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

In Europe, Gabka began with prophylactic measures in families with clefts in their history, using vitamins and an oxygen stimulating agent. The latter Solcoseryl®, Actihae-

Correspondenceshouldbe directed to Dr. Dr. Johannes Schubert, Klinik und Poliklinik für Stomatologie, der Martin-Luther-Universität Halle-Wittenberg Abteilung für Chirurgische Stomatologie und Kiefer-Gesichtschirurgie, DDR-4020 Halle (S.), Große Steinstraße 19, Wittenberg, West Germany.

The pellets contain: 27% maize, 3% dextrine, 8% soybean meal, 8% fish-flour, 4% feed 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

<table>
<thead>
<tr>
<th>Group</th>
<th>Day of Pregnancy</th>
<th>Treatment</th>
<th>No. of Litters</th>
<th>Living Fetuses</th>
<th>Mean Litter Size</th>
<th>Resorptions and Dead Fetuses</th>
<th>Fetuses with CP</th>
<th>Mean Body Weight (m ± S) in mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>CPA alone</td>
<td>14</td>
<td>83</td>
<td>5.9</td>
<td>45</td>
<td>75</td>
<td>90.4</td>
</tr>
<tr>
<td>2</td>
<td>10.5</td>
<td>+ 0.3 ml Solcoseryl/mouse</td>
<td>6</td>
<td>62</td>
<td>10.3</td>
<td>1</td>
<td>44</td>
<td>71.0**</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>CPA alone</td>
<td>23</td>
<td>183</td>
<td>8.0</td>
<td>44</td>
<td>119</td>
<td>65.0</td>
</tr>
<tr>
<td>4</td>
<td>11.5</td>
<td>+ 0.1 ml Solcoseryl/mouse</td>
<td>15</td>
<td>103</td>
<td>6.9</td>
<td>27</td>
<td>60</td>
<td>58.0**</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>+ 0.3 ml Solcoseryl/mouse</td>
<td>23</td>
<td>196</td>
<td>8.5</td>
<td>14</td>
<td>42</td>
<td>21.4***</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>+ 0.3 ml Solcoseryl/mouse</td>
<td>19</td>
<td>169</td>
<td>8.9</td>
<td>26</td>
<td>71</td>
<td>42.0***</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>+ 0.3 ml Solcoseryl/mouse</td>
<td>21</td>
<td>163</td>
<td>7.8</td>
<td>46</td>
<td>35</td>
<td>21.5***</td>
</tr>
<tr>
<td>8</td>
<td>12.5</td>
<td>CPA alone</td>
<td>21</td>
<td>203</td>
<td>9.7</td>
<td>12</td>
<td>71</td>
<td>35.0</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>+ 0.3 ml Solcoseryl/mouse</td>
<td>18</td>
<td>165</td>
<td>9.2</td>
<td>11</td>
<td>46</td>
<td>27.8**</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>+ 0.3 ml Solcoseryl/mouse</td>
<td>19</td>
<td>156</td>
<td>8.2</td>
<td>25</td>
<td>22</td>
<td>14.1***</td>
</tr>
<tr>
<td></td>
<td>control +)</td>
<td></td>
<td>89</td>
<td>1011</td>
<td>9.3</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

+) data from Dr. sc. Schmidt, Institute of Biology of the Martin-Luther-University Halle/S. (GDR)
+++ no significance
+++ p < 0.01
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 Versuchproduktion der DDR 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 Jena-pharm, 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 polivitamin 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).

Acknowledgment: I would like to thank Prof. Dr. H. A. Freye, Institute of Biology of the Martin-Luther-Universität Halle-Wittenberg, DDR for donating and keeping the mice in his institute.

References


CHAMBERLAIN, J.G. and GOLDYNE, M.E., Intraamniotic injection of pyridine nucleotides or adenosine triphosphate as countertherapy for 6-aminonicotinamide (6-AN) teratogenesis, Teratology, 3: 11–16, 1970.


KREYBIG, Th., The critical sensitivity of the developmental phase and the organotropic action of different teratogenic agents; receptors of morphogenesis in the mammalian embryo, In: Teratology, Excerpta Medica Foundation, Amsterdam, 152–159, 1969.


PEEFER, G., Personal communication, 1080.


