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Tetraploidy Induction of Mouse Embryos by *In Vitro* Culture with Cytochalasin B

Dong Il Jin

Division of Applied Biological Science, Sun Moon University

Cytochalasin B를 이용한 체외배양에 의한 생쥐 수정란의 4배체 유도

진 동 일

선문대학교 응용생물과학부

요 약

효율적인 homozygous 동물을 생산하기 위한 실험의 단계로 염색체가 4배체인 수정란의 이용성을 타진하기 위해 생쥐 수정란과 cytochalasin B를 사용하여 4배체 유도에 관한 실험을 수행하였다. 생쥐 2-세포기 수정란을 10 μg/ml 농도의 cytochalasin B로 약 20시간 배양하였을 때 모든 수정란은 발육을 거의 멈추었으나, 이 수정란을 cytochalasin B-free medium에 체외배양하였을 때 발육이 재개되어 48시간 후 상실기나 배반포기까지 약 74%의 발육율을 나타내었다. 그러나 발육된 수정란의 세포수는 대조구에 비해 훨씬 적은 것으로 나타났다. 염색체 분석결과 cytochalasin B로 처리한 대부분의 수정란은 4배체인 것으로 나타났고 약간의 수정란은 mosaicism과 다배체를 나타내기도 하였다. 그러므로 cytochalasin B를 이용하여 효과적으로 4배체의 수정란을 유도할 수 있는 것으로 나타났다.

(Key words : tetraploidy induction, mouse embryo, *in vitro* culture, cytochalasin)

INTRODUCTION

Cytochalasim B (CB) has been extensively applied in studies on the mechanism of cell division in various type of cells, including oocytes and cleaving eggs. In 1967, Carter found that CB can inhibit cytokinesis of cell without interferring with karyokinesis. CB-treated cells generally resume cleavage upon removal from CB and reculturing. It has been shown that preimplantation mouse embryos treated with CB at the stage of 1- or 2-cell, can develop into the blastocyst stage or beyond; the treatment with CB induced polyploidy in most of the embryos (Niemierko, 1975; Perry and Snow, 1975; Snow, 1973; Snow 1975; Tarkowski et al., 1975). Pups from tetraploid embryos has not been reported by far probably due to extraembryonic contribution of tetraploidy embryos during organogensis (Lu and Markert, 1980). However, CB has been utilized to double the haploid genome to produce homozygous mouse embryos (Markert and Petters, 1977). Recently, tetraploid embryos were used with embryonic stem (ES) cells for the production of the only ES cell-derived fetuses (Nagy et al., 1990). This study reports the treatment of cleaving mouse embryos with CB and the establishment of tetraploidy in mouse embryos by suppressing the second

cleavage division with CB.

MATERIALS AND METHODS

Female mice were superovulated with intraperitoneal injections of 5 i.u. PMSG followed 48 hours later by the injection of 2.5 i.u. HCG. Embryos were flushed from the oviducts of mated females approximately 48~50 hours post HCG. Flushing and culture medium for mouse embryos in this experiment was BMOC-3. After being separated from 3- and 4-cell embryos, 2-cell embryos were divided into 2 groups; CB-treated group (10μg/ml of CB, overnight culture) and control (BMOC-3). These embryos were then removed from the CB medium and cultured *in vitro*. About 48 hours later, embryos in the CB-treated group and the control group were transferred to medium containing 1 μg/ml colcemid and returned to the incubator. After 3~5 hours the embryos were isolated and the number of cells and ploidy were determined by air-drying technique (Kleinfeld and Sisken, 1966: Tarkowski, 1966). The embryos were put into hypotonic (1%) sodium citrate and let to stand at room temperature for 20~40 minutes. A microdrop of this solution together with the embryos was placed on a grease-free slide. One drop (0.02 ml) of acetic alcohol (3 parts absolute ethyl alcohol, 1 part of glacial acetic acid) was immediately expelled from another pipette, whose tip was brought just over the microdrop. After the embryos began to flatten, a few more drops of acetic alcohol were applied to give adequate spreading of nuclei and metaphase plates and good stainability of chromosomes. Final scattering of nuclei and spreading of chromosomes was achieved during air-drying. After the slides were dry, a few drops of stain (2% aceto-orcein) were placed on the slide for 30 minutes, and then excess stain rinsed off with ethyl alcohol. The slides were mounted in permount. The karyotypes of embryos were examined through the optical microscope.

RESULTS

After 20 hour treatment of CB, 47.6% of 2-cell embryos still remained at 2-cell stage, while more than 80% of the control embryos had cleaved to 4~8 cell and morula stage (Table 1). Of the embryos removed from CB medium and cultured in CB-free medium for another 48 hours, about 85% appeared to be recovered and continued to cleave even if their development were delayed (Table 2). However, these embryos

Table 1. Effect of CB on 2-cell embryos after 20 hrs of culture

	Total no. embryos	Stage of embryos (%)			
		2 cell	4~8 cell	Morula	Abnormal
CB-treated	103	49 (47.6)	54 (52.4)		
Control	106	4 (3.8)	80 (75.5)	9 (8.5)	10 (9.4)

Table 2. Development *in vitro* of CB-treated and control 2-cell embryos following 48hrs of culture

	Total no. embryos	Stage of embryos (%)				
		2 cell	4~8 cell	Morula	Blastocyst	Degenerated
CB-treated[a]	90	8 (8.9)	10 (11.1)	12 (13.3)	55 (61.1)	5 (5.6)
Control	94	2 (2.1)	3 (13.9)	8 (85.1)	77 (81.9)	4 (4.3)

[a] Embryos were exposed to CB for 20 hours and then cultured in CB-free medium for 48 hours.

Table 3. Karyotype of 2 day-cultured embryos

	No. embryos examined	No. cells in embryos (average)	Ploidy (%)			
			2n	2N/4N	4N	Polyploidy
CB-treated	44	16~40 (28)	0	2 (4.5)	37 (84.1)	5 (11.4)
Control	47	18~58 (40)	47 (100.0)			

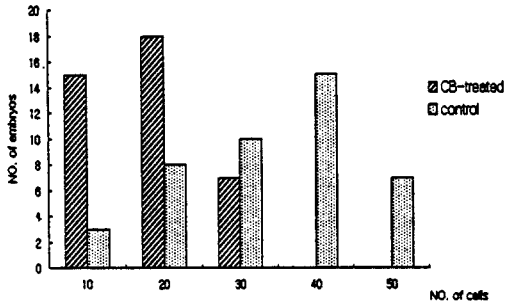

Fig. 1. Number of cells in 72 hour-cultured embryos.

contained the considerably reduced cell numbers compared with control embryos (Table 3, Fig. 1). Almost all the CB-treated embryos were tetraploid. Several embryos treated with CB appeared to be mosaic or polyploidy while 100% of control embryos were dipoloid (Table 3). One group of CB-treated 4-cell embryos showed similar results as CB-treated 2-cell embryos (data not shown).

DISCUSSION

Snow (1973, 1975) and Tarkowski, et. al. (1977) showed that CB at a concentration of 10 ug/ml was highly effective in producing tetraploidy by suppression of the second cleavage. About 40~75% of the initial CB-treated 2-cell embryos developed to blastocysts, and in contrast with diploid in control embryos. the ploidy of CB-treated embryos were 4n, 4n/2n and high degree of ploidy. in this study, all the embryos in CB-treated groups might not culture long enough to develop into the blastocyst stage. About 74% of CB-treated 2-cell embryos developed normally up to morula and blastocyst stage with significantly reduced cell numbers (Table 2, Fig. 1). Because some of embryos did not spread well during the air-drying chromosome preparation, the number of chromosome was hard to estimate. Almost all the CB-treated embryos were tetraploid, only several embryos were mosaic or polyploidy. The main factor of the mosaic or polyploid embryos is probably due to the asynchrony of second cleavage, both between eggs or between sister blastomeres (Tarkowski et al., 1977). Some embryos failed to recover the ability to cleave after removal from CB although their nuclei continue dividing, each time contributing to a common spindle, and the cells become highly polyploid (Snow, 1973; Siracusa et al., 1980).

SUMMARY

By subjecting 2-cell mouse embryos to 10 ug/ml of cytochalasin B (CB) for 20 hours, all the embryos remained at 2- or 4-cell stage. After transferring to CB-free medium and culturing *in vitro*, 74% of the CB-treated embryos appeared to continue to cleave and develop into morula and blastocyst stage. But, the cell number of CB-treated embryos was significantly reduced. Almost all the CB-treated embryos were tetraploid, while only several embryos were mosaic or polyploidy. CB can effectively induce tetraploidy by suppressing the second cleavage of mouse embryos.

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