Thalidomide-Like Malformations Caused by a Tween Surfactant in Mice

Ursula Kocher-Becker, Walter Kocher

Institut für Toxikologie und Embryonalpharmakologie, Freie Universität Berlin, Garystr. 9, D-1000 Berlin 33

Heinrich Ockenfels

Abteilung Physiologische Chemie, Universität Ulm

Z. Naturforsch. **36 c**, 904–906 (1981); received May 8, 1981

Nonionic Surfactant Tween 20, Embryotoxicity, Teratogenicity, Thalidomide-like Malformations, Mouse

The nonionic surfactant Tween 20 was applied intraperitoneally to NMRI and Swiss mice on day 9 of gestation. It caused embryonic death and malformations of the vertebrae, ribs and, in lower frequency, of the limbs.

The nonionic surfactant Tween 20 [polyoxyethylene(20)sorbitan monolaurate] is one of the more frequently encountered xenobiotics. Surface-active agents of the Tween type are used as constituents of cleaning agents, as food additives and as vehicles in the drug and cosmetic industry [1]. Because of its good solubilizing properties Tween 20 has been used in teratological experiments as a solubilizer of poorly soluble substances, *e.g.* thalidomide [2], its hydrolytic metabolites [3] and structurally related compounds [4]. In these investigations, performed with Swiss mice, Tween 20 was found to have no effect on developing embryos when given alone in doses of 10 ml/kg (diluted with physiological saline).

However, observations made some years ago casted doubt on the assumption that Tween 20 can be regarded as an inert vehicle in teratological experiments. In an attempt to apply a thalidomide metabolite to CBA- and C57BL-mice we found that Tween 20, given intraperitoneally in a dose of 2.5 ml/kg (diluted with physiological saline) killed more than 50% of the animals. Similar results were described by Scott *et al.* for another mouse strain [5]. Another nonionic surfactant, Triton W. R. 1339, had proved to be teratogenic in Swiss mice [6]. Further investigations on the effect of several Tween types on developing sea urchins showed serious developmental defects in the embryos [7]. In the following we refer to experiments partly performed in connection with investigations on a hydrolytic thalidomide metabolite. They clearly show that Tween 20 in lower doses has not only teratogenic properties but in addition produces malformations with striking similarities to those produced by thalidomide.

NMRI and Swiss mice were mated for 3 h in the early morning (day 0 of gestation). Pregnant animals received a single injection of Tween 20 (Serva, Heidelberg, batch Nr. 37470) intraperitoneally on day 9 of gestation. The doses applied varied from 1–5 ml/kg of the commercially available concentrate (made up with physiological saline to 10 ml/kg or, in the case of the highest dose, to 20 ml/kg). Pregnancy was terminated on day 16 or 18 of gestation. Fetuses were inspected for external malformations, sacrified and double stained by alcian blue and alizarin red for examination of the skeleton. Statistical analysis was performed by the χ^2-test for malformed fetuses and the number of litters with malformed fetuses (without considering variations from replicated experiments).

5 ml/kg Tween 20, the highest dose applied, was toxic to the pregnant mothers to such an extent that 5 of 6 animals in the NMRI strain and 9 of 11 animals in the Swiss strain died. 3.3 ml/kg Tween 20 caused death of 3 out of 12 NMRI animals and abortion of the litters in a further 3 cases. All doses of Tween 20 shown in Table I caused malformations in NMRI and Swiss fetuses (the 2 cases of exencephaly in the normal controls were atypical for the spectrum of malformations found after Tween treatment). Dose dependency was found for the numbers of malformed fetuses ($p < 0.01$), but not for those of litters with malformed fetuses, in the Swiss strain; the same applies to the results obtained in NMRI mice when the 2.5 ml/kg dose is compared with the two lower doses. The percentage of malformed fetuses was higher in the Swiss than in the NMRI strain ($p < 0.01$ for 2.5 ml/kg, $p < 0.05$ for 1 ml/kg.

The type of malformations was the same in all groups. In most cases wedge-shaped and incomplete vertebrae, fused vertebral arches in the thoracic and lumbar region, and/or rib fusions of varying severity in length of fusion and numbers of ribs involved were found (Fig. 1 a, b). Limb malformations occurred in a sporadic manner and most of them were of the thalidomide type. Among others,

Reprint requests to Dr. U. Kocher-Becker.

0341-0382/81/0900-0904 $ 01.00/0

Table I. Embryotoxicity and teratogenicity following treatment with Tween 20 to pregnant mice.

	NMRI					Swiss		
Tween 20 content	1.0 ml/kg	1.7 ml/kg	2.5 ml/kg	3.3 ml/kg	controls	1.0 ml/kg	2.5 ml/kg	controls
pregnant mothers	7	10	35	12	25	15	21	8
maternal deaths	0	0	2	3	0	1	4	0
litters aborted	0	0	2	3	0	0	1	0
litters with at least one malformed fetus	2	2	17	2	2*	7	9	0
implantations	83	118	361	79	278	192	237	141
fetuses: resorbed	6	11	71	23	18	23	55	8
fetuses: (%)	(7.2)	(9.3)	(19.7)	(29.1)	(6.5)	(12.0)	(23.2)	(5.7)
fetuses: dead	0	0	3	0	1	0	6	3
fetuses: living	77	107	287	56	259	169	176	130
fetuses: malformed	2	4	41	6	2*	20	57	0
fetuses: (%)	(2.6)	(3.7)	(14.3)	(10.7)	(0.8)	(11.8)	(32.4)	
malformations: limbs alone or combined with others	0	0	6	0	0	3	4	0
malformations: vertebrae and ribs without limb involvement	1	4	33	6	0	17	53	0
malformations: others	1	0	2	0	2*	0	0	0

* atypical: exencephaly.

Fig. 1a, b. Skeletons of two 18 day fetuses treated with 2.5 ml/kg Tween 20 on day 9 of gestation. Malformations of thoracic and lumbar vertebrae and fusion of ribs. Magn. a) 9 ×, b) 12 ×.

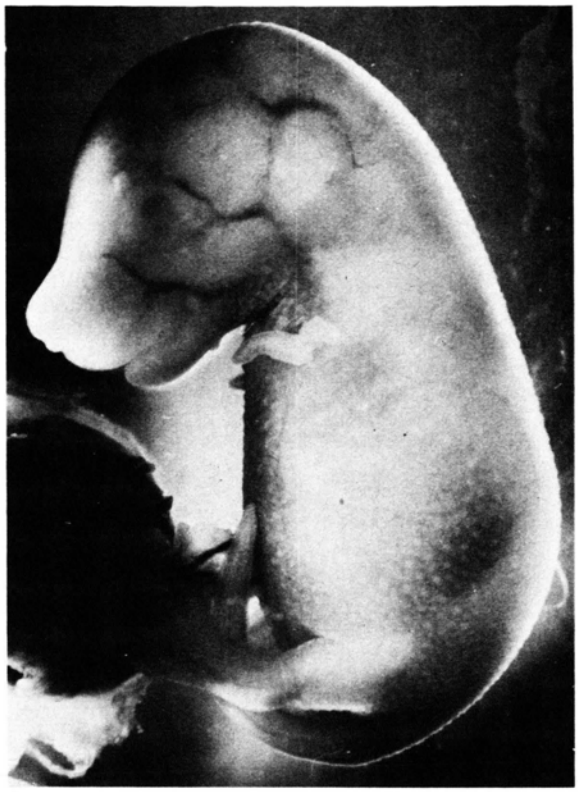

Fig. 2. 16 day fetus after treatment with 2.5 ml/kg Tween 20 on day 9 of gestation: phocomelia of both forelimbs. Magn. 7×.

even classic phocomelia was seen (Fig. 2). Severe limb malformations were not restricted to the higher doses: even with 1 ml/kg a case of phocomelia of the left hind limb was obtained.

The malformations caused by thalidomide are not confined to the limbs. Other organs are affected too. Malformations of the vertebral column were found in about 50% of the thalidomide cases observed in the human [8]. They were similar to and located in the same region of the spine as the anomalies of the vertebrae caused by Tween 20 in the mouse. Our results show that Tween 20 is not merely a solubilizing agent but a teratogen in itself. The similarity of the malformations produced by Tween 20 and thalidomide raises the question of a similar teratological action of both agents.

Acknowledgements

In the publication cited under [3] the responsibility of one of the authors (H. O.) was confined to the synthesis of some of the thalidomide metabolites used in the study. We thank Mrs. M. Sheikzadeh and Mrs. L. Becker for their expert technical assistance and Dr. B. Grover for critical revision of the manuscript. Supported by grants of the Deutsche Forschungsgemeinschaft to the SFB 29 Berlin.

[1] N. Schönfeldt, Grenzflächenaktive Äthylenoxid-Addukte (Wissenschaftliche Verlagsgesellschaft, Stuttgart, 1976).
[2] F. Köhler and H. Koch, Arzneim. Forsch. (Drug Res.) **24,** 1616–1619 (1974).
[3] W. Meise, H. Ockenfels, and F. Köhler, Experientia **29,** 423–424 (1973).
[4] K. Fickentscher, A. Kirfel, G. Will, and F. Köhler, Mol. Pharmacol. **13,** 133–141 (1977).
[5] W. J. Scott, R. Fradkin, and J. G. Wilson, Teratology **16,** 333–335 (1977).
[6] C. Roussel and H. Tuchmann-Duplessis, C. r. hebd. Séanc. Acad. Sci. D **266,** 2171–2174 (1968).
[7] H. Bresch and H. Ockenfels, Naturwissenschaften **64,** 593–594 (1977).
[8] H. G. Willert and H. L. Henkel, Klinik und Pathologie der Dysmelie (Springer, Berlin and New York, 1969).