TERATOGENIC EFFECTS OF CYTOCHALASIN B ON 9.5 DAY RAT EMBRYOS.

Rosa Mª MIRAPEIX, Pere J. FABREGAS, Josep Mª DOMENECH.
Departamento de Ciencias Morfológicas. Facultad de Medicina. Universidad Autónoma de Barcelona. E-08193. Bellaterra (Barcelona). Spain.

Cytochalasin B (CB) is a mould metabolite which grows on plants and certain cereal grains. It is known to affect cellular motility and the cytoskeleton. Moreover, it produces a teratogenic effect on several animal embryos (chick, hamster, mouse). The aim of this study is based on the effect of CB during the organogenetic period.

Rat embryos were explanted at 9.5 days, placed in a 1.5 ml of culture medium (75% rat serum, 25% DMEM (Gibco) and CB or dimethyl sulphoxide (DMSO)) and cultured for 48 hours at 37ºC. CB was dissolved in 1% DMSO and added to the medium to give the following final concentrations: 0.05 μg/ml; 0.2 μg/ml; 0.4 μg/ml and 0.5 μg/ml. In control embryos, DMSO was added in a volume equal to CB. Thus, the only variable in the culture media was the presence or absence of a particular concentration of CB. At the end of the 48-hour period, the embryos were examined in order to determine survival rate and gross malformations. Embryo growth was assessed using yolk sac diameter, crown-rump and head length, whereas embryo development was assessed using morphological score (Brown and Fabro, 1981). Thereafter, the embryos were fixed in Bouin's fluid, serially sectioned at 5μm and stained with haematoxylin-eosine. Developmental parameters were expressed as mean ± standard error of the mean. Control and CB-treated embryos were compared by using Student's t-test ($p < 0.001$ level of significance was chosen). The survival rate and the incidence of malformations were expressed in percentages. Control and treated-embryos were compared by using Fisher's exact test ($p<0.01$ level of significance were chosen).

Control embryos: Developmental parameters are summarized in Table I. At the end of the culture period, the embryos had rotated. We could observe the limb buds and three branchial arches. The first branchial bud of the embryos were still separate. The optic cup was formed. The neural tube was completely closed. The prosencephalon had laterally expanded. The cells of the optic vesicles were wedge-shaped with their nuclei located in the basal half of the cell and mitotic figures near the lumen. The overlying ectoderm had thickened and would become the lens placode. On both sides of the rhombencephalon a closed otic pit could be observed. The otocyst presented a dorsal recess and some neuroblasts were clustered, rostrally to it,forming the VIII cranial ganglion. The cranial ganglia V, VII, IX and could also be distinguished.

CB Treated embryos: Embryonic growth and development were significantly reduced ($p<0.001$ for all parameters). The higher the doses, the lower the morphometric parameter score (see Table I). Some embryos became so stunted that it was impossible to count the somites with accuracy. At the lowest doses (CB 0.05 μg/ml), neural tube defects and survival rate were not significantly different from controls, whereas some embryos (3/8) showed a delay in axial rotations ($p<0.01$) (see Table II). At concentration ≥ 0.2 μg/ml, there was significant reduction in the survival rate ($p<0.001$). This became more evident when the highest doses were used (see Table II). The number of malformed embryos increased at high doses and there were statistical differences from the controls ($p<0.001$). Open neural tube defects were the most common gross malformation (ranged from a failure of closure in the cephalic region of the neural tube to a total craniorrhachischisis). There was a relatively high incidence in CB-treated embryos of abnormal rotation.The higher the doses, the more unturned embryos (see Table II). At concentration of CB 0.05 μg/ml, the prosencephalon (the most affected) was hypoplastic. The neuroepithelium consisted of three or four prismatic cell layers in which mitotic images could be distinguished near the lumen. At 0.2 μg/ml, 33% of embryos showed exencephaly until the mesen-rhombencephalic union. Some neuro-epithelial cells were in the lumen, while others formed a protrusion into the ventriculus. Mitotic figures were seen in the middle of the neuroepithelium. At the optic primordia , mitoses were more numerous than in the controls. At 0.4 μg/ml we observed almost total exencephaly and craniorrhachischisis. Optic vesicles were severely affected. They were collapsed (there was an adhesion of their lateral walls) and some embryos showed anophthalmia. The mesencephalon had many cephalic folds. The rhombencephalon was the least affected structure. The otic primordia was structurally normal, but showed little development since the dorsal recess could not be found. At the highest dose (CB 0.5 μg/ml), embryos presented all the above-mentioned anomalies and moreover the optic vesicles were all collapsed. The otic primordia were not observed.

Treatment	No	No Somites	C-R Length (mm)	Head length mm)	Yolk Sac (mm)	Morph Score
Control	33	28.86±1.40	4.28±0.45	2.12±0.20	4.83±0.44	44.13±1.83
CB 0.05	8	24.00±2.72	3.12±0.58	1.81±0.22	3.43±0.66	39.93±1.11
CB 0.2	33	22.33±0.47	2.33±0.47	1.16±0.23	2.50±0.71	26.77±2.99
CB 0.4	30	20.00±0.83	2.25±0.35	1.41±0.31	2.50±0.41	20.21±1.39
CB 0.5	14	21.00±0.25	1.62±0.12	1.25±0.25	1.25±0.36	18.64±0.71

TABLE I. Growth mesurements and score of 9.5 day rat embryos cultured for 48 hours. Results are given as mean ± S.E.M. All CB-treated embryos are significantly differents from control-embryos, $p< 0.001$ (Student´s T-test).

Treatment	No Embryos	Survival Rate(%)	Neural T. Defects (%)	Failure of Rotation
Control	33	100	0	0
CB 0.05	8	100	0	37.50*
CB 0.2	33	63.63**	33.33**	57.14**
CB 0.4	30	50.00**	60*	100**
CB 0.5	14	21.42**	100*	100**

TABLE II. Effects of cytochalasin B on 9.5 day rat embryo. * - Significantly different p<0.01 (Fisher´s exact test). ** - Significantly different p<0.001 (Fisher´s exact test)

Our results show that CB has teratogenic effects on rat embryos cultured in vitro during organogenesis. The main mechanisms affected by CB are embryonic rotation and neurulation. Regarding axial rotation, we should bear in mind that at the beginning the embryonic disc is concave dorsally until day 10.5, when the embryo starts axial rotation at the same time that the amniotic cavity expands. About day 11, the embryo reaches its final position. Some factors have been suggested to evoke rotation of the embryo: the heart-beat that would cause dipping movements of the head towards the right side and the increased asymmetry of the heart (Deuchar and Parker, 1975), the changes in the shape of the mid-region somites (Matsuda, 1991), the growth of the foregut and hindgut (Rough,1968), the asymmetry of the mitotic activity within the neuroectoderm located close to the rotating part (Poelmann et al.,1987) and the extraembryonic membranes (yolk sac and amnion) which would plan an important factor during rotation (Deuchar and Parker 1975; Poelmann et al.,1987), in such a way that detachment or extrusion of the embryos from their membranes would imply a non-correct rotation of the embryo. In CB-treated embryos we have observed abnormal body shape, which suggests some kind of interaction in axial rotation. Those embryos which were most severely affected were ventrally convex whereas those which were least affected had abnormal caudal body rotation. Although the absence of embryos turning has been reported in rat embryos treated with cytochalasin D (Matsuda,1991), we have found no information in the literature consulted concerning axial rotation defects in rat embryos cultured with CB.

Neurulation was the other mechanism affected by CB (Table II). Almost all the CB treated embryos showed some sort of defects involving the head region, such as reduction of the prosen-mesencephalic development or failure of the neural tube closure at the anterior neuropore. The pattern of these anomalies was similar to that already reported in chick (Linville and Shepard,1972) and in mice (O'Shea,1981). Serial sections revealed severe damage in the neuroepithelium.

Some authors have related neural tube defects with axial rotation delays. In this study, we have observed that the embryos which presented a failure in the axial rotation showed the posterior part of the trunk bent back, and there was abnormal fusion of the neural folds. According to Deuchar and Parker (1975) the existence of cephalic malformations prior to the beginning of the inversion would annul or impede the transmission of the initial stimulus coming from the cephalic region. Cole and Trasler (1980) observed that extensive delays in turning lead to abnormal body shape and that the former could provide a mechanical basis for the failure of the neural tube to close. Poelman et al (1987) postulated that mitotic asymmetry of the neural tube was involved in rotation. Moreover, we have observed that all the embryos with non closure of the neural tube had failure in axial rotation but the reverse was not true, since not all the embryos with delays in axial rotation had neural tube defects. Thus, there is a direct relationship between neural tube defects and failure of the embryonic rotation. We therefore suggest that, although it might not be the most important mechanism, alterations in the closing of the neural tube can provoke alterations in rotation.

In the present in vitro study, effects of CB on initial stages of organogenesis in rat embryos have been observed. The results have showed that CB caused various neural tube defects (particularly abnormalities in the closure of the cephalic region) as well as interaction in axial rotations.

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