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The fate of [^{14}C]thalidomide in the pregnant hamster

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The embryotoxicity and fate of [^{14}C]thalidomide in the pregnant European golden hamster have been investigated. Daily administration of thalidomide (1 or 2 g/kg orally) to pregnant hamsters on days 4–12 inclusive of pregnancy was not embryotoxic. [^{14}C]Thalidomide (150 mg/kg) administered on the 204th hr of pregnancy is well absorbed and about 84% of the ^{14}C is excreted in the urine and 9% in the faeces in the 3 days after dosing. The urinary ^{14}C consists of thalidomide (3% of dose), α-(*o*-carboxybenzamido)glutarimide (26%), 2- and 4-phthalimidoglutaramic acids (8%), 2-phthalimidoglutaric acid (0·2%) and 2- and 4-(*o*-carboxybenzamido)-glutaramic acids plus 2-(*o*-carboxybenzamido)glutaric acid (27%). ^{14}C is present in the embryo and the relative concentrations of radioactivity in the embryo and plasma are about the same at 4, 12 and 24 hr after dosing. At 4 hr after dosing the embryo contains mainly thalidomide, but at 12 hr this has largely disappeared and the ^{14}C consists of seven hydrolysis products. The lack of embryotoxicity of thalidomide in the hamster is thus not due to an inability of the teratogen to penetrate to the conceptus.

THALIDOMIDE is teratogenic in man (Lenz, 1961, 1962; McBride, 1961), monkey (Delahunt & Lassen, 1964), rabbit (Felisati, 1962; Giroud, Tuchmann-Duplessis & Mercier-Parot, 1962; Seller, 1962; Somers, 1962; Spencer, 1962), rat (Bignami, Bovet & others, 1962; King & Kendrick, 1962; Bignami, Bovet-Nitti & Rosnati, 1964), mouse (Giroud & others, 1962; Di Paolo, 1963), and chicken (Kemper, 1962; Boylen, Horne & Johnson, 1963; Ehmann, 1963; Yang, Yang & Liang, 1963). However, thalidomide does not appear to be teratogenic in the hamster (Somers, 1963; Fratta, Sigg & Maiorana, 1965) although Homburger, Chaube & others (1965) have reported some embryotoxicity in certain inbred strains of hamsters, but not in randomly bred strains.

Thalidomide is teratogenic in the pregnant New Zealand white rabbit when administered only during the morphogenetic phases of embryonic development (Fabro & Smith, 1966). Furthermore, when thalidomide is administered to the mother it penetrates into the conceptus and the teratogenicity appears to be due to thalidomide itself rather than to one of its metabolites (Fabro, Smith & Williams, 1967). The resistance of the hamster to the teratogenic effects of thalidomide could be due to an inability of the teratogen to penetrate into the embryonic tissues when given to the mother; we have therefore investigated the fate of [^{14}C]-thalidomide when administered orally to pregnant hamsters.

Experimental

MATERIALS AND METHODS

Thalidomide, m.p. 272°, was a gift from the Lilly Research Laboratories Ltd. [*Carbonyl*-$^{14}C_1$]thalidomide (m.p. 270°; specific activity 0·75 μc/mg) was synthesized from [*carbonyl*-$^{14}C_1$]phthalic anhydride according to the method of Beckmann (1962). (±)-α-(*o*-Carboxybenzamido)glutarimide, (±)-2- and 4-phthalimidoglutaramic acids, (±)-2-phthalimidoglutaric

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acid, (±)-2- and 4-(*o*-carboxybenzamido)glutaramic acids and 2-(*o*-carboxybenzamido)glutaric acid were samples previously prepared (Schumacher, Smith & Williams, 1965; Fabro & others, 1967).

ANIMALS

Pregnant European golden hamsters (random breed; 7 years closed colony; 100–150 g) were purchased (A. F. Longmoor, 63, Sherrard Road, Forest Gate, London, E.11). They were mated at the breeding centre at night between 11 p.m.–2 a.m. and were sent to our animal department the following morning. They were kept in individual cages and maintained on Diet No. 41B (E. Dixon & Son, Ware) with water *ad lib.*

For the teratogenic testing, thalidomide was administered orally each day as a suspension (in 0·5% carboxymethylcellulose) at dose levels of 1 or 2 g/kg to groups of pregnant hamsters from the 4th to the 12th day inclusive, of gestation. The controls were given 1 ml 0·5% carboxymethylcellulose orally. On the 15th day the hamsters were killed and the number of implantations, resorptions and viable foetuses, both normal and abnormal, were counted. Viable foetuses were weighed and examined for external malformations and then fixed in 95% ethanol. After one week the foetuses were dissected for internal malformations. The skeletons were stained with alizarin according to the method of Chaube (1965).

[^{14}C]Thalidomide (150 mg/kg; 20 μc/kg) was also administered orally as a single dose to 19 pregnant hamsters on the 204th hr of pregnancy. At this time morphogenesis is occurring and 15–20 pairs of somites are present (Waterman, 1948). One group of three treated animals were housed individually in metabolism cages and their urine and faeces collected. Blood samples (0·4 ml) were withdrawn by heart puncture from a second group (4 animals) at 4, 8, 12 and 24 hr after dosing, and the plasma separated by centrifugation at 2000 rev/min for 10 min. Three groups each of 4 treated animals were killed at 4, 12 and 24 hr respectively after dosing, and blood samples and embryos removed. Embryos free of blood and uterine tissue were isolated by placing the uterus containing the embryos in hexane at −40°. The embryo and trophoblast were then isolated by peeling off the uterine tissue. The embryos were quickly washed in ice-cold saline, dried on blotting paper, weighed and homogenized in groups of three in a mixture of equal parts of methanol and dioxan and the volume adjusted to 10 ml. Portions (2 ml) were transferred to counting vials containing a scintillation fluid consisting of a mixture of dioxan–ethylene glycol–methanol (88:2:10 by vol.) containing naphthalene (6%), 2,5-diphenyloxazole (0·4%), 1,4-bis-(5-phenyloxazolyl)benzene (0·02%) and 5% thixotropic gel powder (Cab-o-sil). ^{14}C in urine, faeces and blood was estimated as previously described (Fabro & others, 1967). ^{14}C was counted in a Packard Tricarb Liquid Scintillation Spectrometer (Model No. 3214) and counting efficiency was measured by the twin-channel ratio method (Bush, 1963) or by internal standards. The nature of the ^{14}C compounds in the urine, plasma and embryo was determined as previously described (Fabro & others, 1967).

Results and discussion

EFFECT OF THALIDOMIDE ON THE HAMSTER PREGNANCY

Table 1 shows that in the European golden hamster the average number of implantation sites, the litter size, the mean body-weight of the

TABLE 1. EFFECT OF THALIDOMIDE IN PREGNANT HAMSTERS. Thalidomide was orally administered on days 4–12 inclusive of pregnancy. Animals were killed on the 15th day of pregnancy. Foetuses were examined for external and internal malformations.

Treatment	No. of animals	Total no. of implantations	Average no. of implantations ± s.d.	Resorptions	Average litter size ± s.d.	Mean foetal body wt (g ± s.d.)	Normal foetuses	Malformed foetuses
Controls* ..	44	370	8·4 ± 3·0	62 (16·7%)	7·0 ± 2·4	1·8 ± 0·4	308	0
Thalidomide (1 g/kg) ..	24	224	9·3 ± 2·7	30 (13%)	8·1 ± 3·2	1·7 ± 0·3	194	0
Thalidomide (2 g/kg) ..	22	188	7·7 ± 3·1	26 (16%)	7·4 ± 2·5	2·0 ± 0·5	162	0

* Controls were given 1 ml 0·5% carboxymethylcellulose.

15-day old foetus, and the incidence of resorptions and malformed foetuses was not affected by oral administration of thalidomide (1 or 2 g/kg) daily on days 4–12 of pregnancy. These findings are similar to those found by Somers (1963) who administered doses of up to 8 g/kg throughout pregnancy without causing a reduction in litter size, malformations of the young or a significant increase in resorptions. Similar results have been found by Fratta & others (1965) and by Homburger & others (1965) in certain random bred strains of hamster.

FATE OF [^{14}C]THALIDOMIDE IN THE PREGNANT HAMSTER

Table 2 shows that the greater part of an oral dose of thalidomide in pregnant hamsters is absorbed and excreted mainly (84%) in the urine

TABLE 2. EXCRETION OF ^{14}C BY PREGNANT HAMSTERS AFTER A SINGLE ORAL DOSE OF [^{14}C]THALIDOMIDE. [^{14}C]Thalidomide (150 mg/kg; 20 μc/kg) was administered orally to pregnant hamsters on the 204th hr of pregnancy.

	% of doses of ^{14}C found in				
	Urine		Faeces		
Hamster no.	0–24 hr	24–72 hr	0–24 hr	24–72 hr	Total
18	78·4	7·4	2·8	5·3	93·9
19	68·5	2·7	2·8	9·0	83·0
20	77·0	10·4	0	7·6	95·0

with only about 9% in the faeces in the 3 days after dosing. The urinary ^{14}C in the 24 hr urine consists of thalidomide (3% of dose), α-(*o*-carboxybenzamido)glutarimide (26%), 2- and 4-phthalimidoglutaramic acids (8%), 2-phthalimidoglutaric acid (0·2%) and 2- and 4-(*o*-carboxybenzamido)glutaramic acids plus 2-(*o*-carboxybenzamido)glutaric acid (27%). These values are the means of three experiments. Fig. 1 shows the ^{14}C plasma levels at various times after an oral dose of [^{14}C]thalidomide.

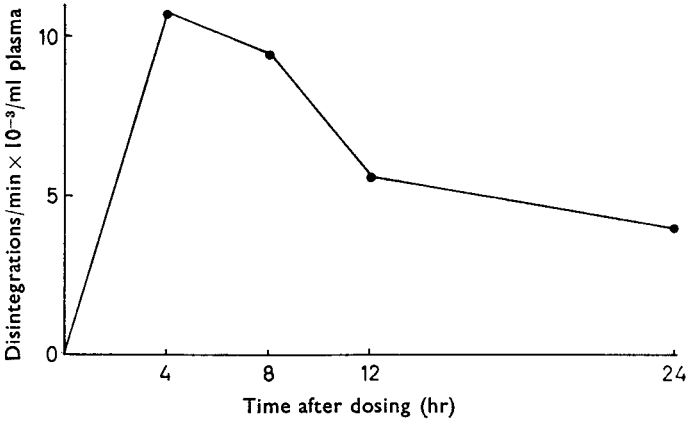

FIG. 1. Plasma ^{14}C levels after a single oral dose of [^{14}C]thalidomide (150 mg/kg) to pregnant hamsters.

It reaches a maximum at about 4 hr after dosing and then steadily declines; at 24 hr the level is about 40% of that at 4 hr. It can be seen from Table 3 that radioactivity passes to the embryo and that the relative concentrations of ^{14}C in the embryo and plasma are about the same at 4, 12 and 24 hr after dosing. At 4 hr after dosing about 50% of the plasma ^{14}C is thalido-

TABLE 3. DISTRIBUTION OF ^{14}C IN THE MATERNAL PLASMA AND EMBRYO AFTER THE ORAL ADMINISTRATION OF [^{14}C]THALIDOMIDE TO PREGNANT HAMSTERS. [^{14}C]Thalidomide (150 mg/kg; 20 μc/kg) was administered orally to groups of pregnant hamsters on the 204th hr of pregnancy and they were killed at the times shown. Values are means; figures in parentheses refer to ranges.

Time after dosing (hr)	No. of animals	Disintegrations/min/g	
		Plasma	Embryo
4	4	10,176 (8,532–14,820)	11,363 (4,992–19,980)
12	4	6,762 (5,956–6,044)	7,741 (6,213–7,992)
24	4	3,190 (2,850–3,624)	2,921 (2,679–3,079)

TABLE 4. CONCENTRATION OF THALIDOMIDE AND ITS METABOLITES IN THE EMBRYO AND MATERNAL PLASMA AFTER THE ADMINISTRATION OF [^{14}C]THALIDOMIDE TO THE PREGNANT HAMSTER. See Table 3 for dose. Values are the means of three experiments.

Compound	Concentration (μg/g) in			
	plasma		embryo	
	4 hr	12 hr	4 hr	12 hr
Thalidomide	17·2	5·7	19·1	4·4
α-(*o*-Carboxybenzamido)glutarimide	14·1	8·3	15·0	7·2
2- and 4-Phthalimidoglutaramic acids	1·3	4·4	0·1	2·5
2-Phthalimidoglutaric acid	<0·1	<0·1	<0·1	<0·1
2- and 4-(*o*-Carboxybenzamido)glutaramic acids + 2-(*o*-carboxybenzamido)glutaric acid	0·8	2·2	0·1	10·8

mide, the rest is largely α-(*o*-carboxybenzamido)glutarimide with small amounts of other hydrolysis products. At 12 hr after dosing the plasma level of thalidomide has declined to about 30% of that at 4 hr, and the rest of the ^{14}C consists of hydrolysis products. Similarly, the embryo contains at 4 hr mainly thalidomide and α-(*o*-carboxybenzamido)-glutarimide but at 12 hr the level of thalidomide has declined to 20% of that at 4 hr and the rest of the ^{14}C consists of seven hydrolysis products (Table 4).

It is thus clear that an oral dose of thalidomide given to a pregnant hamster during the period of morphogenesis is able to penetrate to the conceptus and to persist for more than 12 hr as such. Therefore, the lack of embryotoxicity of thalidomide in the hamster is not due to the inability of the teratogen to reach the embryo.

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