TERATOLOGICAL STUDIES ON COLLAGEN WOUND DRESSING (CAS) IN MICE

Ueto TAKADA, Masahide MORIGUCHI, Toshiaki HATA
and Atsuko YAMAMOTO

Central Research Laboratories, Meiji Seika Kaisha, Ltd.

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Abstract.....We subcutaneously injected collagen wound dressing (CAS) extracts equivalent to the area covering human body surface by 35 and 70%, respectively, into mouse dams from days 6 to 15 of pregnancy.

No toxicity was observed in dams and no external, skeletal and visceral anomalies related to CAS treatment were observed in fetuses.

It is, therefore, concluded that CAS extracts produces no teratogenesis after subcutaneous injection during organogenesis in mice.

Key words : Collagen wound dressing, teratogenesis, mice.

INTRODUCTION

Collagen wound dressing (CAS) is an unwoven wound dressing made of purified atelocollagen from bovine derma.

It has already been reported that CAS exhibited favorable therapeutic effects on the experimental burn or skin excision in rabbits (Shibata et al. 1981, Takeda et al. 1982). In the present paper, we will report on the teratogenicity of CAS in mice.

MATERIALS AND METHODS

1. Test substance

CAS was used as an extract with physiological saline. The extract was prepared as follows :

CAS (100 sheet of 10×10 cm) cut into pieces, approximately 2 to 3 cm long, was perfused with physiological saline at a 50-fold volume and kept in a water bath at 37°C for 24 hours. Then, CAS was homogenized in ice at 10,000 rpm for 10 minutes and filtered with a stainless filter of 60 mesh. The resultant filtrate was centrifuged at 4°C at 30,000 G for 60 minutes. The resulting supernatant was lyophilized, desalted by dialysis and condensed to obtain an undiluted CAS extract (580 g protein/ml by Lowry's method).

2. Animals

Jcl-ICR strain mice were purchased from a commercial breeder (Japan Clea. Co.) in

連絡先 : ☎ 222 横浜市港北区師岡町 760 明治製菓(株)中央研究所 森 口 政 英

this experiment. Females weighing about 31 g were mated with adult males of the same strain. Day 0 of pregnancy was determined to be the day of vaginal plug appearance in the dams. The animals were housed at room temperature ($23\pm1^{\circ}\text{C}$) and at a relative humidity of $55\pm5\%$ and given animal chow (Oriental Yeast Co., Ltd.) and tap water ad libitum.

3. Procedures

Twenty and ten fold diluted CAS extracts were subcutaneously injected, respectively into animals from groups A and B by 10 ml/kg once daily for 10 days from days 6 to 15 of pregnancy. General condition, and food and water consumption were recorded at day 0 and from days 6 to 15, and on day 18 of pregnancy. On day 18 of pregnancy, all dams were laparotomized to grossly observe thoracic and visceral organs and tissues. Ovaries and uteruses, corpora lutea and implantations were also observed. Surviving fetuses were examined to determine their sex, body weight and the existence of any gross anomalies. One-third of the fetuses were fixed in Bouin solution and prepared by free hand razor blade section, according to Wilson's method, to observe visceral anomalies (Wilson J. G. et al. 1965), two-thirds were fixed in alcohol and stained with alizarin red S according to Dawson's modified method, to observe skeletal malformations (Dawson A. B. 1926).

RESULTS

1. Effects on dams

Table 1. Laparotomy findings on mouse dams treated with CAS extract during organogenesis

Groups	Control	CAS extract	
	saline	A ($\times 20$ diluted)	B ($\times 10$ diluted)
No. of examined	21	20	21
No. of dead or aborted	0	0	0
Corpora lutea (mean)	280(13.3 ± 1.39)	260(13.0 ± 1.21)	278(13.2 ± 2.19)
Implantations (mean)	268(12.8 ± 1.84)	251(12.6 ± 1.43)	272(13.0 ± 2.09)
Live fetuses (mean)	243(11.6 ± 2.44)	232(11.6 ± 1.54)	248(11.8 ± 3.27)
Males	118	117	131
(sex ratio)	(0.94)	(1.02)	(1.12)
Females	125	115	117
Male fetal body weight	1.3 ± 0.11	1.4 ± 0.09	1.4 ± 0.11
Female fetal body weight	1.3 ± 0.10	1.3 ± 0.10	1.3 ± 0.11
Early resorptions (%)	10(3.7)	6(2.4)	18(6.6)
Later resorption (%)	14(5.2)	9(3.6)	3(1.1)
Dead fetuses (%)	1(0.4)	4(1.6)	3(1.1)
Gross anomalies (%)			
Open eyelids	6(2.5)	5(2.2)	7(2.8)
Cleft palate	1(0.4)	0	0
Exencephaly	0	1(0.4)	0

No dams died nor did they experience spontaneous abortion. No marked changes were observed in the general conditions from commencement of medication to laparotomy. The mean body weight and food and water consumption were almost the same as

Table 2. Visceral and skeletal findings on mouse fetuses from dams treated with CAS extract during organogenesis

Groups	Control saline	CAS extract	
		A ($\times 20$ diluted)	B ($\times 10$ diluted)
Visceral			
No. of examined	76	73	77
Cleft palate (%)	1(1.3)	0	0
Hydronephrosis (%)	2(1.3)	2(2.7)	1(1.3)
Skeletal			
No. of examined	167	159	171
Malformation			
Exencephaly (%)	0	1(0.6)	0
Defect of lumbar verteb. (%)	0	0	1(0.6)
Waved rib (%)	0	0	1(0.6)
Asymmetrric sternebrae (%)	10(6.0)	17(10.7)	10(5.8)
Variation			
Delayed ossification of processus xiphoideus (%)	4(2.4)	2(1.3)	1(1.2)
of cervical vertebrae (%)	16(9.6)	26(16.4)	22(12.9)
of sternebrae (%)	27(16.2)	18(11.3)	14(8.2)
Cervical ribs (%)	2(1.2)	0	9(5.3)
XIV ribs (%)	1(0.6)	1(0.6)	0
Lumbar ribs (%)	19(11.4)	19(11.9)	24(14.0)

for A and B group dams for the control.

2. Laparotomy findings

There were no significant differences in the mean corpora lutea, implantations, surviving fetuses and fetal body weights between A and B groups and the control.

In the control group, gross anomalies were found in six fetuses (2.5%) with open eyelids and one (0.4%) of cleft palate; in A group, 5 (2.2%) had open eyelids and one (0.4%) of exencephalia; and in group B, 7 (2.8%) had open eyelids. In general, no significant intergroup differences were observed.

Such visceral anomalies as hydronephrosis in one fetus (1.3%) were found in the control group, 2 (2.7%) from A group, and one (1.3%) from B group.

Such skeletal malformations as asymmetric sternebrae were found in 10 fetuses (6.0%) from control group, one (0.4%) with an incomplete defect of frontale and parietale bones corresponding, to already mentioned exencephalus; and 17 (10.7%) with asymmetric sternebrae from A group. In group B, each animal had lumbar defect and waved ribs and 10 (5.8%) had asymmetric sternebrae, however, intergroup differences,

Table 3. Ossification of mouse fetuses from dams treated with CAS extract during organogenesis

Groups	Control saline	CAS extract	
		A ($\times 20$ diluted)	B ($\times 10$ diluted)
Sternebrae (mean $\pm$ SD)	5.8 $\pm$ 0.52	5.8 $\pm$ 0.65	5.9 $\pm$ 0.44
Forelimb			
Proximal phalanx I (%)	0	0	0
II (%)	96.1	100.0	98.8
III (%)	96.7	100.0	100.0
IV (%)	95.5	100.0	99.4
V (%)	80.8	92.8	87.4
Middle phalanx I (%)	0	0	0
II (%)	31.1	41.2	27.8
III (%)	59.6	79.3	63.5
IV (%)	42.8	67.3	55.6
V (%)	1.5	1.3	0.9
Hindlimb			
Proximal phalanx I (%)	30.2	47.2	38.9
II (%)	87.4	98.1	95.9
III (%)	93.1	98.7	96.2
IV (%)	85.9	98.7	95.9
V (%)	75.8	95.3	88.9
Middle phalanx I (%)	0	0	0
II (%)	12.0	16.0	14.0
III (%)	16.8	29.9	21.1
IV (%)	10.8	19.8	13.7
V (%)	0	0	0

were insignificant.

Skeletal observations were infrequently found as retarded ossification of processus xiphoidieus, cervical vertebrae, sternebrae, cervical ribs, 14th ribs and lumbar ribs with approximately the same incidences in all groups.

There were no significant differences in ossification of sternebrae and digitus.

DISCUSSION

Generally, it is considered that pregnant mice receiving a test substance during organogenesis from days 6 to 15 of pregnancy would be most suitable for extrapolation of experimental results into possible teratogenesis in human fetuses or newborns. (Nishimura, H. 1977)

In the present study, we subcutaneously injected 10- and 20-fold diluted CAS extracts equivalent to the area covering human body surface by 35 and 70%, respective-

ly, into mouse dams once daily from days 6 to 15 of pregnancy.

As a result, no toxicity was observed in the general conditions and body weights of dams. CAS extracts had no influence on the embryonic development after implantation, in view of the rate of surviving fetuses and fetal body weight.

No fetuses were found to have any particular visceral or skeletal anomalies. Such observed gross anomalies as open eyelid, cleft palate and exencephalus are largely insignificant against our back ground data.

It is, therefore, concluded that CAS extracts would give no influences on mouse dams or fetuses, nor would it cause teratogenesis after subcutaneous injection during organogenesis.

REFERENCES

- DAWSON, A. B. (1926) : Note on the staining of the skeleton of cleared specimens with alizarin red S. *Stain Technology*, **1**, 123-124.
- NISHIMURA, H. (1977) : Summary of comparative embryology and teratology. In : *Handbook of Teratology 3* : J. G. Wilson and F. Clarke Fraser, ed. Plenum press, New York and London, pp. 119-154.
- SHIBATA, U. et al. (1981) : The efficacy and safety of collagen wound dressing (CAS) in animal experiments. *Saishin-Igaku*, **36** (9), 1834.
- TAKEDA, U. et al. (1982) : Laboratory studies on collagen wound dressing, Part-I : Experimental use on skin excisions and burns. *J. Dermatology*, to be published.
- EO ; DPM. J. G. (1965) : Methods for administering agents and detecting malformations in experimental animals. In : *Teratology : Principles and Techniques*. J. G. Wilson and J. Warkany, ed. University of Chicago press.