Sphingosine-1-phosphate inhibits ceramide-induced apoptosis during murine preimplantation embryonic development

Lei Guo [a,1], Xujing Geng [a,1], Lan Ma [a], Chen Luo [a], Weisen Zeng [b], Xianghong Ou [a], Leining Chen [a], Song Quan [a,*], Hong Li [a,*]

[a] Center for Reproductive Medicine, Department of Obstetrics and Gynecology, Nanfang Hospital, Southern Medical University, Guangzhou, China
[b] Department of Cell Biology, Southern Medical University, Guangzhou, China

ARTICLE INFO

ABSTRACT

Sphingolipids are a complex family of naturally occurring molecules enriched with lipid rafts that contribute to their unique biochemical properties. Sphingolipid metabolites, including ceramide (Cer) and sphingosine-1-phosphate (S1P), are bioactive signaling molecules that regulate cell movement, differentiation, survival, and apoptosis, but their effects on preimplantation development of murine embryos are not well-characterized. In this study, murine zygotes were collected, cultured *in vitro*, and treated with 50 μM C2-Cer plus various concentrations of S1P. The blastocyst formation rate was decreased in the C2-Cer–treated group, compared with that in the control group and the group treated with 50 μM C2-Cer plus 25, 50, or 100 nM S1P ($P < 0.05$), respectively. The total cell number of the blastocysts from various treatment groups was similar at 110 hours post-hCG treatment, but that from the group treated with 50 μM C2-Cer was significantly decreased at 120 hours post-hCG treatment, compared with the control group and the group treated with 50 μM C2-Cer plus 50 nM S1P. However, the apoptotic cell number of blastocysts from the group treated with 50 μM C2-Cer was significantly increased at 110 and 120 hours post-hCG treatment, compared with the control group and the group treated with 50 μM C2-Cer plus 50 nM S1P. Moreover, expression of *p53* in the group treated with 50 μM C2-Cer was higher than that in the control group and the group treated with 50 μM C2-Cer plus 50 nM S1P ($P < 0.05$). In conclusion, Cer decreases the blastocyst formation rate and induces embryonic cell apoptosis, but S1P partly inhibits the effects of Cer during preimplantation development of murine embryos.

1. Introduction

It is well known that a dynamic balance between apoptosis and proliferation is essential to maintain cell growth and development. Thus, excessive apoptosis in early embryos have negative effects on embryonic development and viability. Levy et al. [1] reported that mammalian embryonic apoptosis had important implications for embryonic fragmentation and preimplantation embryonic survival. Furthermore, it was also reported that apoptosis arrested embryonic development and formation of fragments [2,3].

Sphingolipid metabolites are emerging as important lipid signaling molecules in health and disease [4,5]. Among them, sphingosine-1-phosphate (S1P) functions as a growth and survival factor, and acts as a ligand for a family of G protein–coupled receptors, whereas ceramide (Cer) activates the intrinsic and extrinsic apoptotic pathways through receptor-independent mechanisms [6]. Therefore, the cellular balance

Table 1
Sequence of primers used for RT-PCR.

Gene name	Accession no.	Primer sequence (5′–3′)	Product size (bp)
p53	NM_011640	For-TTATGAGCCACCCGAGGC	195
		Rev-GTACGGCGGTCTCTCCCAG	
Gapdh	NM_008084	For-TGGCAAAGTGGAGATTGTTGCC	156
		Rev-AAGATGGTGATGGGCTTCCCG	

of these sphingolipid metabolites forms the "sphingolipid rheostat," which addresses the importance of balance of these mediators and not the absolute amount of metabolites in determining cell fate [7]. Morita et al. [8] reported that oocytes lacking the gene for acid sphingomyelinase or wild-type oocytes treated with S1P resisted developmental apoptosis and apoptosis induced by anti-cancer therapy, confirming cell autonomy of the death defect. Moreover, radiation-induced oocytes loss in adult wild-type female mice was completely prevented by *in vivo* therapy with S1P. Jee et al. [9] reported that when immature murine oocytes were incubated in maturation medium with S1P, the blastocyst formation rate was higher and the percentage of apoptotic blastomeres was significantly lower, compared with the control group. Roth et al. [10] reported that S1P protected the bovine oocytes from a physiologically relevant heat shock and affected oocyte maturation, even in the absence of heat shock. Furthermore, they also reported that the S1P-treated oocytes that survived heat shock and became blastocysts had normal developmental potential. However, there were few studies regarding the roles of S1P in preimplantation development of murine embryos.

In this study, we determine the effects of S1P and C2-Cer on the formation of murine blastocysts and apoptosis of blastomeres at the blastocyst stage.

2. Materials and methods

All chemicals and media were purchased from Sigma Chemical Company (St. Louis, MO, USA) except for those specifically mentioned. All animals were maintained in accordance with the Animal Experiment Standard of Southern Medical University.

2.1. Collection of mouse embryos

Embryos were collected from 4-week-old CD-1 female mice. Female mice were injected with 5 IU of pregnant mare serum gonadotropin, after 48 hours later by 5 IU of hCG, and then mated with CD-1 male mice (males >10 week old). Zygotes were collected from the oviduct 20 hours post-hCG treatment and cumulus cells were removed by 0.1% hyaluronidase treatment [11,12].

Embryos were cultured in CZB (MR-019-D, Millipore Co., Billerica, MA, USA) with 50 μM C2-ceramide (C2-Cer) or with 50 μM C2-Cer plus 12.5, 25, 50, or 100 nM S1P, respectively. The embryos were cultured in CZB, with or without an equal volume of the carrier of SIP (methanol, 0.01%, vol/vol) as the sham control. For these studies, 20 to 30 zygotes were cultured in a 50-μL culture drop under mineral oil at 37 °C under 5% CO_2.

2.2. RNA isolation, reverse-transcription, and quantitative real-time polymerase chain reaction

Total RNA was isolated from 50 preimplantation embryos of each group at the morula stage (85 hours post-hCG treatment) using the RNeasy Micro kit (74004, Qiagen Co., Hilden, Germany) following the manufacturer's instructions. Embryos were transferred to lysis buffer and treated with the RNase-free DNase supplied in the kit. The mRNA was reverse transcribed into cDNA using oligo (dT) primers and the PrimeScript first strand cDNA synthesis kit (D6110, TAKARA, TAKARA Biotechnology (Dalian) CO., Dalian, China), according to the manufacturer's instructions.

To quantify mRNA, real-time polymerase chain reaction (RT-PCR) analyses were performed using the ABI Prism 7500 RT-PCR system (Applied Biosystems, Life Technologies Co., Carlsbad, CA, USA). The SYBR Premix Ex Taq reagents (DRR041, TaKaRa, TAKARA Biotechnology (Dalian) CO.) were used for monitoring amplification, and the results were evaluated with the 7500 software program (version 2.0.1, Applied Biosystems, Life Technologies Co.). RT-PCR primers were designed using the PrimerExpress software (Applied Biosystems, Life Technologies Co.) purchased from Invitrogen (Life Technologies Co.). All

Table 2
Effects of C2-Cer and S1P on the formation of murine blastocysts.

Treatment	No. of embryos (%)			
	Total	≤8 cell	Morula	Blastocyst
Control	90	6 (6.7)	4 (4.4)[a]	80 (88.9)[a]
0.01% methanol (vol/vol)	90	5 (5.5)	4 (4.4)[a]	81 (90.0)[a]
50 μM C2-Cer	100	10 (10.0)	61 (61.0)[b]	29 (29.0)[b,c]
50 μM C2-Cer + 0.01% methanol	97	9 (9.3)	60 (61.9)[b]	28 (28.9)[b,c]
50 μM C2-Cer + 12.5 nM S1P	94	10 (10.6)	49 (52.1)[b]	35 (37.2)[b,c]
50 μM C2-Cer + 25 nM S1P	98	10 (10.2)	40 (40.8)[b]	48 (49.0)[b,d]
50 μM C2-Cer + 50 nM S1P	102	9 (8.8)	31 (30.4)[b]	62 (60.8)[b,d]
50 μM C2-Cer + 100 nM S1P	100	8 (8.0)	41 (41.0)[b]	51 (51.0)[b,d]

Embryonic developmental data were analyzed from four independent experimental replicates.
[a,b] Within a column, means without a common superscript differed (P < 0.01).
[c,d] Within a column, means without a common superscript differed (P < 0.01).