Teratogenic Effects of 5-Chlorodeoxyuridine on the Rat Fetus; Protection by Physiological Pyrimidines*

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SUMMARY

Pregnant Wistar rats received single intraperitoneal injections of 5-chlorodeoxyuridine (C1UdR) on the 12th day of gestation. They were sacrificed on the 21st day and the fetuses examined for gross malformations. For study of skeletal malformations, specimens were fixed in 95 per cent ethanol, cleared and stained in alizarin red, and examined in 100 per cent glycerine. Single doses of 125, 250, 500, and 1000 mg/kg of C1UdR, which did not cause an excessive number of fetal resorptions, produced malformations such as clubbed appendages, poly- and ectrodactylous fore and rear paws, encephaly, cleft palate, and retarded kinky tail in 27, 78, 91, and 100 per cent of the survivors at each of these doses, respectively.

In experiments in which pregnant rats received 500 mg/kg of C1UdR and varying amounts of TdR simultaneously, doses of TdR below 125 mg/kg did not protect against the teratogenic action of C1UdR; but at 250 mg/kg or more of TdR complete protection occurred. In other experiments equal amounts (500 mg/kg) of the two compounds were given separately at time intervals ranging from 15 to 240 minutes. In the group of animals which received the C1UdR after TdR, complete protection occurred up to 30 minutes and partial protection thereafter. Resulting abnormalities were confined exclusively to the rear feet. When C1UdR was given prior to TdR, only 50 per cent of the surviving embryos were normal at 15 minutes (partial protection) and none at 90 minutes.

It is adduced that decline in protective activity of TdR is presumably proportional to the rate of incorporation of C1UdR into the replicating DNA in place of TdR and its rapid degradation in embryonic tissue.

Recent investigations in a variety of experimental systems indicate that the type of halogen, fluorine, bromine, iodine, or chlorine, substituted at the 5-position of uracil deoxyriboside greatly influences the activity and the type of inhibition of cell proliferation. 5-Fluorodeoxyuridine (FUdR) inhibits deoxyribonucleic acid (DNA) synthesis by blocking the methylation of deoxyuridylic acid to form thymidylic acid (10, 19, 20), whereas bromodeoxyuridine (BUdR), iododeoxyuridine (IUdR), and chlorodeoxyuridine (C1UdR) inhibit the incorporation of thymidine (TdR) into DNA (12, 15, 16, 20, 38, 39, 42). In addition, 5-bromouracil (BU), iodouracil (IU), and chlorouracil (CIU) substitute for the thymine of DNA (14, 19, 35, 43, 44), whereas 5-fluorouracil (FU) and chlorouracil (CIU) are incorporated in place of uracil into bacterial ribonucleic acid (RNA) (9, 13, 19, 21, 44). The use of radioactive forms of the abnormal pyrimidines on mammalian cell lines in tissue culture (15, 18, 31) in vitro on E. coli (2, 4, 10) in mouse leukemia (34) and in rats and mice in vivo (32) indicate that BUdR, IUdR, and CIUdR may be incorporated in place of TdR in DNA replication; hence, their inhibitory effects may be partially or completely reversed in the presence of exogenous TdR in these systems (2, 4, 10, 15, 18, 31, 34, 40).

The 5-fluoro analogs of uracil, xeric acid, the iodo-, bromo-, derivatives of deoxyuridine (IUdR, BUdR), and 5-fluorodeoxycytidine were shown to be teratogenic in rat, chick (11, 28), and mouse embryos.1 Thymidine will prevent the toxicity of FUdR in several in vitro and embryonic systems (19, 27, 29, 34, 40). In intact mice, however, TdR enhanced the toxicity of FUdR (7) whereas timed administration slightly decreased its teratogenic effect on the mouse embryo.2 This may be explained by

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TdR's inhibiting the degradation of FUdR in vivo and thus prolonging its action. In this study the teratogenic activity of CIUdR in the developing rat fetus is demonstrated, and TdR is shown to protect the 12th-day fetus against fetal malformations produced by 500 mg/kg of CIUdR.

MATERIALS AND METHODS

One hundred and thirty-eight female rats of the CF Wistar strain, weighing from 170 to 250 gm., were mated during estrus by exposure to males of the same strain. Pregnant females were caged separately and fed the standard laboratory chow pellet with water ad libitum. On the 12th day of gestation the rats were given intraperitoneal injections of varying single doses (on a mg/kg of body weight basis) of CIUdR or TdR alone or in combination. When the two compounds were combined, 500 mg/kg of CIUdR was injected simultaneously with doses of TdR ranging in amounts from 7 to 2000 mg/kg. Timed interaction of the two compounds was also investigated. In these experiments 500 mg/kg of CIUdR was injected at times varying from 0 to 240 minutes before or after a single dose of 250 or 500 mg/kg of TdR. The animals were sacrificed on the 21st day of gestation; surviving fetuses were removed from the uteri, weighed and examined for malformations, and approximately half the number from each litter were fixed in 95 per cent ethanol, cleared, and subsequently stained in alizarin red. Stained specimens were examined in 100 per cent glycerine for the presence of skeletal anomalies. The number of dead and resorbed fetuses in each litter was also recorded. CIUdR and TdR were dissolved in distilled water and used within 1 hour after preparation.

RESULTS

The per cent of fetal mortality observed and the per cent of abnormal survivors that were obtained at different doses of CIUdR are shown in Chart 1. Fetal mortality for all the doses of CIUdR shown in the graph (62—1000 mg/kg) were within the normal (n) control range of 0—10 per cent. A dose of 62 mg/kg did not produce any malformations, but an increasingly higher percentage of abnormal survivors was recovered at 21 days from litters whose mothers were treated with 125—1000 mg/kg on the 12th day. The types of abnormalities frequently seen at these doses are listed in Table 1; there is an increasing incidence of specific abnormalities and a more extensive involvement of different parts of the body with higher doses of the drug. At 125 and 250 mg/kg, abnormalities were predominantly of the rear appendages, whereas at 500 and 700 mg/kg the entire embryo was deformed. Representative embryos from litters treated with 500 and 1000 mg/kg of CIUdR are shown in Figures 1 and 2 and associated skeletal deformities in Figures 3 and 4.

By comparison, 250 mg/kg of CIUdR produced no teratogenic effect on the 11th, and 500 mg/kg gave only 35 per cent abnormal survivors, which showed only minor tail defects and polydactyly of the rear paws. At 1000 mg/kg, 95 per cent of the fetuses at 21 days were abnormal, with additional cleft palate and lip.

While selecting doses for the study, it was noted that 40-gm. rats showed no ill effects from 2000 mg/kg and pregnant females tolerated 1000 mg/kg but fetuses were very small. Thymidine alone, at single doses of 500, 1000, or 2000 mg/kg, injected into the 12th-day pregnant rat did not cause fetal mortality or malformations.

CIUdR and TdR were given in various time sequences. The results of experiments in which 500 mg/kg of CIUdR was injected simultaneously with varying amounts of TdR on the 12th day of gestation are summarized in Table 2. From these data it is evident that a minimal dose of 250 mg/kg of TdR is required to provide complete protection of the fetus against 500 mg/kg of CIUdR, but lower doses (7—125 mg/kg) also appear to be partially effective in reducing the number of fetuses with all abnormalities listed in Table 2 except that of the rear paw. This is particularly evident when 7 mg/kg of thymidine was given. Fetal mortality in this experimental series was within the 0—10 per cent normal range.

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**CHART 1.—Lethal and teratogenic effects produced by single intraperitoneal injections of CIUdR on the 12th day pregnant rat, sacrificed on the 21st day.**

**TABLE 1**

<table>
<thead>
<tr>
<th>Fetal Malformations Observed on 21st Day of Gestation</th>
<th>CIUdR injected (mg/kg):</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per cent with specific abnormalities</td>
<td>62</td>
</tr>
<tr>
<td>-----------------------------------------------------</td>
<td>---</td>
</tr>
<tr>
<td>Poly- and ectrodactyly rear paw</td>
<td>0</td>
</tr>
<tr>
<td>Retarded, rear leg and clubbed fore leg</td>
<td>0</td>
</tr>
<tr>
<td>Retarded, kinky tail</td>
<td>0</td>
</tr>
<tr>
<td>Ectrodactyly fore paw</td>
<td>0</td>
</tr>
<tr>
<td>Cleft palate</td>
<td>0</td>
</tr>
<tr>
<td>Encephaly</td>
<td>0</td>
</tr>
</tbody>
</table>
TABLE 2

PROTECTIVE EFFECTS OF VARYING AMOUNTS OF TdR AGAINST 500 MG/KG OF CIUdR WHEN INJECTED SIMULTANEOUSLY INTO 12-DAY PREGNANT RAT

<table>
<thead>
<tr>
<th>TdR (mg/kg)</th>
<th>Total survivors:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per cent abnormal:</td>
</tr>
<tr>
<td>0</td>
<td>80 68 100 100 100</td>
</tr>
<tr>
<td>7</td>
<td>64 91 17 31 30 35</td>
</tr>
<tr>
<td>15</td>
<td>31 30 16 15 17 30</td>
</tr>
<tr>
<td>31</td>
<td>62 35 82 92 35 15</td>
</tr>
<tr>
<td>62</td>
<td>38 0 0 0 0 0</td>
</tr>
<tr>
<td>125</td>
<td>38 0 0 0 0 0</td>
</tr>
<tr>
<td>250</td>
<td>35 0 0 0 0 0</td>
</tr>
<tr>
<td>500</td>
<td>33 0 0 0 0 0</td>
</tr>
<tr>
<td>700</td>
<td>31 0 0 0 0 0</td>
</tr>
<tr>
<td>1000</td>
<td>30 0 0 0 0 0</td>
</tr>
<tr>
<td>2000</td>
<td>30 0 0 0 0 0</td>
</tr>
</tbody>
</table>

The mortality was between 0 and 10 per cent in all groups (control value).

CHART 2.—Fetal effects produced by timed interaction of a single dose of 500 mg/kg of CIUdR and 250 or 500 mg/kg of TdR in the 12th-day pregnant rat. Sacrificed on the 21st day.

Further evidence in support of the protective role of TdR was obtained from a series of experiments in which a single dose of 250 or 500 mg/kg of TdR was injected into rats prior to or after an injection of 500 mg/kg of CIUdR at time intervals of 15, 30, 60, 90, 150, 210, or 240 minutes. The results are represented graphically in Chart 2. A dose of 500 mg/kg of TdR, given prior to CIUdR, provided a complete protection against the teratogenic effect of the latter up to 30 minutes and only partial protection (50 per cent at 90 minutes and 25 per cent at 240 minutes) thereafter; with 250 mg/kg of TdR 64 per cent of the survivors were normal at 15 minutes and none at 90 minutes. When 500 mg/kg of TdR was given up to 30 minutes after CIUdR, only 50 per cent of the survivors were normal and none at 90 minutes. Abnormalities in all partially protected fetuses were confined exclusively to the rear appendages (Table 3).

DISCUSSION

The data presented demonstrate that the rat fetus can be severely injured by brief periods of exposure to the action of CIUdR. The proportion of abnormal embryos obtained with single injections of CIUdR given to pregnant rats on the 12th day varied with the dose of the drug, and the widest range of fetal abnormalities occurred with 500 and 1000 mg/kg. With 500 mg/kg consistent abnormalities of the appendages and tail occurred in over 90 per cent of the survivors at 21 days, whereas with 62.5 mg/kg all treated litters were normal. A dose of 1000 mg/kg of TdR did not produce any fetal malformations.

The protective role of exogenous TdR against halogenated pyrimidines is well established in several systems (2, 4, 6, 10, 12, 18, 31, 34, 40). Our experiments show that 250 mg/kg of TdR given at zero time protects the 12th-day fetus against the teratogenic effects of 500 mg/kg of CIUdR, thus indicating that this amount of TdR counteracts at least 400 mg/kg of CIUdR. This effectiveness of TdR to protect the embryo against CIUdR, however, declines gradually with increasing time intervals between administration of the two compounds. Thus at 15 minutes 500 mg/kg of TdR (in experiments in which TdR is given after CIUdR) gives only partial protection (50 per cent abnormal survivors), which is equivalent to the effect seen with 125 mg/kg of CIUdR alone; this suggests that about 25 per cent of CIUdR effect has already been completed. In the same experimental series 500 mg/kg of TdR at 60 minutes after CIUdR gives about 95 per cent abnormal fetuses, and this is equivalent to the effect seen between 250 and 500 mg/kg (78–92 per cent abnormal fetuses) of CIUdR alone. This implies that the teratogenic effect of CIUdR is almost completed by
60 minutes and, once completed, is irreversible. The rate of decline of protective activity of TdR is thus presumably proportional to the rate at which significant amounts of CIUdR are incorporated into the replicating DNA of the embryonic cells in place of TdR. A further suggestion of the rate of catabolism of CIUdR may be adduced from the data on the decreasing protective effect with time following CIUdR. Despite the fact that CIUdR is available for only a short time, its damaging effect on the fetus is irreversible. When TdR is given 60 or more minutes before CIUdR there is a rapid loss of protective activity against CIUdR. This observation is consistent with a report of the availability of exogenous thymidine. C14-labeled thymidine was cleared rapidly from blood of rats; only 2 per cent of an intravenous dose remained at 1 hour (32). TdR did not potentiate the effect of CIUdR, whereas this effect occurs with FUdR (7); this supports the impression that FUdR and CIUdR act by different mechanisms.

Tracer experiments have demonstrated that DNA is biochemically inactive in tissues where little or no cell division is occurring. However, in proliferating tissues, such as tumors (1) and regenerating liver (22–24, 26, 36), labeled precursors are rapidly incorporated into the DNA. In vitro dividing cells utilize nucleosides added to the medium for nucleic acid synthesis (41). It has been suggested that thymidine kinase may be playing an important role in controlling DNA synthesis in regenerating liver, as well as in tissues of fetal and neonatal rats, by controlling the synthesis of dTTP (3, 8, 25, 33, 37). Furthermore, stimulation of mitotic activity (17) and elevation of thymidine kinase specificity (25) were observed in normal rat tissues following TdR injection. The rat embryo as a rapidly proliferating tissue appears to be more sensitive to CIUdR than the pregnant rat, presumably by its relative requirements for TdR and kinase. Reports concerning cellular inhibition produced by anti-cancer drugs suggest that cells in general fail to distinguish between the natural metabolite and certain analogs. CIUdR acted as an effective antimetabolite in the embryonic system under study and was presumably incorporated into the DNA of the cells in place of TdR and also acted to inhibit pyrimidine utilization. Embryonic damage is probably due to alteration of DNA by CIUdR incorporation, since a delay in TdR incorporation into DNA would not be expected to result in such severe injury, irreversible within 60 minutes by TdR.

### Table 3

**Protective Effects of TdR When Given at Various Times Before and After 500 mg/kg of CIUdR to the 12-Day Pregnant Rat**

<table>
<thead>
<tr>
<th>MINUTES PRIOR</th>
<th>MINUTES AFTER</th>
</tr>
</thead>
<tbody>
<tr>
<td>90-240</td>
<td>15-30 60-90</td>
</tr>
<tr>
<td>CIUdR (500 mg/kg)</td>
<td>TdR (500 mg/kg)</td>
</tr>
<tr>
<td>Per cent abnormal survivors</td>
<td>90-240</td>
</tr>
<tr>
<td>Per cent appendicular malformations: Poly- and ectodactyly (rear paw) Retarded and/or clubbed: Rear leg Fore leg</td>
<td>100</td>
</tr>
<tr>
<td>TdR (250 mg/kg): Per cent abnormal survivors</td>
<td>100</td>
</tr>
<tr>
<td>Per cent appendicular malformations: Poly- and ectodactyly (rear paw) Retarded and clubbed (rear leg)</td>
<td>100</td>
</tr>
</tbody>
</table>

**References**


22. ———. Nucleic Acid Metabolism in Regenerating Rat Liver. V. Comparison of Results in Vivo and in Tissue Slices. Ibid., 16:180–92, 1956.


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