The Distribution and Fate of Iododeoxycytidine in the Mouse and Rat*

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SUMMARY

The distribution and fate of iododeoxycytidine (ICdR) and iododeoxyuridine (IUdR) have been compared. ICdR was more slowly degraded in the rat than IUdR. IUdR and iodouracil, but not iodocytosine, were identified as degradation products. Following the administration of ICdR labeled with I131 or I125 to rats or mice, there was incorporation of the radioactive label in the DNA extracted from various organs. In the rat, concentration of radioactivity in the DNA of each organ was appreciably higher at 24 hours than at 1 hour after injection and was increased at the latter interval by pretreatment with 5-fluorodeoxyuridine.

Incorporation of ICdR into DNA of the rat was quantitatively less than that previously reported for bromodeoxycytidine (BCdR) and, except in the testis, less than that of IUdR. In the mouse the amounts of ICdR and IUdR incorporated into DNA were similar.

The incorporation of halogenated analogs of thymidine, 5-bromodeoxyuridine (BUdR) and 5-iododeoxyuridine (IUdR), into the DNA of mammalian cells in vitro is associated with an increased susceptibility of the cells to the lethal effects of x-radiation (6). However, owing to the extensive and rapid degradation of BUdR or IUdR when administered in vitro, only a minor part of the compound is available for incorporation into the cells of various tissues (7, 11, 13, 14, 17, 18, 21). Extensive degradation of these agents, therefore, presents a major obstacle in using them to enhance radiosensitivity in vitro. Bromodeoxycytidine (BCdR) is much more slowly degraded than BUdR in the rat (13) and is incorporated into DNA, presumably after an obligatory enzymatic deamination to BUdR-monophosphate (4). The pattern of incorporation of BCdR in the DNA of various tissues differed from that of BUdR and thymidine (18). It seemed important to determine whether those mechanisms responsible for differences between the catabolism of BCdR and BUdR would produce comparable differences between iododeoxycytidine (ICdR) and IUdR. The present study was, therefore, undertaken to compare the rates of degradation of ICdR and IUdR and the extent of their incorporation into the DNA of normal and neoplastic tissue in the rodent.

MATERIALS AND METHODS

**Laboratory animals.**—Male Long-Evans rats, weighing approximately 200 gm., and C57BL/Ka male mice, average weight of 25 gm., were used. Animals were fed Purina chow and allowed water ad libitum. Tumors in the mice were rapidly growing sarcomas first induced in July, 1959, by the subcutaneous injection of methylcholanthrene. Since its induction, the tumor has been carried in this laboratory by transplantation into males of successive generations.

**Labeled compounds.**—ICdR-I131 and ICdR-I125 were prepared by a modification of the method of synthesis of ICdR-H3 of Chang and Welch (2). The modification involved the substitution of stable deoxycytidine for deoxycytidine-H3 and the introduction of 20 mc. I131 or 10 mc. of I125 as iodine in carbon tetrachloride at the step which calls for addition of I2-CCl4 to deoxycytidine and iodic acid. The necessary amount of I131 or I125 as sodium iodide, together with 1 mg. of stable NaI, in a volume of 0.5 ml. or less, was converted to iodine in a small test tube by addition of a few drops of concentrated H2SO4 in the presence of a pinch of MnO2 and about 0.1 ml. of CCl4.
agitation, and occasionally by gentle heat, iodine was trapped in the CCl₄ phase, which changed to a violet color. IUdR-I₁³¹ and IUdR-I₁²⁶ were prepared in a manner similar to that of preparing labeled ICdR, except that deoxyuridine was used as a starting material in place of deoxycytidine. The purity of the compounds after their elution from Dowex-1 formate columns and lyophilization was determined by descending paper chromatography with butanol: H₂O : ammonia (86:14:5 per cent) with Whatman #3 paper, and methanol: HCl: H₂O (70:20:10) with Whatman #1, for 16 hours.

Specific activities were determined from measurements of radioactivity against a calibrated appropriate radioactive source and measurement of ultraviolet absorption at the wavelength of maximum absorbance for each compound. In addition to those compounds which were synthesized, C¹⁴-labeled thymidine (TdR) and H²-labeled deoxycytidine (CdR) were also used.

**Blood clearance.**—The method used for blood clearance experiments in the rat is that described in a previous report from this laboratory (13).

**Analytic methods.**—Descending paper chromatography of plasma samples and standards was routinely performed with the same two solvent systems described above for the identification and determination of purity of synthesized labeled compounds. Stable IUDR, 5-iodouracil (IU), 5-iodocytosine (IC), ICdR, and radioactive iodide-I₁³¹ or iodide-I₁²⁶ were used as standards.

The methods used for the measurement of the content and radioactivity of DNA of tissues were reported in a previous study (12). The determination of the radioactivity in whole organs followed the general technics described previously for the counting of Br²⁶ (13), and the same report also indicated the method employed for the determination of the activity of an isotope when present in a mixture of three isotopes. In order to count I₁³¹ or I₁²⁶ efficiently, either alone or in the presence of C¹⁴ and H², it was, of course, necessary to select suitably different discriminator settings of the spectrometers. The marked differences in the energy of the chief photopeaks of the gamma rays of I₁²⁶ (35 kv.) and I₁³¹ (360 kv.) permitted the measurements of the amount of each in tissues containing mixtures of these isotopes of iodine, by employing pulse height analysis in counting the gamma ray activity of the samples.

1 New England Nuclear Corp., Boston, Mass., 1 mc/mM.
2 Schwarz Laboratories, Inc., Mt. Vernon, N.Y., 1.1 c/mM.
3 California Corporation for Biochemical Research, Los Angeles, California.
4 Synthesized according to the method of Chang and Welch (7).

Scanning of the radioactivity of the paper chromatograms was performed by using an automatic windowless gas-flow counter and recorder. The areas under the recorded peaks of radioactivity on the chromatographed paper strips were measured with a planimeter. The percentage of the total radioactivity due to an identified component was calculated from the ratio of the area under its respective peak to that of the total area of all radioactive components.

**RESULTS**

**Fate of ICdR-I₁³¹ in the rat.**—The distribution and fate of parenterally administered ICdR in the rat was studied in two experiments. In Experiment 1, a solution mixture containing 1.38 µmoles each of ICdR-I₁³¹, TdR-C¹⁴, and CdR-H² of activity 20.3 µc., 1.98 µc., and 109 µc., respectively, was injected intravenously into each of two 200-gm. rats. Samples of 200 µl. of arterial blood were obtained at 1, 2, 3, 4, 6, 8, 10, 15, 20, 30, 40, and 60 minutes. These samples were centrifuged, and the total I₁³¹ activity of 50 µl. of each plasma, respectively, was measured in a well-type scintillation counter, while 10 µl. of each plasma was chromatographed on paper with butanol: H₂O : ammonia used as solvent. The percentage of the total radioactivity due to ICdR-I₁³¹ was calculated from planimetric measurement of peak areas on the recordings of radioactivity of the chromatographed paper strips. From this figure, corrected values for net ICdR-I₁³¹ activity in the various serum samples were obtained. The results in both animals were similar and are shown in Chart 1. Total I₁³¹ activity fell slowly for about 20 minutes, then remained essentially unchanged up to 60 minutes. ICdR-I₁³¹ activity fell slowly and progressively during the 60 minutes. By the end of the experiment, 60-70 per cent of the original activity was still in the form of ICdR-I₁³¹. IUDR-I₁³¹ was not detected in any of the samples. At the end of the clearance portion of the experiment the animal was sacrificed, and varying sized portions of the kidney, liver, spleen, testis, thymus, bone marrow, and of the mucosa of the colon, stomach, and intestine were analyzed for their DNA content and DNA radioactivity. The results, expressed as per cent of administered dose/mg DNA for each isotope, are shown in Chart 2. Note that a different scale was used to plot the I₁³¹ data. When compared with the amounts of C¹⁴ and H² present, the quantity of I₁³¹ is, therefore, only one-tenth as great as is apparent by the length of its corresponding bar on the graph. The results indicate that (a) although I₁³¹ radioactivity was detected in
the DNA, the amount, compared with that of C\textsuperscript{14} and H\textsuperscript{3}, was very low; (b) I\textsuperscript{131} activity was greatest in the marrow and stomach (results for liver were inconsistent); (c) H\textsuperscript{3} activity was clearly greatest in the stomach and relatively high in marrow, colon, and spleen.

In Experiment 2, the relative clearance rates and extent of incorporation into DNA of ICDR\textsuperscript{125} and IUdR, with and without pretreatment with 5-fluorodeoxyuridine (FUdR), were compared, with the use of ICDR-I\textsuperscript{125} and IUdR-I\textsuperscript{131}. Each of four 200-gm. rats received a mixture containing 0.2 \textmu mol of each of ICDR-I\textsuperscript{125} and IUdR-I\textsuperscript{131} in a volume of 1.2 ml. One pair of animals was chosen for a clearance experiment and sacrificed at 1 hour, and the other pair was sacrificed at 24 hours. One rat of each pair received 5-FUdR, 0.05 mg/kg, at 18 hours and again at 4 hours prior to injection of the labeled compounds. Arterial blood samples were obtained from the first pair of animals at 1, 2, 4, 6, 8, 10, 15, 20, 30, 40, and 60 minutes after the injection of the mixture. Aliquots of 100 \textmu l of the respective plasmas were used to determine total I\textsuperscript{125} and I\textsuperscript{131} activity, and aliquots of 20 \textmu l were chromatographed. The results are indicated in Chart 3. Although the initial plasma levels of radioactivity were slightly different, plasma I\textsuperscript{131} activity in the control and 5-FUdR-treated rat fell gradually at about the same rate for 20 minutes and then remained essentially constant for the remainder of the hour. Total plasma I\textsuperscript{131} activities in the two animals were also similar but differed from the I\textsuperscript{125} curves in that they showed a slight rise during the first 4 minutes and thereafter showed a slow and progressive fall up to 60 minutes. The percentage of the total activity of areas of the paper strip due to I\textsuperscript{125} and I\textsuperscript{131}, respectively, was determined by exciting portions of the strip which corresponded to the position of chromatographed standards and determining their I\textsuperscript{125} and I\textsuperscript{131} activity with a liquid scintillation spectrometer,\textsuperscript{4} adapting the method of Geiger and Wright (10) for the counting of these activities. From these values and the total radioactivity of I\textsuperscript{125} and I\textsuperscript{131}, respectively, corrected values for plasma ICDR-I\textsuperscript{125} and IUdR-I\textsuperscript{131} were calculated. The results are shown in Chart 4. A marked discrepancy is now seen between the curves showing ICDR-I\textsuperscript{125} and IUdR-I\textsuperscript{131} plasma levels at the various time intervals. This discrepancy is due to the much more rapid and extensive degradation of IUdR than ICDR. Chromatographic evidence of rapid degradation of IUdR to IU and iodide was obtained. Variable and small amounts (0–8 per cent) of the original ICDR-I\textsuperscript{125} were found as IUdR, the other degradation products being IU and iodide. No significant differences were observed between the controls and the animals treated with 5-FUdR.

The DNA content and DNA I\textsuperscript{131} and I\textsuperscript{125} radioactivity were determined in portions of marrow, spleen, thymus, testis, liver, kidney, and the mucosa of the colon, intestine, and stomach from the two animals killed at 1 hour, and compared with similar data obtained from the two animals sacrificed at 24 hours. The results are shown in Chart 5. For visual purposes the scale for I\textsuperscript{131} activity (ICDR) has been adjusted so as to magnify the length of the bars tenfold as compared with those

\textsuperscript{4}Tri-Carb liquid scintillation spectrometer, Packard Instrument Co., La Grange, Ill.
the stomach and liver in the latter case; (f) by 24 hours the initial discrepancies between the amounts of I\textsuperscript{126} and I\textsuperscript{131} incorporated were reduced, and in some instances—e.g., thymus and testis—were abolished or reversed; and (g) the effect of 5-FUdR on increasing the amount of I\textsuperscript{126} incorporated was appreciable in the animal killed 1 hour after the injection of ICdR, but slight or absent in the animal killed 24 hours later.

**Comparative study of the fate of ICdR and IUdR in the mouse.**—A comparison of the distribution of radioactivity in the organs of normal and tumor-bearing mice was made at varying intervals after injection of IUdR-I\textsuperscript{125} and ICdR-I\textsuperscript{131}. Ten normal C57BL/Ka mice weighing about 25 gm., and ten mice of the same strain bearing a 5-day-old transplanted sarcoma, each received a series of ten injections of equal volume of an IUdR-I\textsuperscript{125} solution,
given morning and afternoon for 5 days (total dose, 0.078 μmole, 21 μc). Another group of ten normal mice and another group of ten tumor-bearing mice each received ICdR-\(^{131}\) according to a similar schedule (total dose, 0.066 μmole, 14.3 μc.). Five animals in each of the four groups of ten animals were killed 48 hours after the last injection, two mice in each group were killed at 72 hours, and the remaining three in each group at 96 hours. When the mice were killed, the following organs or portions of the body were weighed and the radioactivity determined: colon, intestine, kidney, liver, hind legs, spleen, stomach, skin, thymus, tumor, and carcass. The results, expressed as per cent of administered dose of each isotope per gram wet tissue, are presented in Chart 6. The DNA content and DNA radioactivity of portions of some of the organs of two of the five animals in each group killed at 48 hours were determined after completion of the counting of the respective whole organ. The mean values, expressed as (% of dose/mg. DNA) \( \times 10^{-3} \), are indicated by the numbers opposite the corresponding bars representing total activity at the 48-hour interval. The results show that (a) the ranges of concentration of radioactivity in the organs after administration of IUdR and ICdR were similar; (b) the organs with the highest concentration of each compound were thymus, intestine, colon, spleen, and skin than after the respective concentrations of \(^{125}\) after IUdR-\(^{125}\) injection; (f) the concentration of \(^{131}\) and \(^{125}\) in DNA were highest in colon and intestine, intermediate in stomach, thymus, spleen, and tumor, and lowest in liver and kidney; and (g) only a rough correlation was found between the total isotopic activity of an organ and the activity of the same isotope in the DNA extracted from that organ. Discrepancies in this correlation are especially apparent on comparing the respective figures for thymus and colon, or for colon and tumor.

**DISCUSSION**

These results indicate that ICdR, like BCdR, is slowly degraded in the rat, and that when iodine-
labeled ICdR is administered to the mouse and rat the radioactive label is to some extent incorporated into the DNA of various tissues. Quantitatively, however, the amount of incorporation of radioiodine into DNA after ICdR-I" or ICdR-I" administration was only about 1/50 of the incorporation of Br" reported after administration of BCdR-Br" (13), and about 1/20 that of I" after administration of IUdR-I", when the animal was sacrificed 1 hour after the injection of the labeled compound. Owing to the slow degradation rate of ICdR, greater amounts of the compound were found to have been incorporated if a longer time was allowed to elapse before the animal was killed. Thus, the amount of the label of ICdR incorporated into DNA after 24 hours was about two- to tenfold greater than that incorporated after 1 hour. When the time of sacrifice was extended to 5 days after the first of a series of daily injections of IUdR-I" and ICdR-I" to tumor-bearing mice, no significant differences between the amounts of I" and I", respectively, in DNA extracted from various organs or tumor were observed.

The slow degradation of BCdR and ICdR in the rat indicates that those mechanisms responsible for the rapid cleavage of BUdR and IUdR to bromouracil and iodouracil, respectively, do not result in comparable cleavage of BCdR and ICdR. The formation of bromocytosine was not observed after administration of BCdR to the rat (13), nor

<table>
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<tr>
<th>Tissue</th>
<th>1125 IUdR</th>
<th>1125 control</th>
<th>1131 IUdR</th>
<th>1131 control</th>
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<td>Tumor</td>
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CHART 6.—Distribution of I" and I" in organs of tumor-bearing C57BL mice at various intervals after a 5-day series of intraperitoneal injections of IUdR-I" and ICdR-I".
was iodocytosine detected in any of the present experiments after administration of ICdR as predicted by Welch (20). On the other hand, the finding of both IUDr and IU in the plasma after ICdR administration indicates that deamination of the latter to IUdR is a probable initial obligatory step. A preliminary report by Cramer et al. (4) states that ICdR is incorporated into DNA of mastocytoma cells in vitro as IUDr-5-phosphate. Evidence for an analogous incorporation of BCdR as BUdR-5-phosphate has been presented (4).

After injection of ICdR-I131 in the rat, the incorporation of I131 into the DNA of bone marrow exceeded that in other organs. Relatively high bone marrow concentration of Br28 and H1 were also found after administration of BCdR-Br28 and CdR-H1, respectively (13). These findings are probably adequately explained by the known presence of deoxycytidylic deaminase in adult rat bone marrows (16). In the mouse, ICdR and IUdR appear to be incorporated into DNA in approximately equal amounts and with essentially similar patterns of distribution in different organs, suggesting the possibility that in this species deoxycytidylic deaminase is more ubiquitously distributed. Of interest in this regard was the relatively high incorporation of ICdR into the rapidly growing mouse sarcoma, since neoplastic tissue may also contain relatively large amounts of deaminase (16).

The enhanced incorporation into DNA of both I131 and I125 after the administration of IUDR-I131 and ICdR-I125 in animals pretreated with 5-FUdR is also consistent with a prior deamination of ICdR to IUdR. However, although this effect of 5-FUdR was noted in animals killed 1 hour after injection of the labeled compounds, no such effect was apparent when the animals were sacrificed after 24 hours. Perhaps the failure to observe an effect of 5-FUdR at the later interval was due to inability of the small dose used to maintain a sufficiently high local concentration of F UdR for many hours. Increased incorporation of exogenous thymidine or thymidine analogues under the influence of 5-fluorouracil or 5-FUdR has been previously reported from this and other laboratories (5, 8, 9, 12, 15, 19).

Because the amount of ICdR incorporated into DNA is less than that of BCdR, there is probably no advantage to be gained in using ICdR over BCdR as a radiosensitizing agent. On the contrary, BCdR would seem to be the preferable drug. However, studies of the relative fates of these two compounds and their relative radiosensitizing potencies in man are needed before definite conclusions may be reached regarding their potential clinical usefulness as adjuncts in the radiotherapy of cancer. Preliminary findings (1) indicate that in man the clearances of ICdR and IUDR from the blood are similar. With regard to their possible use in radioactive form in the demonstration and localization of tumors using external detectors, ICdR-I125 or ICdR-I131 is likely to be found preferable to BCdR-Br28 because, for such purposes, Br28 possesses an undesirably high energy and short life and because any released bromide is widely distributed and, unlike iodide, is difficult to eliminate from the body.

REFERENCES


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