Prevention of Experimentally Induced Cleft Palate in Mice

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Cleft palate was induced by giving cyclophosphamide (CPA) to pregnant mice on different days of pregnancy. Application of Solcoseryl®, an oxygen stimulating, protein-free extract of the blood of calves, modified the teratogenic and embryolethal effect of CPA and significantly decreased the frequency of cleft palate (from over 70% to about 20% in some groups). This study supports the clinical findings of a reduction in the incidence of facial clefting in man following application of Solcoseryl® and vitamins (Gabka, 1975).

KEY WORDS: Prevention, animal experiments

Cleft lip and palate is considered to be of multifactorial origin (Fraser, 1971; Burdi, 1977; and many others). Genetic factors alone account for about 20% of all cleft cases (Pfeifer and Schuchardt, 1980), while the percentage of cleft palate with purely genetic etiology is much lower (Spriesterbach et al., 1973). If different causative factors are necessary to bring about a malformation, then it may be possible that one of these factors could be influenced in some way, thus avoiding the malformation (Warkany, 1972; Poswillo, 1980). The assessment of recurrence risk (Rudd, 1977), protection of mothers against environmental damage (Smithells et al., 1980; Poswillo, 1980) and diagnosis in utero are all practical preventive or interceptive methods (Nevin, 1976).

In Europe, Gabka began with prophylactic measures in families with clefts in their history, using vitamins and an oxygen stimulating agent. The latter Solcoseryl®, Actihaemyl®) is a protein free extract from the blood of calves which stimulates oxidative metabolism and improves oxygen utilization under hypoxic conditions (Gabka, 1975, 1978; Gabka and Jorgensen, 1971, 1973). Another German team in Hamburg used vitamin B_1 for prophylactic treatment (v. Kreybig and Schmitz, 1978). These teams have successfully treated more than 400 pregnancies in cleft families (Pfeifer, 1980).

Studies with polyvitamins have been carried out in both laboratory animals and man (Conway, 1958; Peer et al., 1958, 1964; Strean and Peer, 1956; Wollam et al., 1957; Briggs, 1976). Recently Smithells et al. (1980) have apparently prevented neural tube defects by periconceptional polyvitamin supplementation. Clinical studies, however, are based on empiric data: the real individual risk of recurrence is not known. Cleft prevention in animal experiments offers better statistical control (Schubert, 1972, 1973, 1977). There have been investigations of cleft prevention using specific antidotes to various teratogens (Chamberlain, 1967; Chamberlain and Goldyne, 1970; Chaube and Murphy, 1973; Ferm and Carpenter, 1967; Miller, 1973; Posner et al., 1967; Schubert, 1973; Woollam and Miller, 1958). Schubert used the oxygen stimulating drug Solcoseryl® and in later experiments combined this with vitamin administration (Schubert, 1980).

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The pellets contain: 27% maize, 3% dextrine, 8% soybean meal, 8% fish-flour, 4% fead yeast, 2% skim-milk powder, 3% green meal, 5% barley, 35% wheat, 2% premix of active principles, 3% mineral substances.

TABLE 1. Embryotoxic and teratogenic effects of 20 mg/kg body weight cyclophosphamide and the influence of Solcoseryl® in various dosage

Group	*Day of Pregnancy*	*Treatment*	*No. of Litters*	*Living Fetuses*	*Mean Litter Size*	*Resorptions and Dead Fetuses*	*Fetuses with CP*		*Mean Body Weight (m ± S) in mg*
							n	*%*	
1		CPA alone	14	83	5.9	45	75	90.4	499 ± 93
2	10.5	+ 0.3 ml Solcoseryl/mouse	6	62	10.3	1	44	71.0+++)	607 ± 24
3		CPA alone	23	183	8.0	44	119	65.0	641 ± 124
4		+ 0.1 ml Solcoseryl/mouse	15	103	6.9	27	60	58.3++)	726 ± 135
5	11.5	+ 0.1 ml Solcoseryl/mouse from 11.5 to 13.5 day	23	196	8.5	14	42	21.4+++)	794 ± 92
6		+ 0.3 ml Solcoseryl/mouse	19	169	8.9	26	71	42.0+++)	737 ± 112
7		+ 0.3 ml Solcoseryl/mouse from 11.5 to 13.5 day	21	163	7.8	46	35	21.5+++)	914 ± 165
8		CPA alone	21	203	9.7	12	71	35.0	772 ± 76
9	12.5	+ 0.3 ml Solcoseryl/mouse	18	165	9.2	11	46	27.8++)	821 ± 42
10		+ 0.3 ml Solcoseryl/mouse from 11.5 to 13.5 day	19	156	8.2	25	22	14.1+++)	783 ± 115
11		control +)	89	1011	9.3	100	0	0	1040

+) data from Dr. sc. Schmidt, Institute of Biology of the Martin-Luther-University Halle/S. (GDR)
++) no significance
+++) $p < 0,01$

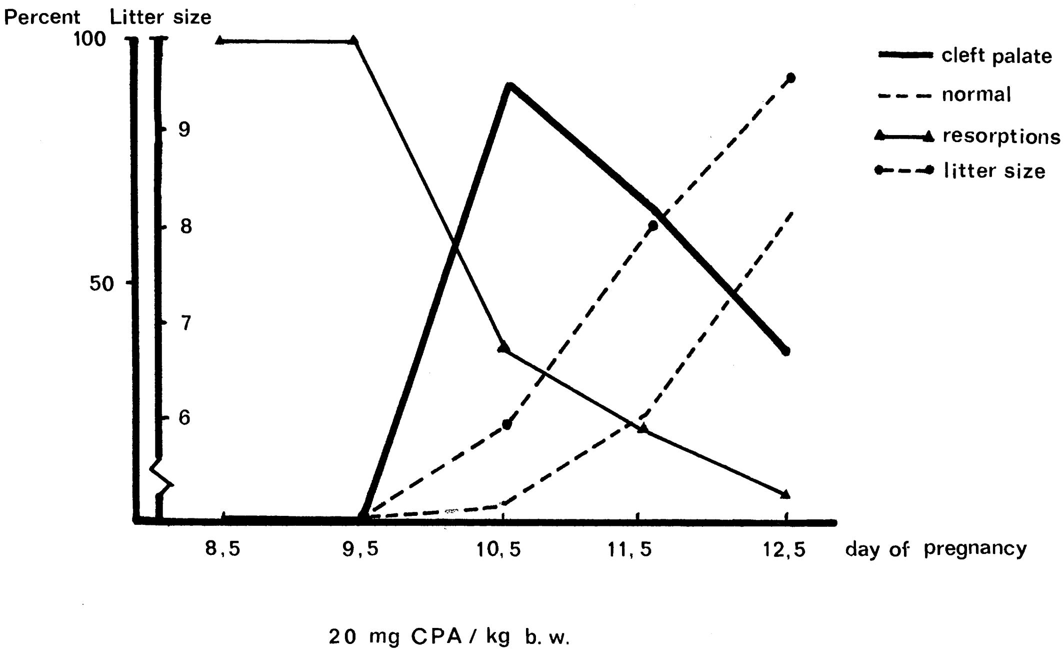

FIGURE 1. Frequency of the teratogenic and embryotoxic effects of 20 mg/kg body weight CPA i.p. in AB/Jena-Halle II mice on different days of pregnancy.

Methods

The AB/Jena-Halle II strain of mice were used in this study. If, in female mice (nullipara) of 25–30 g body weight, a vaginal plug was found at 7 a.m., that day was called day 0 of pregnancy. The females had free access to food (Pellets from VEB Versuchsproduktion derDDR Schönwalde, GDR)* and water. Five animals were kept in each box. Natural light cycles were used, and the investigations were performed in the winter. The temperature of the room was 22 ± 2°C and approximately 70% humidity.

At 1 p.m. on different days of pregnancy groups of randomized female mice (body weight 32 ± 4 g) received injections of the following drugs intraperitoneally: 20 mg/kg body weight cyclophosphamide (VEB Jenapharm, Ankerwerk Rudolstadt, GDR) and various doses of Solcoseryl® (Solco-AG, Basel, Switzerland). The mice were sacrificed at the end of the 18th day of pregnancy and the fetuses removed from the uterus and investigated for macroscopic malformations.

Results

The optimal stage for the induction of cleft palate by cyclophosphamide (CPA) is indicated in Table 1. The embryotoxic and teratogenic effects of cyclophosphamide are illustrated in Figure 1.

The preventive medicaments indicated in Table 1 produced the results illustrated in Figure 2.

Discussion

The embryotoxic and teratogenic effects of CPA in this study were similar to those previously described by Schubert (1977).

The most effective day of administration for production of cleft palate was 10.5, but we chose 11.5, because there was a much greater litter size with a lower embryolethal effect. Both the good clinical results of Gabka and the previous findings of Schubert (1972, 1973, 1977) and Metah et al. (1976) were supported by this experimental animal study. We do not know the mechanism of action of this preventive measure, but we postulate that Solcoseryl® administration could cause cells damaged by CPA to repair or regenerate more rapidly. The toxic effects of CPA reach their maximum after 24 hours (v. Kreybig, 1969), after which cellular repair begins. This is in keeping with the better results obtained after repeated treatment with Solcoseryl®. Moreover, the effect of a single application of Sol-

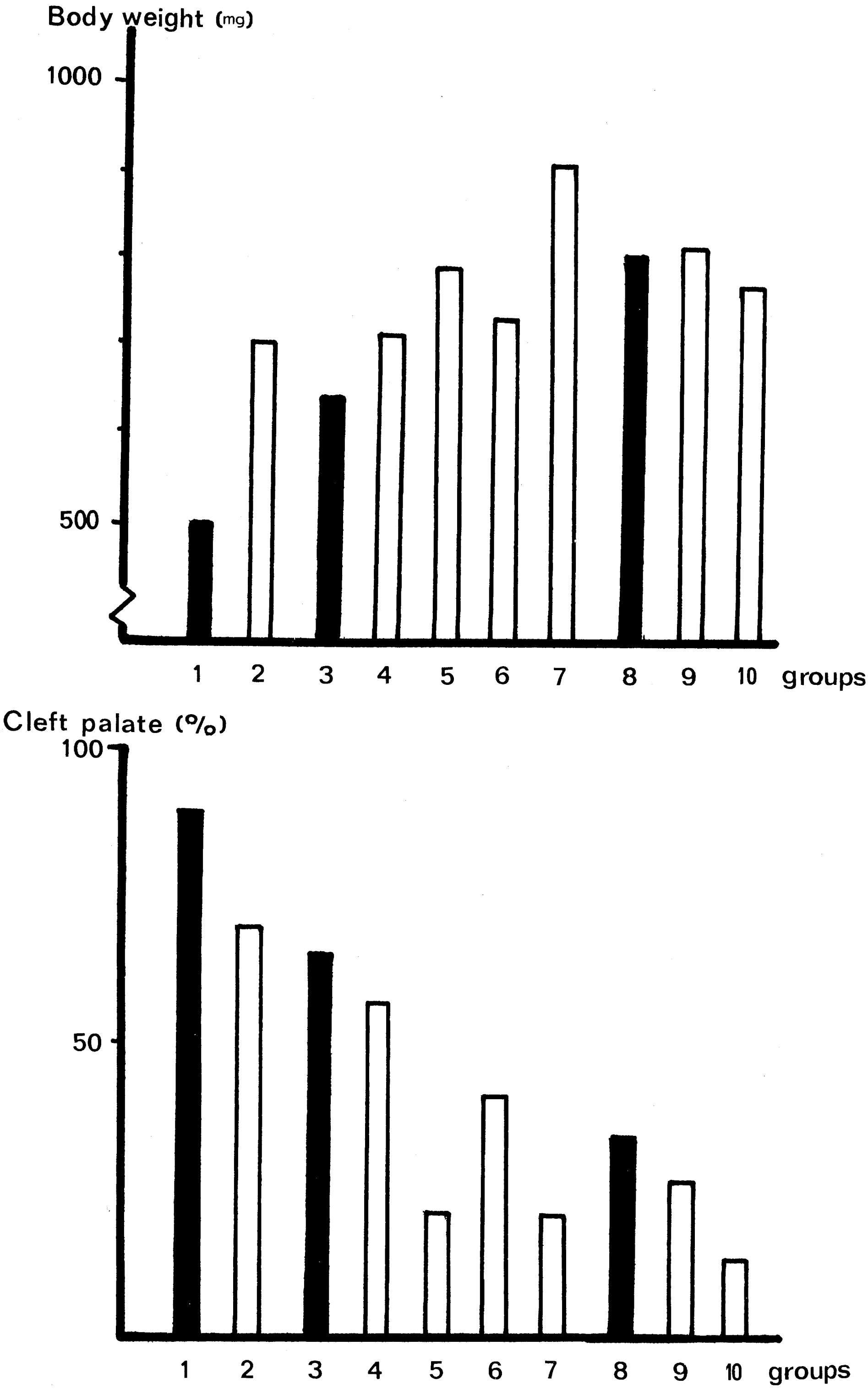

FIGURE 2. Effect of various preventive treatment on cyclophosphamide—induced cleft palate in AB/Jena-Halle II mice on the 10.5 (groups 1–2), 11.5 (groups 3–7) and 12.5 (groups 8–10) days of pregnancy (black—not prophylactically treated).

coseryl® seems to be dose-dependent whilst repeated applications are dose independent (groups 5 and 7).

However the above hypothesis does not explain either the better preventive effects seen when Solcoseryl® is administered some days before the application of CPA or the absence of any effect when it is administered for 3 days beginning one day after CPA administration (Schubert, 1980). Interestingly, Smithells et al. (1980) observed a similar decrease in the incidence of human neural tube defects following polyvitamin supplementation for some months before and during pregnancy.

The preventive treatment did not selectively kill the malformed fetuses. The percentage of resorptions and dead fetuses showed no significant increase with the exception of group 10. In the other groups, there was either a significant decrease of embryotoxicity (groups 2 and 5) or no significant difference from the control group. Before comparing the present results with those of other investigators, it is important to take into account possible strain differences, environmental conditions and chronobiological influences (v. Mayersbach, 1977).

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