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Low dose Cyclosporin A treatment increases live birth rate of unexplained recurrent abortion - initial cohort study

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Summary

Background and objective: Pregnancy is similar to allogeneic transplantation. Eighty percent of unexplained recurrent spontaneous abortions (URSA) relate to disturbances of immune regulation. Cyclosporin A (CsA) is a immunosuppressant widely used after organ transplantation and to treat autoimmune disease. Animal studies show that low dose of CsA could induce maternal-fetal immunity tolerance while enhance trophoblast invasion. So far no clinical trial reported on the effect and safety of cyclosporin A treatment for URSA has been published. The objective of this study was to explore the effect, safety, and mechanism of low-dose CsA treatment in human patients in order to find a novel therapy to treat URSA. *Materials and Methods*: Eighty-six patients with eligible URSA treated at the clinic of the present hospital were included in this study from December 2009 to December 2012. The research was approved by the Ethics committee. Through a clinical study with prospective non-randomized controlled trials, the patients were divided into CsA treatment group (n = 66 cases) and in control group (n = 20 cases) based on the patients' choice. Both groups started treatment as soon as the pregnancy test was positive. Patients in the treatment group were treated with oral CsA 100 mg/day for 30 days. Patients in the control group were treated with progesterone 20 mg im per day until 12 weeks of gestation. Cytoimmunology test of CD3, CD4, CD8, CD4/8, CD4/25, CD19/21, and Th/Ts were examined before and after the treatment in both groups. Clinical consequences of mothers and fetuses were followed up and recorded. Live birth rate and cytoimmunology markers and their change before and after the treatment were analyzed and compared between the two groups. *Results:* The live birth rate was significantly higher in study group (41/66, 62.1%) than in the control group (6/20, 30.0%) ($p < 0.001$). There was no obvious side effect and adverse consequence in the pregnancy women. No IUGR or birth defect was observed in fetus in this study. After CsA treatment, CD3 level in maternal blood was higher in successful group than abortion group but CD8 level was decreased after CsA treatment. *Conclusions:* Low-dose CsA treatment increases live birth rate of unexplained recurrent abortion. No maternal-fetal adverse consequence was observed in this study and it is safe in clinic use. The mechanism of CsA therapy may be related to immune regulation which may favor the success of pregnancy.

Key words: Cyclosporin A; Unexplained recurrent spontaneous abortion (URSA); Progesterone; Live birth rate; Cytoimmunology markers.

Introduction

Unexplained recurrent spontaneous abortion (URSA) is defined as unexplained, continuous, and more than twice spontaneous abortion.There is no effective solution at present [1]. Pregnancy is similar to the allogeneic transplantation and 80% of URSA relate to disturbances of immune regulation. Cyclosporin A (CsA) is an immunosuppressant widely used after organ transplantation [2-5] and to treat autoimmune disease [6-8]. Previous report shows pregnancies in transplants women under immunosuppressive treatment can be managed with a positive outcome [9-11]; thus CsA as the most common used immunosuppressant attracts the interest in URSA therapy.

Previous series animal studies have shown that low-dose CsA could induce maternal-fetal immunity tolerance while enhancing proliferation, movement, migration, invasion of trophoblasts, and inhibit apoptosis of trophoblasts [12, 13]. CsA promotes the migration and invasion of trophoblasts in first trimester. CsA improves murine pregnancy outcome in abortion-prone matings: involvement of CD80/86 and CD28/CTLA-4. CsA induces titin expression via MAPK/ERK signalling and improves proliferative and invasive potential of human trophoblast cells. Further experiment show CsA to enhance the activated human umbilical vein endothelial cell (HUVEC) monolayers through different downstream targets, and ultimately, improve the transformation and remodeling of spiral arteries [14-16].

The effect of CsA treatment in mice abortion model has been confirmed, yet main concerns about fetal and postnatal development are IUGR and preeclampsia [17-18]. The key point is the dosage of CsA, one to two mg/kg, shows the best results [18, 19]. Although animal model show that large-dose CsA has embryo renal toxicity, yet in human post-transplantation pregnancy with the CsA treatment, there is no neonatal malformation report except IUGR and preeclampsia, nor long-term development problems. CsA is reported with good effects on the treatment of pregnancy with immune disease such as SLE. Interestingly in previous study, low-dose CsA not only reduced abortion rate, it even increased the growth of the placenta and fetus in a mice model [20, 21].

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So far there is no clinical trial reported on the effect and safety of CsA treatment for URSA. The objective of this study was to explore the effects, safety, and cell-immune monitoring markers of low-dose CsA treatment in patients, in order to find a novel therapy to treat URSA, and further potential application in reproductive medicine.

Materials and Methods

Patients

A total of 86 patients with eligible URSA treated at the clinic of the present hospital were included in this study from December 2009 to December 2012. The research was approved by the hospital ethical committee. Informed consent was obtained from every participant in each group. Including criteria: (1) URSA is defined as unexplained and continuous more than twice spontaneous abortion; (2) volunteered to participate in this study, willing to take drug treatment and follow-up, and sign the informed consent; (3) healthy, no addition physical examination finding nor abnormal laboratory findings except for recurrent spontaneous abortion (RSA) related criteria; (4) aged 20-40 years; (5) regular menstruation; (6) normal endocrine hormone levels; (7) negative antiphospholipid antibody; (8) TORCH IgM (-); (9) blood group antibody < 1: 128; (10) normal chromosome karyotype. Excluding criteria: (1) patient that suffered from respiratory system, endocrine, neurological, severe cardiovascular system diseases, suffering from digestive system or genitourinary system disease; (3) having CsA contraindications (severe infection, tumor, immune deficiency syndrome). Through a clinical study with prospective non-randomized controlled trials, the patients were divided into CsA treatment group (n=66 cases) and in control group (n=20 cases), based on whether they would like the CsA treatment. (4) Due to an ethical consideration, by the mid-term of the study, due to the significant higher successful rate in CsA group, all the patients entered CsA treatment.

Treatment protocol included both groups that began treatment as soon as the pregnancy test was positive. Patients in the treatment group were treated with oral CsA 100mg/day for 30 days. Patients in the control group were treated with progesterone 20 mg im per day until 12 weeks of gestation. Cytoimmunology test of the percentages of $CD3^+$, $CD4^+$, $CD8^+$,CD4/CD8, CD4/CD25, and CD19/CD21 in the total peripheral lymphocytes before and after the treatment were examined before and after the treatment in both groups.

Observing measures included: (1) pregnancy outcome: including delivery mode, birth weight, gestational age, and pregnancy complications; (2) fetal growth; (3) neonatal well-being; (4) pregnancy process; (5) cytoimmunology condition: the laboratory examination of the percentages of $CD3^+$, $CD4^+$, $CD8^+$,CD4/CD8, CD4/CD25, and CD19/CD21 in the total peripheral lymphocytes before and after the treatment.

Observation of effective degrees included treatment by CsA only and successful delivery of termed healthy baby; (2) treatment with CsA only and successful delivery of premature baby without marked abnormality in growth; (3) very early premature delivery as 28 weeks gestation and neonatal death; (4) failed pregnancy and failed treatment.

Evaluation of side effects included: (1) clinical adverse event: the complications and signs of clinical change of the treated subjects were recorded carefully during the visit; (2) routine laboratory examinations of blood and urine, liver, and kidney function and endocrine hormone levels.

Table 1. — *Demographics in the the studied groups.*

Parameters	Study group	Control group	*t*	*p*
Maternal age (years)	32.90±5.01	30.45±4.51	1.96	0.05
Paternal age (weeks)	35.34±4.95	32.55±4.34	2.27	0.02
Pregnant frequency	4.21±1.59	3.10±1.37	2.74	0.00
Abortion frequency	3.43±1.37	2.63±0.68	2.47	0.01
Birth weight (g)	3296.428 ± 623.410	3335.000 ± 407.71	0.246	0.807

Table 2. — *The pregnant process between the two groups.*

Pregnant Process	CsA (66)%	Progesterone (20)%	χ^2	*p*	OR (95% CI)
~12	22 (33.33)	13 (65.0)	6.876	0.009	0.258 (0.090 0.736)
~28	3 (4.54)	1 (5.00)	2.202	0.138	4.385 (0.533 36.042)
~37	3 (4.54)	0 (0.00)	1.000	0.447	
~42	38 (57.57)	6 (30.00)	4.671	0.031	3.167 (1.082 9.267)
Live birth	41 (62.12)	6 (30)	6.391	0.011	3.827 (1.302 11.245)
Success pregnant(>12)	46 (66.67)	7 (35)	6.876	0.009	0.258 (0.090 0.736)
Baby take home (%)	45 (68.18) (4 twins)	6 (30.00)	16.929	0.000	9.545 (2.988 30.497)

Statistical analysis

The SPSS package (16.0) was used to undertake the analysis. The indicators of the rate of live birth and of abortion were analyzed using Pearson's *chi*-square test, and relative risk ratios and odds ratios with confidence intervals. The student's *t*-test was applied to compare indicators of gestational weeks, maternal age, and neonatal birth weight between groups. Results were expressed as mean ± standard deviation. A p-value < 0.05 was considered as significant, and all inferential tests were two-tailed.

Results

Demographics in the studied groups

There were altogether 86 patients with eligible URSA treated at the clinic of the present hospital which were included in this study from December 2009 to December 2012. There were 66 patients in study group (44 cases of natural conception, 22 cases of IVF) and 20 patients in control group (all were natural conceptions). No significant differences in maternal and paternal age, birth weight, pregnant and abortion frequency were found between the two groups (Table 1).

Pregnancy process and outcomes

In the CsA study group, there were 66 cases, 45 cases were successful pregnancies (gestational age above 12 weeks); the success rate was 66.67%, and live birth rate was 62.1%. Among these, 38 cases were singletons, four were twins, and three cases were inevitable abortions between 12-28 weeks. There were no congenital defects. All

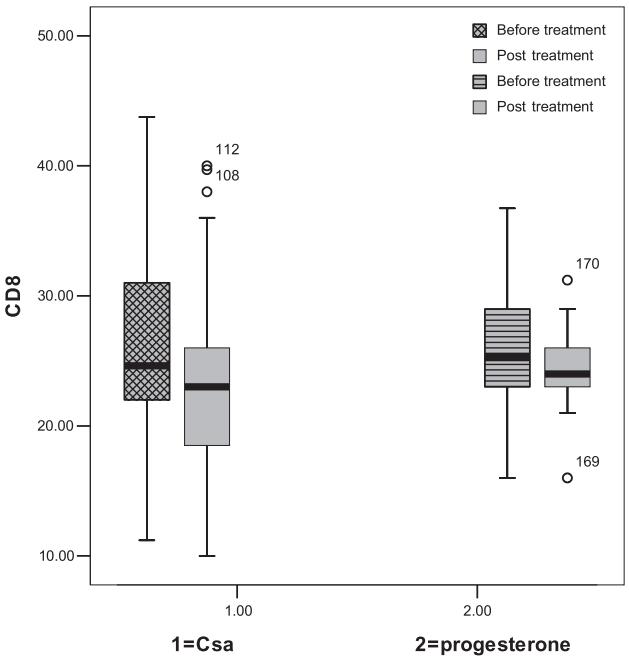

Figure 1. — Post-treatment CD 8 level significantly decreased in study group while there was no change in control group.

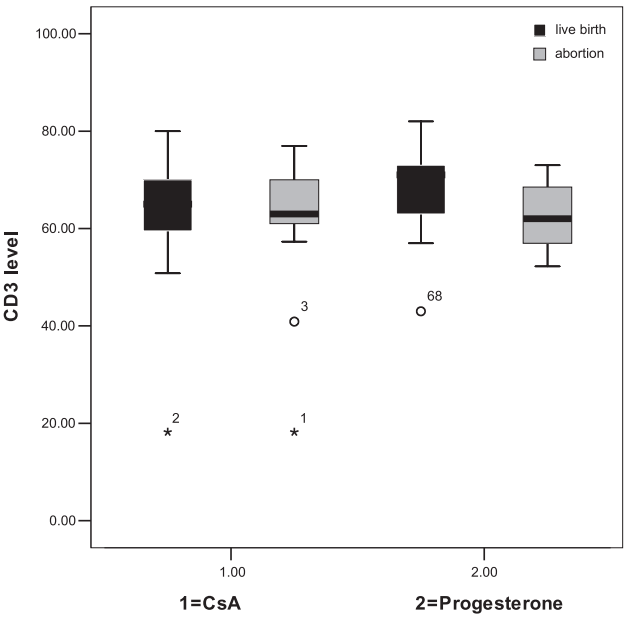

Figure 2. — In the study group, there was significantly higher post-treatment CD3 level in the pregnancy success group than in the abortion group.

had normal birth weight. Pregnancy loss rate in early pregnancy (gestational age less than 12 weeks) was 33.34%. The therapy was successful in both IVF and natural conception; there was no difference in the success rate between IVF and natural conception group (16/22 *vs*. 25/44, χ^2=1.578; p = 0.209).

In the progesterone control group, there were altogether 20 cases: six cases with successful pregnancy (30.00%). Among them six cases of success termed singleton deliveries resulted in six live babies. All had normal birth weight and showed no obvious defect. Fourteen cases had early abortion and the pregnancy loss rate in early pregnancy (gestational age less than 12 weeks) was 70% (Table 2). The pregnancy successful and delivery rates were significantly higher in study group.

Cytoimmunology condition

There was no difference in the cytoimmunology condition before the treatment between two groups. Post-treatment CD 8 level was significantly decreased in study group while there was no change in control group (Figure 1). In the study group, there was significantly higher post-treatment CD3 level in the pregnancy success group than in the abortion group (Figure 2). In the control group, there was significantly higher pre-treatment CD19/CD20 level in the successful pregnancy group than that in the abortion group (Figure 3).

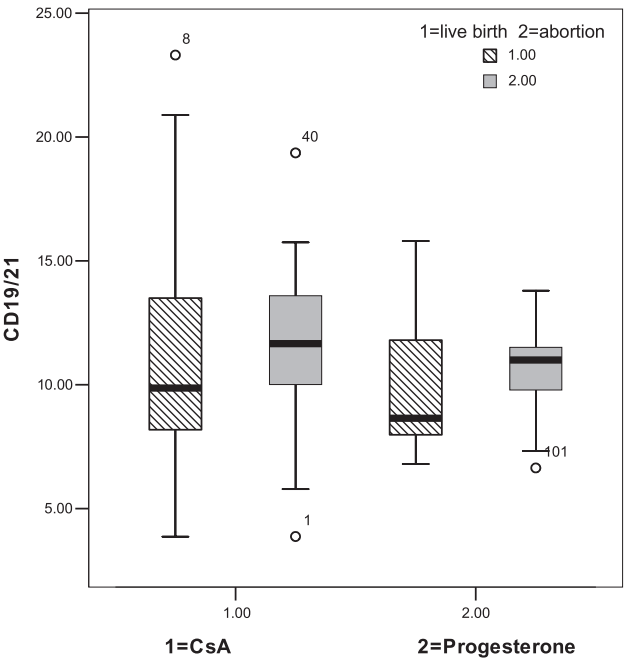

Figure 3. — In the control group there was significantly lower pre-treatment CD19/CD21 level in the in the pregnancy success group than in the abortion group.

Evaluation of side effects

There were no complications and signs of clinical changes in the treated patients. Nether there was neither adverse pregnant outcome as IUGR nor neonatal mobility.

Discussion

This is the first prospective clinical trial of low-dose CsA treatment for URSA. CsA treatment group had significantly higher live birth rate (41/66, 62.1% than in the control group (6/20, 30.0%). These result correspond with the previous animal study [21-23]. The reason is the effect of low dosage of CsA which can induce maternal-fetal immunity tolerance while enhancing trophoblast invasion. The present authors designed the treatment to begin as soon as the pregnancy test was positive and it lasted for 30 days, which corresponds to the entire embryology period, which is key for a successful pregnancy.

CsA is a powerful immunosuppressant that is widely used to prevent organ rejection and to treat several autoimmune diseases [20, 24]. Previous study has demonstrated that CsA inhibits Th1-type cytokine production but has no effect on Th2-type cytokine in DICs, either cultured alone or co-cultured with other cell types. CsA can differently regulate cytokine production in these cells and induce a Th2 bias at the maternal-fetal interface. Th2 bias by CsA at the maternal-fetal interface requires a coordinated interaction between embryonic trophoblasts and maternal decidual stromal cells (DSC) and decidual leukocyte (DLC). DSCs and DICs might amplify the effect of CsA on the Th2 bias in trophoblasts at the maternal-fetal interface. Treatment with CsA did not change the production of any of the examined cytokines in trophoblasts or DSCs alone. It is very interesting that different levels of cytokine production in a certain cell were observed when all cell types are co-cultured, suggesting that the cytokine production was regulated in the co-culture [25, 26]. The above study demostrates that CsA induce Th2 bias at the maternal-fetal interface induces maternal-fetal immune tolerance and improves pregnant outcomes.

Du *et al.* have described that the MAPK3/MAPK1 and Ca2t/calcineurin/NFAT pathways are deferentially involved in the regulation of growth and invasion of human trophoblast cells by CsA [27-29]. CsA promoted growth of trophoblast cells through activating the MAPK3/MAPK1 pathway while CsA enhancing invasiveness of the cells via activating MAPK3/MAPK1 and inhibiting Ca2t/calcineurin/NFAT pathways. CsA-induced MAPK3/MAPK1-mediated transactivation of AP1 is responsible for the growth and invasion of trophoblasts [30, 31]. This model depicting the role of CsA in regulating growth and invasion of human trophoblasts and contribute to the understanding of mechanisms of trophoblast cell growth and invasion regulated by CsA.

Regarding low-dose CsA treatment with oral CsA 100 mg/day for 30 days, the present authors minimized and optimized the lowest dosage and appreciated the duration. From the animal study [18], the best dosage of CsA is one to two mg/kg/day. In the present study the authors began with 100 mg/day (similar to two mg/kg/day) and have improves the successful rate by two times (62.1% *vs.* 30.0%) without side effect. Further clinical trials can be designed to achieve an optimized dosage. Patients in the control group were treated with progesterone 20 mg im per day until 12 weeks of gestation. The present authors did not use placebo due to the ethic issue that all the patients should be treated. Low-dose CsA could improve the trophoblasts' biological function and induce maternal tolerance during early pregnancy thus improving pregnancy outcomes by even one dosage during peri-implantation.

It is very promising that there was no obvious side effect and adverse consequences during pregnant women and in infants in this study. Previous studies report side effects of post-transplantation with therapeutic dosage of CsA treatment that were mainly were IUGR and preeclampsia which are caused by placenta malfunction [2, 3]. Here in this study low-dose CsA can induce maternal-fetal immunity tolerance while enhancing trophoblasts invasion. There was no report on fetal malformation after CsA treatment neither in the previous report nor in this study. Larger number of clinical trials are needed for further confirmation of fetal malformation. Also longer period follow-ups are needed for long-term outcomes.

By monitoring the cytoimmunology condition before and after the treatment, there were several findings in subgroup on the percentages of $CD3^+$, $CD4^+$, $CD8^+$,CD4/CD8, CD4/CD25, and CD19/CD21 in the total peripheral lymphocytes. $CD8^+$ level was decreased after CsA treatment. After CsA treatment, CD3+ level was higher in successful subgroup than in abortion subgroup; in the control group, there was significantly higher pre-treatment CD19/CD21 level in the pregnancy success subgroup than that in the abortion subgroup. The present authors could not reach a conclusion due to the limitation of sample size. CsA may work through immunologic mechanism and more evidence is needed with future study [32, 33].

Several studies have indicated that normal pregnancy is associated with an elevation in the number of $CD4^+CD25^+$ Treg cells, which may be important in maintaining maternal-fetal tolerance [24]. From recent animal studies, the maternal $CD4^+CD25^+$ regulatory T-cell pool was systemically expanded during mice pregnancy, and the expanded population of splenic $CD4^+CD25^+$ T cells contains suppressor activity, which could abolish the rejection of the fetus [34-36].

Pregnancy failure is caused by maternal immune rejection of the fetus. Circulating Treg cells increase during early pregnancy and reach their peak in the second trimester, and then gradually decline in postpartum. The isolated $CD4^+CD25^+$ cells express FoxP3 mRNA and inhibit proliferative responses of autologous $CD4^+CD25^+$ T cells to allogeneic dendritic cells [37, 38]. The above studies provide some insight in future study regarding cytoimmunology condition during CsA treatment.

In conclusion, low-dose CsA treatment increases live

birth rate of unexplained recurrent abortion. No maternal-fetal adverse consequence was observed and it is safe in clinic use. The mechanism is that low-dose CsA could induce maternal-fetal immunity tolerance while enhancing proliferation, movement, and invasion of trophoblasts. It also has the potential for therapeutic intervention for certain pathological pregnancies, such as IUGR, preeclampsia, and IVF. It may lead to a new therapeutic field in reproductive and maternal-fetal medicine.

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