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The Effect of Different Concentrations of GM-CSF on the Development of Pre-Implantation Embryos in Mice (Pilot Study)

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The cytokine of granulocyte macrophage colony stimulating factor (GM-CSF) is a glycoprotein, which is synthesized in the female reproductive tract and has embryonic trophic effect in mammals. The objective of this study was to examine the optimal dosage of GM-CSF to improve the mouse embryo development in vitro. To collect two and eight cells embryos, the pregnant NMRI mice were sacrificed by cervical dislocation at 48 h and 72 h post hCG injections, respectively. The embryos were cultured randomlly in T6 medium supplemented with 5 mg /ml bovine serum albumin (BSA) and 0, 2, and 10 ng / ml human rGM-CSF. The data of blastocyst formation and hatching in different groups of embryo culture were compared by chi-square analysis. The results showed that the developmental rates of 2 and 8 cells embryos to hatching blastocyst in the presence of 2 ng/ml of GM-CSF their control groups (51.5% and 49.7%, respectively) were more than those in the other groups, but insignificant. It seems more researches are necessary to confirm this suggestion that the GM-CSF with 2 ng/ml concentration may have a better potential, not only to enhance the developmental rates of 2 and 8 cells embryos but also for decreasing the degeneration of those embryos.

Key words: *Granulocyte Macrophage Colony Stimulating Factor, Embryo Development*

Introduction

The GM-CSF is a glycoprotein with 23- kDa molecular weight and has some effects on immune and non-immune cells (Ruef and Coleman, 1990). This cytokine was produce by several cells in extramedullary sites such as mesangial cells (Budde *et al*., 1989), osteoblast (Horowitz *et al*., 1989), tracheal epithelial cells (Smith *et al.*, 1990), and keratinocytes (Chodakewitz *et al*., 1988). It is also synthesized in the female reproductive tract and its targets in this site are myeloid leukocytes, preimplantation embryos and trophoblast cells (Robertson and Seamark, 1992; Chegini *et al*., 1999). Steroid hormones such as estrogen and progesterone affect the secretion of GM-CSF in the uterus (Robertson *et al*., 1996).

Several investigators showed that GM-CSF has embryotrophic effect in mammals (Moraes and Hansen 1997; Sjoblom *et al*., 1999; Robertson *et al*. 2001). Moraes and Hansen (1997) indicated that GM-CSF may play a role in the early development of bovine embryos and might be useful for incrasing blastocyst production rates in serum free culture media.

Robertsone *et al*. (1999) showed that fetal growth and viability were jeopardized in the absence of maternal GM-CSF, suggesting that GM-CSF of either maternal or fetal origin is required for optimal growth and survival of the fetus in mice.

To examine the optimal dosage of GM-CSF for improving the development of mouse embryo, we examined its different concentrations (0, 2, and 10 ng /ml) in 2 cells and eight cells mouse embryo culture media. The developmental rates of those embryos were assesed and compared to hatching blastocysts.

Materials and Methods

For embryo collection, superovulation of NMRI mice (6-10 weeks old) were performed using 10 IU injection of pregnant Mare Serum Gonadotropin (PMSG) followed with another injections of 10 IU Human Chorionic Gonadotropin (hCG) hormone 48 hours later. Following hCG injection, one or two females were caged with male mouse (8-10 weeks old). Female mice were examined for the vaginal plug on the following morning and the presence of vaginal plug was considered as pregnancy. To collect two cells and eight cells embryos, the pregnant females were sacrificed by cervical dislocation at 48 h and 72 h post hCG injections, respectively. The

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Table I. Development of 2 and 8 cells embryos in the presence or absence of GM-CSF 168 h after hCG injection.

Groups	GM-CSF (ng/ml)	Repeat	No. embryo	Murolla (%)	Blastocyst (%)	Hatched embryo (%)	Degenerated embryo (%)
2 cell	0	4	42	5(11.9)	8 (19.4)	15 (37.8)	14 (33.3)
2 cell	2	4	38	4 (15.2)	7 (18.3)	18 (51.5)	9 (23.7)
2 cell	10	4	41	6 (12)	8 (20)	13 (31.8)	14 (34.1)
8 cell	0	3	35	6 (17.1)	10 (28.5)	10 (31.2)	9 (25.7)
8 cell	2	3	29	3 (10.3	6 (20.7)	14 (49.7)	6 (20.7)
8 cell	10	3	32	5 (15.6)	9 (28.1)	10 (31.2)	8 (25.7)

collected embryos at two and eight cells developmental stages were cultured randomly in T6 medium supplemented with 5 mg /ml BSA and 0, 2, and 10 ng / ml concentrations of rGM-CSF. The developmental rates of embryos were assessed daily under inverted microscope until 120 h (for 2 cells embryos) and 96 h (for 8 cells embryos) after culturing to hatching blastocyst stage. The data of blastocyst formation and blastocyct hatching in different groups of embryo culture were compared by chi-square analysis.

Results

The development of two cells and eight cells embryos in the presence of different concentrations of recombinant GM-CSF at four repeat experiences are presented in the table I. From the total of 42, 38 and 41 two cells embryos which were cultured in the medium containing 0, 2, and 10 ng/ ml GM-CSF, 5 (11.9%), 4 (15.2%) and 6 (12%) reached to murolla, 8 (19.4%), 7(18.3%) and 8 (20%) reached to blastocyst stage and 15 (37.8%), 18 (51.5%) and 13 (31.8%) reached to hatched blastocyst stage at 120 h after culturing, respectively. The degenerated embryos in those groups were 14 (33.3%), 9 (23.7%) and 14 (34.1%), respectively.

From the total of 35, 29 and 32 eight cells embryos, which were cultured in the different concentrations of GM-CSF (0, 2, and 10 ng/ml), 6 (17.1%), 3 (10.3%) and 5 (15.6%) reached to murolla and 10 (28.5%), 6 (20.7%) and 9 (28.1%) reached to blastocyst stage and 10 (28.5%), 14 (49.7%) and 10 (31.2%) reached to hatched blastocyst stage, respectively. The degenerated embryos in those groups were 9 (25.7%), 6 (20.7%) and 8 (25.7%), respectively. However, the developmental rates of embryos in the presence of 2 ng/ml of GM-CSF were higher than the other groups, but insignificant between the experimental groups and their controls.

Discussion

The data of the present study showed that the GM-CSF is one of the cytokine that increases the embryo development in mice. It has the greatest effect to enhance the hatching rate of embryos on both 2 cells and 8 cells stages at 2 ng /ml concentration. The hatching rate of 2 cells embryos was 51.5% and for 8 cells embryos was 49.7%. However, in this regards in comparison with the other groups there were not significantly differences. Also, after 120 h of culturing the number of degenerated embryos at 2 ng /ml concentration of GM-CSF was lower than the other groups (23.7% V.S. 33.3% and 34.1% for 2 cell embryos, also 20.7% V.S. 25.7% and 25.7% for 8 cell embryos). Further study is needed on the larger number of embryos to confirm these differences.

Thus, it seems that the GM-CSF with 2 ng/ml concentration has better potential not only to enhance the developmental rates of 2 cells and 8 cells embryos, but also for decreasing the degeneration of those embryos. Finally, because this research was only a pilot study, more researches are necessary to confirm our findings.

It has been shown that GM-CSF is a physiologically important regulator of embryonic development in vivo and it present in the uterus as a product of the maternal tissue or embryo (Chegini et al., 1999; Moraes et al., 1999). After mating in mice, the GM-CSF concentration in uterus was increased (Semark and Robertson 1990; Robertson et al., 1992). It is possible that activation of specific lymphoid cell populations in the uterus by serum or conceptus antigens could increase GM-CSF production (Jokhi et al., 1994). More studies should be performed to evaluate the effects of GM-CSF on the embryo quality and pregnancy rates.

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