Effect of Cyclosporine on Weight and Crown-rump Length of Mice Embryo

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Cyclosporine A is an immunosuppressive agent, which is widely used for organ transplantation, autoimmune and other diseases. Cyclosporine inhibits the calcineurin and thereby causes inhibition of chondrogenesis. Cyclosporine crosses the placenta and reaches to the embryo, and thereby may affect them. Many females, that should receive cyclosporine, are in childbearing age. The objective of this study was to determine whether cyclosporine administration has any effect on weight and crown-rump length of mouse embryos. Many out bred adult mice were selected. Female mice were mated overnight with males, checked in the morning for vaginal plug. Pregnant mice were randomly assigned into experimental and control groups. The experimental group received cyclosporine and control group received an equivalent amount of olive oil on days 7, 8, and 9 of gestation. Pregnant mice were killed and embryos were removed on days 13, 16, and 18 of gestation. The weight and crown-rump length of embryos were recorded. Comparison between two groups was made using *t.* test. There were highly significant differences between crown-rump length of experimental and control groups on days 16 and 18 (P <0.05), but on day 13, crown-rump length difference was not significant. In all ages of gestation, the experimental group had less weight than the control group (P<0.05). Results of this study indicate that cyclosporine causes low weight and low crown-rump length in mice embryos.

Key words: Cyclosporine, weight, crown-rump length, pregnancy, mouse embryo

Cyclosporine A (CsA) is a selective and powerful immunosuppressive agent which is widely used for organ transplantation[1-5], autoimmune diseases[6-8], and also for other diseases[9]. CsA inhibits the activation of calcineurin[10,11] and thereby causes inhibition of chondrogenesis[12], and muscle mass change[13]. CsA crosses the placenta blood barrier reaches to the embryo[2,4,14,15]. Many female patients that they should receive CsA, are in childbearing age or pregnant[1,9,16]. No systematic overview of the use of CsA in pregnancy has been conducted so far, and there are few controlled studies on CsA administration during pregnancy[1,2], although one case of CsA-induced osseous malformation, including limb defects, has been reported[17]. Also few studies address the weight or height of embryos. Furthermore, many studies have used a combination of medications and effects of individual agents on pregnancy have not been well established[1,2,4,5,18-20]. Other problems exist exclusively in organ transplant recipients (or other diseases), such as hypertension that may have also same impacts on birth weight and crown-rump length (CRL)[1,21].

The primary objective of this study was to determine whether CsA exposure in uterus is associated with low weight and low CRL of embryos.

All efforts were made to minimize both the suffering and the number of the animal used. In this study, the humane killing of the mice and embryos collection was carried out after approval by the Ethics Committees of Shiraz University of Medical Sciences.

In this experimental study, many out bred adult (male and female) mice were kept in a standard condition in separate cages[22,23] for one month. Female mice (50) were mated overnight with males, checked in the morning for vaginal plugs. The first day of gestation was considered to the day after plug was found[23]. CsA was dissolved in olive oil at concentration of 15 mg/ml[24]. Two groups of pregnant mice were randomly selected and were assigned into experimental and control groups. The experimental group received an intraperitoneal injection of CsA at dosage of 50 mg/kg/day of body weight[25] and control group received an equivalent amount of olive oil on days 7, 8, and 9 of gestation.

First, the mice were anesthetized with ether inhalation and then killed by cervical dislocation[23] on days 13, 16, and 18 of gestation. The uterus and its contents were exposed by laparatomy. The weight and CRL of embryos were recorded. A comparison between two groups was made using Student's t test. Comparisons resulting in P<0.05 were considered significant.

There were 235 embryos in the experimental group and 95 in the control group. The number of embryos decreased in CsA treated group and there were 120 embryo absorption sites in experimental group[25]. In all ages of gestation, the experimental group had less CRL than the control group (fig. 1), but on day 13, there was not significant difference between experimental and control groups. On days 16 and 18, there were highly significant differences between experimental and control groups (P<0.05). The ratio of CRL in experimental to control group was 0.97, 0.89, and 0.85 on days 13, 16, and 18 of gestation, respectively. Therefore, CsA effect on CRL was higher

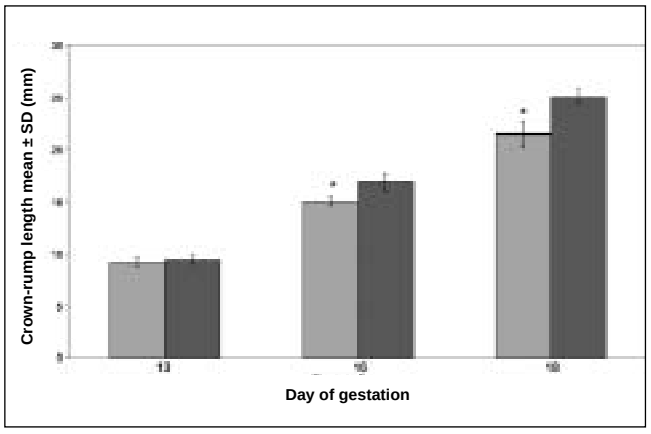

Fig. 1: Comparison between embryos' mean crown-rump length
Comparison between embryos' mean crown-rump length in the experimental (CsA, □) and control (oil, ■) mouse groups on days 13, 16 and 18 of gestation. *P <0.05

in the last days of pregnancy than earlier days.

Fig. 2 illustrates the comparison of CsA effect on the weight of embryos. In all ages of gestation, the experimental group embryos had less weight than the control group and there were highly significant differences between experimental and control groups (P <0.05). The ratio of weight in experimental to control group was 0.56, 0.72, and 0.85 on days 13, 16, and 18 of gestation, respectively. Thus, CsA effect on weight was higher in the early days of gestation than last days. As shown in figs. 1 and 2, these results revealed that the mice treated with CsA had a significantly higher frequency of low weight and low CRL than the mice treated with olive oil alone.

According to our data, CsA decreases CRL and weight of embryos. It supports the findings of other studies related to CsA effect on pregnancy outcomes.

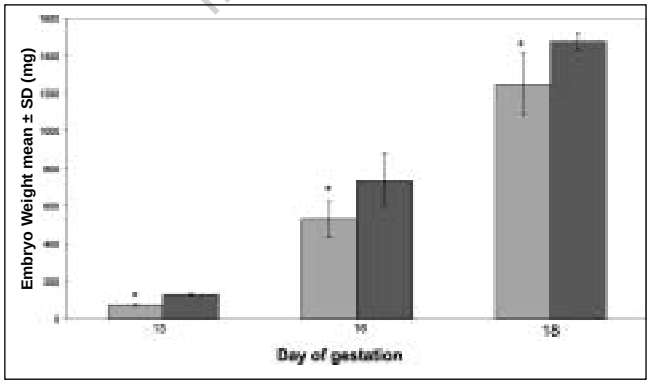

Fig. 2: Mean weight of embryos
Mean weight of embryos in experimental (CsA, □) and control (■Oil,) mouse groups on days 13, 16, and 18 of gestation. *P< 0.05

There is little research on CsA effect on the height or CRL of embryos. Sgro *et al.*[19] showed that the embryo's length was significantly lower in the study group as compared to the control group. Also, Pujals *et al.*[17] have reported that CsA decreased length of lower limb. In the present study, CsA effect on CRL was not significant in early days of gestation. Also other researchers have shown that *in vitro* treatment of CsA can interfere with mesenchyme and DNA content and inhibit chondrogenesis on limb bud[26,27] or can induce some osseous malformations[17] and bone loss[20]. CsA, via inhibition of calcineurin, can inhibit chondrogenesis process[12] and skeletal muscles mass change[13]. CsA effects on cartilage and muscles can cause, direct or indirect, shortening of limbs and body, although more researches are necessary to prove this subject.

Several reports have shown that children born by mothers who had taken CsA often present with the low birth weight (LBW), intrauterine growth retardation or small for gestation age[17,24]. Our data accords with other studies on the effect of CsA on weight of embryos. Rahbar and Forghani[28] reported that 77% of newborns, whose mothers used CsA, had LBW. There are similar reports about LBW in infants whose mothers used CsA alone or with combination to other drugs[29]. For example, LBW percentage reported by Lamarque *et al.*[14] was 44.3%, Armenti *et al.*[21] 44-65%, Janssen and Genta[7] 50%, and Moon *et al.*[30] 63.5%. Moon *et al.*[30] have reported that LBW of neonates was related to their mother's hypertension. They compared infants of hypertensive and non-hypertensive mothers, although there was LBW in infants whose mothers did not have hypertension but received CsA.

The dosage used in this study considerably exceeds what is normally given to transplant recipients and other patients, although some patients have been given as much as 25 mg/kg/day or also 30 mg/kg/day in rats and 100 mg/kg/day in rabbits[31]. Gasser *et al.*[24] also used 50 mg/kg/day in mice for teratogenicity. The results of this study indicate that CsA causes low weight and low CRL in mice embryos, as compared with those published in the recent literature with CsA. CsA mostly decreased the weight and CRL of mouse embryos in the earlier and last days of pregnancy, respectively. It is recommended that the drug should not be taken during pregnancy, unless the benefit to the patient outweighs the risk for the fetus. Obviously,

more research should be done on the subject.

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