Effects of α -momorcharin on preimplantation development in the mouse

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Summary. When α -momorcharin was injected intraperitoneally (0.2 mg/25 g body weight) into pregnant mice on Days 1–3 of pregnancy, over 50 % of the mice failed to support an implantation. In-vitro study of the effects of the protein on preimplantation embryos showed that the protein did not significantly disturb embryonic development from the 2-cell to compacting morula stage except when high concentrations (≥ 0.5 μ g/ml) of protein were present. In many embryos, compaction of blastomeres was incomplete and subsequent blastocyst formation was impaired. Other protein-treated embryos that formed compacted morulae and early blastocysts later showed decompaction and degenerated. The protein-treated embryos generally had fewer numbers of cells because cell division beyond the morula stage was impaired. The poor development of morulae may be the cause of inhibition of early pregnancy in the mouse by α -momorcharin.

Introduction

The basic glycoprotein α -momorcharin has recently been isolated and purified from the seeds of *Momordica charantia*. This protein shows an inhibitory effect on pregnancy in the mouse during the implantation period (Law, Tam & Yeung, 1983) and induces abortion during mid-gestation, i.e. Days 10–14 (unpublished observation). When compacted morulae and early blastocysts were exposed to this protein *in vitro*, development of the embryos beyond the blastocyst stage was impaired. The incidence of complete hatching from the zona pellucida and of successful attachment of the trophoblast to the plastic culture substrate both decreased. The extent to which trophoblast outgrew on the substrate also diminished. Inner cell mass development was less affected than the trophoblast. It was concluded that the in-vivo inhibition of implantation may be a consequence of the deleterious effect of α -momorcharin on the trophoblast (Law *et al.*, 1983). In the present study, we have investigated the effect of α -momorcharin on the development of preimplantation embryos *in vitro* and attempted to correlate the results with the in-vivo inhibition of early pregnancy in the mouse.

Materials and Methods

α -Momorcharin was isolated from the crude extract of decorticated seeds of *Momordica charantia* by acetone fractionation, and this fraction was further purified on CM Sepharose CL 6B and Sephadex G100 (fine) columns (Pharmacia, Uppsala, Sweden). The final preparation was tested for its abortifacient activity in pregnant mice at Days 4–14 of gestation and was shown to be

chemically homogeneous by SDS-PA gel electrophoresis and immunoelectrophoresis. Preliminary analyses indicated that this protein has a molecular weight of 31 000, contains PAS-positive sugar moieties, carries aspartic acid in its N-terminal sequence but no half-cystine and little methionine, and is separated as a basic molecule during electrophoresis.

Random bred ICR mice were caged with males overnight. The presence of vaginal plugs was checked the following morning (Day 1 of pregnancy). Pregnant mice were injected intraperitoneally with α -momorcharin on Days 1-3 (see Table 1). The inhibitory effect of the protein on implantation was expressed as the percentage of non-pregnant animals per treatment.

Two-cell, 4-cell and compacting (8-16-cell) embryos were obtained from the oviducts of mice on Days 2-3 of pregnancy and the embryos were collected in PB1 solution (Whittingham & Wales, 1969). The embryos were then washed once in fresh Medium 16 (Whittingham, 1971) and were cultured in groups of 5-10 in 0.5 ml Medium 16 under 5% CO₂ in air. α -Momorcharin was added in small volumes of phosphate-buffered saline to the culture medium. Embryos of different developmental stages were exposed to the protein either continuously or over a limited period in culture (see Tables 2 & 3). The embryos were examined under phase-contrast optics at 24-h intervals for 3-4 days until most of the control embryos had reached the blastocyst stage. The cell number of pre-compaction cleavage-stage embryos was estimated by visual counting and that of morulae and blastocysts was determined on cell spreads prepared by the technique of Tarkowski (1966).

Results

Effect on early pregnancy in vivo

α -Momorcharin given to mice on Days 1-3 of pregnancy resulted in a significant reduction in the incidence of successful implantations at Day 8 (Table 1). The non-pregnant mice showed either a total absence of implantations or degeneration of all the implantations. Amongst the treated mice that remained pregnant the mean $\pm$ s.e.m. number of implantation sites was 13.4 ± 0.5 ($n = 15$) and this was close to the control value of 12.9 ± 0.8 ($n = 18$; not significantly different, t test).

Table 1. Effects of α -momorcharin administered on Day 1-3 on the success of pregnancy in the mouse

Treatment (mg/25 g body wt)	Day of pregnancy at treatment	No. of mice	No. of non-pregnant mice at Day 8 (%)
0 (saline control)	1-3	21	3 (14)
0.2	1	13	*7 (54)
0.2	2	9	*7 (78)
0.2	3	8	*5 (63)

* $P < 0.01$ compared with control value (χ^2 test).

Effects on 2-cell embryos

The development of 2-cell embryos to the compacted morula stage was little affected by α -momorcharin (Table 2); 72-75% of the treated embryos cleaved to the 4-cell stage in the presence of 0.1 or 0.5 μ g α -momorcharin/ml. Only when the protein concentration reached 1.0 μ g/ml, did 49% of the embryos fail to cleave into 4-cell stages. The cell numbers of control and treated embryos were similar (Table 2). When 2-cell embryos were exposed to α -momorcharin (0.1 μ g/ml) during the 2- to 4-cell stages, their subsequent development through cleavage, compaction and blastocyst

formation was not retarded. However, when the period of treatment extended from the 2-cell to the compaction stage, development of embryos beyond the morula stage was impaired and 80% of embryos degenerated (Table 3). The cell number of these treated embryos (14.5 ± 1.9 , $n = 15$) was significantly less ($P < 0.01$, t test) than that of the control embryos (50.8 ± 3.2 , $n = 21$) and cell division seemed to have been arrested at the compacting 16-cell stage. When 2-cell embryos were treated with higher concentrations of protein (0.5–1.0 $\mu\text{g/ml}$) until they reached either the 4-cell stage or the compacting stage, fewer underwent complete compaction and none reached the blastocyst stage. All the treated embryos eventually decompacted and had degenerated by the end of culture.

Table 2. Effects of α -momorcharin on the development of 2-cell stage mouse embryos after 24 h *in vitro*

α -Momorcharin ($\mu\text{g/ml}$)	No. of embryos	No. (%) of embryos with ≥ 1 complete cleavage	Mean $\pm$ s.e.m. cell no. of embryos with ≥ 3 cells†
0 (control)	210	157 (75)	4.1 ± 0.1 (192)
0.1	50	36 (72)	4.1 ± 0.1 (46)
0.5	24	18 (75)	3.9 ± 0.1 (20)
1.0	65	*33 (51)	3.9 ± 0.1 (39)

* $P < 0.01$ compared with control value (χ^2 test).

† No. of embryos in parentheses.

Effects on 4-cell embryos

A continuous presence of α -momorcharin from the 4-cell stage onwards did not appear to affect the progression of development through cleavage stage to morula formation (Table 4a). However, compaction of blastomeres was incomplete in these treated embryos and isolated blastomeres that had been extruded from the embryos were frequently observed. Development from the morula to the blastocyst stage was impaired and at 72 h, when 82% of control embryos had reached the fully expanded blastocyst stage (Pl. 1, Fig. 1), > 80% of the treated embryos had degenerated (Pl. 1, Fig. 2). When 4-cell embryos were treated with α -momorcharin (0.1 $\mu\text{g/ml}$) only during the pre-compaction stages, compaction of morulae was unaffected but significantly more embryos degenerated later and fewer managed to form blastocysts (Table 4b). The treated embryos had significantly ($P < 0.01$) fewer cells (22.8 ± 1.9 , $n = 42$, for 0.1 $\mu\text{g/ml}$; 12.3 ± 2.1 , $n = 13$, for 0.5 $\mu\text{g/ml}$) than did controls (49.2 ± 2.8 , $n = 38$). A more marked inhibitory effect on compaction and blastocyst formation was evident when α -momorcharin was tested at 1 $\mu\text{g/ml}$.

Effects on compacting embryos

A continuous exposure of the compacting 8–16-cell embryo to α -momorcharin only slightly retarded compaction but markedly reduced the incidence of blastocyst formation (Table 4c). A similar effect was produced when α -momorcharin was presented only for 24 h during which over 65% of embryos compacted fully. However, at 48 h these embryos had only one third of the normal cell number (23.6 ± 1.9 , $n = 13$, and 24.3 ± 1.9 , $n = 23$, respectively, compared with controls, 74.6 ± 5.3 , $n = 21$; $P < 0.01$ for both), indicating that cell divisions beyond the morula stage were inhibited. When embryos were exposed to α -momorcharin after most of them had compacted or were early blastocysts, about 40% of embryos degenerated during the next 24 h in culture. Fewer cells were found in these embryos (41.0 ± 2.6 , $n = 20$; $P < 0.01$ compared with controls), but this value was greater than those of the other two treatment groups. While about 80% of the control embryos reached the blastocyst stage after 72 h in culture, nearly all the treated embryos degenerated.

Table 3. Development (%) of 2-cell mouse embryos during culture with α -momorcharin for 72 h

Treatment		24 h				48 h				72 h			
Dose (μ g/ml)	Stages	No. of embryos	$\geq$ 4-cell	No. of embryos	Compacting morula	Compacted morula	Early blastocyst	No. of embryos	Compacted morula	Blastocyst	Degenerate		
0 (control)	—	95	76	65	23	45	15	25	0	80	20		
0.1	2- to 4-cell	20	85	20	15	55	15	20	10	75	15		
	2-cell to compacting	20	95	20	20	50	10	*20	10	0	80		
0.5	2- to 4-cell	25	84	*25	32	48	0	*25	0	0	100		
	2-cell to compacting	25	52	*25	48	8	0	*25	0	0	100		
1.0	2- to 4-cell	25	72	*25	40	4	0	*25	0	0	100		
	2-cell to compacting	25	56	*25	64	4	0	*25	0	0	100		

* $P < 0.01$ for the group of treated embryos compared with control group (χ^2 test on the actual no. of embryos scored for the various developmental stages).

Table 4. Effects of α -momorcharin on the development of (a, b) 4-cell or (c) compacting (8–16-cell) mouse embryos (%) *in vitro*

Treatment		24 h						48 h						72 h					
Dose (μ g/ml)	Stages	No. of embryos		Compacting morula		No. of embryos		Early blastocyst		Compacted morula		No. of embryos		Expanded blastocyst		Degenerated		Blastocyst	
(a) 0 (control)	—	90	96	0	106	41	53	7	56	82	16								
0.05	Continuous	48	81	0	67	36	49	15	*37	14	86								
0.10	Continuous	40	90	0	*63	40	18	43	*33	0	100								
0.50	Continuous	—	—	—	*18	78	0	22	*18	0	100								
(b) 0 (control)	—	42	14	83	2	42	79	5	—	—	—								
0.1	4-cell to compacting	44	30	70	0	*44	16	34	—	—	—								
0.5		*32	72	16	0	*32	0	97	—	—	—								
1.0		*17	65	0	*17	0	0	100	—	—	—								
(c) 0 (control)	—	42	5	71	4	42	48	12	20	80	20								
0.1	Continuous	*31	45	55	0	*31	0	78	*18	0	100								
0.1	Up to compacting morula	*44	32	66	2	*44	5	84	*20	10	90								
0.1	After compaction	30	17	53	30	*41	22	42	*20	0	100								

* $P < 0.01$ for the group of treated embryos compared with control group (χ^2 test on the actual no. of embryos scored for the various developmental stages).

Fig. 1. Control embryos developed from the 4-cell stage for 72 h. Some embryos were hatching from the zona pellucida. Bar = 100 µm.

Fig. 2. Partly decompacted and degenerating embryos developed from the 4-cell stage for 72 h in 0.5 µg α-momorcharin/ml. Bar = 100 µm.

Discussion

We have shown in this study that a single injection of 0.2 mg α -momorcharin/25 g body weight into pregnant mice on Days 1–3 resulted in an inhibition of pregnancy. This inhibitory effect *in vivo* is likely to be the result of a deleterious effect of the protein on the early development of the embryos. Our *in-vitro* observations indicated that the protein prevented the proper compaction of blastomeres into a morula and the α -momorcharin-treated morulae failed to form normal blastocysts. Nearly all treated embryos degenerated at a time when the normal embryo showed an expansion of the blastocyst cavity and hatched from the zona pellucida. Experiments in which the pre-compacted embryos were exposed to α -momorcharin for limited periods strongly suggested that (1) development through the first few cleavage divisions and initial compaction was unaffected by the protein and (2) the defects in compaction and blastocyst formation were manifestations of a subtle deleterious effect imposed by the protein at earlier stages. The possibility that an 'inhibitory' concentration of α -momorcharin may persist in the pregnant mouse until the embryos reach the most susceptible transition stage of morula-blastocyst cannot be excluded until the pattern of disposition of the protein is known. Much less α -momorcharin was required to produce an inhibitory effect on 4-cell or compacting embryos than on 2-cell embryos.

A common feature in the response of the pre-compacted embryo to various inhibitors of nucleic acid and protein synthesis is that continuous treatment during the cleavage to morula stages tends to disturb compaction, blastocyst formation and embryonic viability. Treatment of the embryo at stages of transition from morula to blastocyst tends to affect the differentiation of the inner cell mass and the trophoctoderm of the blastocyst (Thomson & Biggers, 1966; Skalko & Morse, 1969; Tasca & Hillman, 1970; Sherman & Atienza, 1975; Rowinski, Solter & Koprowski, 1975; Pedersen & Spindle, 1977; Spielmann & Jacob-Muller, 1981). Law *et al.* (1983) have demonstrated an impairment of the subsequent differentiation capacity of the trophoblast. α -Momorcharin may also interact with other cell surface glycoproteins on the blastomere and adversely affect compaction. When normal glycosylation of cell membrane protein was inhibited by tunicamycin, the embryos either remained uncompacted or initially underwent partial compaction and later decompaction (Surani, 1979; Surani, Kimber & Handyside, 1981). This is reminiscent of the behaviour of α -momorcharin-treated embryos.

α -Momorcharin has an abortifacient property similar to that of α -trichosanthin in the peri-implantation period and mid-gestation in the mouse (Chang, Saksena, Lau & Wang, 1979; Zhou, Li, Shu, Bao & Chu, 1982; Law *et al.*, 1983). There are, however, very few studies on the effect of these two proteins on preimplantation development. Chang *et al.* (1979) could not observe any effect of α -trichosanthin on pregnancy in mice treated on Days 1–4, while Zhou *et al.* (1982) found a definite abortifacient effect of α -trichosanthin in mice during the implantation period. Ultrastructural evidence of cellular damage has been found in the rabbit blastocyst exposed to trichosanthin *in vitro* (Tseng, Teng, Wu & Chou, 1979). Our study is the first to demonstrate the inhibitory effect of an abortifacient plant protein on preimplantation development in the mouse.

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Received 23 June 1983