine treatment of cystic hygroma colli using OK-432. *Fetal Diagn Ther* 2003;18:391-6.
6. Ogita K, Suita S, Taguchi T, et al. Experience of intrauterine treatment to fetal cystic hygroma: a report of two cases. *Asian J Surg* 2000;23:315-7.
7. Negishi H, Yamada H, Okuyama K, et al. Outcome of non-immune hydrops fetalis and a fetus with hydrothorax and/or ascites: with some trials of intrauterine treatment. *J Perinat Med* 1997;25:71-7.

8. Watari H, Yamada H, Fujino T, et al. A case of intrauterine medical treatment for cystic hygroma. *Eur J Obstet Gynecol Reprod Biol* 1996;70:201-3.
9. The Children's Lymphangioma Study Group, Ogita S, Tsunoda A, Okabe I, et al. Phase III study on OK-432 therapy for lymphangioma in children. *J Jpn Soc Pediatr Surg* 1995;31:29-36. [Abstract]
10. Ogita S, Tsoto T, Deguchi E, et al. OK-432 therapy for unresectable lymphangiomas in children. *J Pediatr Surg* 1991;26:263-70.