PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 187, 488–492 (1988)

Inhibition by 2-Deoxy-D-ribose of DNA Synthesis and Growth in Raji Cells (42693)

FRANK ULRICH

Surgical Research Unit, Boston Veterans Administration Medical Center, Boston, Massachusetts 02130, and the Department of Surgery, Tufts University School of Medicine, Boston, Massachusetts 02111

Abstract. When Raji cells were cultured for 3 days in serum-free medium, addition of 2-deoxy-D-ribose at the start of culture inhibited incorporation of [^{3}H]thymidine and cell division. At deoxyribose concentrations between 1 and 5 m*M*, viability was 80% or greater after 3 days of culture even though 5 m*M* deoxyribose inhibited thymidine incorporation 95–99%. Inhibition by deoxyribose could be completely reversed if the culture medium was replaced with fresh medium up to 8 hr after the start of culture. The inhibition was specific for deoxyribose since other monosaccharides had no effect. Inhibition of DNA synthesis did not appear to be due to depletion of essential nutrients in the medium since the percentage inhibition of thymidine incorporation by cells cultured either in suboptimal serum-free media or in media supplemented with 0.025–5% human AB serum was similar. When DNA repair synthesis was measured as hydroxyurea-resistant thymidine incorporation, addition of deoxyribose to Raji cultures caused increased thymidine incorporation. These results, together with data from others, suggest that deoxyribose damages DNA.

Monosaccharides alter several parameters of the immune system, many of these changes being inhibitory. They include inhibition of natural killer cell cytolysis (1–3); inhibition of mixed lymphocyte culture-induced generation of suppressor cells (4); inhibition of spontaneous cell-mediated cytotoxicity (5–7); inhibition of migration and leukocyte inhibitory factor activity (6, 7); and inhibition of macrophage and neutrophil chemotactic factor activity (7).

In a previous paper it was reported that D-ribose, 2-deoxy-D-ribose, and *N*-acetyl-D-galactosamine—at concentrations of 10–25 m*M*—inhibited by 40% or more the incorporation of [^{3}H]thymidine ([^{3}H]TdR) into rat thymocytes that had been stimulated to divide by concanavalin A, sodium periodate, or Interleukin 2 (IL-2, Ref (8)). To determine whether these sugars could also inhibit the growth of a rapidly growing non-T cell that divides without first having to be stimulated by mitogen, the effects of monosaccharides on the Raji cell, a malignant B cell, were studied (9). The experiments focused on the effects of deoxyribose since data in a previous study from this laboratory showed that inhibition of thymidine incorporation into thymocytes stimulated by IL-2 was significantly greater with this sugar than with either D-ribose or L-ribose (8). While this study was in progress, two papers were published on the effects of D-ribose and deoxyribose in a variety of cells (10, 11).

Materials and Methods. Most of the materials and methods were the same as those in the previous study (8). The Raji cells, a gift from Drs. Leonard Berman and Gordon Blanchard of the Boston V.A. Medical Center, were grown in suspension at 37°C in humidified 5% CO_2 in 250-ml tissue culture flasks (Costar No. 3075) in serum-free Dulbecco's modified Eagle's medium (12) that was supplemented with 2 mg/ml bovine albumin fraction V (Sigma Chemical Co.), 5 μg/ml human transferrin (Sigma), and antibiotics as described previously (8). The cells were subcultured every 3–4 days to $0.05–0.10 \times 10^6$ cells/ml.

Results. *Effects of increasing concentrations of deoxyribose on [^{3}H]TdR incorporation, cell number, and cell viability.* The first experiment in Table I presents data from a typical experiment which shows that increasing concentrations of deoxyribose added to Raji cells at the start of culture cause progressively increased inhibition of thymidine incorporation.

To determine the effects of deoxyribose on cell numbers and viability, parallel cultures were set up. At 3 days, one set was pulsed with [^{3}H]TdR and the number of cells in the

0037-9727/88 $1.50

TABLE I. EFFECTS OF DEOXYRIBOSE ON [^{3}H]TdR INCORPORATION, CELL VIABILITY, AND CELL NUMBER OF RAJI CELLS

Deoxyribose	cpm
m*M*	Experiment 1
0	41,047 ± 965
2.5	21,925 ± 1132
5	559 ± 95
10	179 ± 18
	Experiment 2
0	19,278 ± 226 [0.37 × 10^6]
5	356 ± 109 [0.26 × 10^6]
	Experiment 3
0	— [0.79 × 10^6]
5	— [0.11 × 10^6]
	Experiment 4
0	40,705 ± 1350 (95%)
5	333 ± 137 (83%)

Note. Cells were cultured for 3 days at an initial concentration of 0.05 × 10^6 cells/ml. Figures for cpm are the mean of quadruplicate cultures ± SE. In Experiments 2 and 3, figures in brackets are the number of cells after 3 days determined with a Coulter counter (Experiment 2) and microscopically (Experiment 3). In Experiment 4, figures in parentheses are the viabilities of cells after 3 days of culture.

second set was determined with a Coulter counter. The results of a representative experiment (Table I, Experiment 2) demonstrate that inhibition of thymidine incorporation was accompanied by a decrease in cell number. In the third experiment, the number of cells was determined microscopically. The decrease in cell number in Experiment 2 was less than that in Experiment 3 because with the Coulter counter both live and dead cells were counted.

Experiment 4 shows that although 5 m*M* deoxyribose caused a 99% inhibition of thymidine incorporation, cell viability only decreased from 95 to 83%. When Raji cells were cultured with increasing concentrations of deoxyribose, viability after 3 days was greater than 90% with 1, 2.5, or 5 m*M* sugar (data not shown). Viability decreased to 50% with 10 m*M*, and with 25 m*M* deoxyribose all the cells were dead.

Effects of different monosaccharides. When Raji cells were cultured for 3 days with different sugars, only deoxyribose caused a significant inhibition in [^{3}H]TdR incorporation (Table II). The small inhibition with 5 mM D-ribose was probably not significant. This confirmed my previous observation that the inhibition of [^{3}H]TdR incorporation by D-ribose occurred only at concentrations of 10 m*M* or greater (8).

Effects of adding cold thymidine. To show that changes in [^{3}H]TdR incorporation were not due to alterations either in thymidine transport or in intracellular thymidine pool size, cells and deoxyribose were added to microtiter wells and the suspensions were immediately pulsed with [^{3}H]TdR and harvested after 4 hr of incubation. Under these conditions, deoxyribose had no effect on [^{3}H]TdR incorporation (data not shown).

In another series of experiments, cells were cultured with or without deoxyribose. At 68 hr one set of cells was pulsed with the standard preparation of [^{3}H]TdR which contained 0.0048 μg of thymidine; a second set of cells was pulsed with [^{3}H]TdR to which 0.432 μg of thymidine had been added. Both sets of cells were harvested 4 hr later. The results showed that although the addition of cold thymidine markedly decreased [^{3}H]-TdR incorporation by the cells cultured without sugar (from 31,575 ± 1130 to 8,599

TABLE II. EFFECTS OF VARIOUS MONOSACCHARIDES ON [^{3}H]TdR INCORPORATION INTO RAJI CELLS CULTURED FOR 3 DAYS

5 m*M* sugar	cpm
Experiment 1	
None	48,133 ± 1369
Deoxyribose	390 ± 43
D-Ribose	42,403 ± 559
D-Arabinose	47,658 ± 471
D-Mannose	47,483 ± 522
D-Fucose	49,535 ± 904
Experiment 2	
None	17,497 ± 376
Deoxyribose	1,134 ± 104
D-Xylose	17,090 ± 264
D-Galactose	16,282 ± 788
N-Acetyl-D-galactosamine	22,395 ± 393
α-Methyl-D-mannoside	16,085 ± 494

Note. The initial cell concentration was 0.05 × 10^6 cells/ml. Sugars were added at the start of culture.

± 222 cpm), the inhibition of [^{3}H]TdR incorporation was qualitatively similar with the two thymidine preparations (485 ± 80, no carrier added; 185 ± 38 cpm, carrier added).

Are the effects of deoxyribose reversible? One-milliliter suspensions of Raji cells were cultured in 12 × 75-mm plastic tubes (Falcon No. 2002) with or without 5 m*M* deoxyribose. At different times the suspensions were centrifuged, the supernatant fluids were removed, and the cells were resuspended with medium that had been incubated without sugar for the same length of time as the cells. At 68 hr, four 200-μl aliquots of each suspension were added to microtiter wells, the suspensions were pulse-labeled with [^{3}H]TdR, and harvested 4 hr later. The experiment in Table III demonstrates that replacement of the medium at 8 hr completely reversed the effects of deoxyribose. After 24 hr the effects of the sugar were only partially reversible, and after 48 hr replacement of the medium had little effect on the inhibitory action of deoxyribose.

Effects of deoxyribose on cells cultured in serum-free or serum-supplemented media. The inhibition of DNA synthesis and cell division could have resulted from depletion of essential nutrients in the serum-free medium. To test this, 0.05 × 10^6 cells/ml were cultured either in the standard serum-free medium or in medium in which albumin fraction V and transferrin were replaced with 10% heat-inactivated human AB serum. The results of the first experiment in Table IV show that cells cultured in the serum-supplemented medium incorporated almost six times as much [^{3}H]TdR as cells cultured in the serum-free medium. However, when 5 m*M* deoxyribose was added to the cultures, the percentage inhibition of [^{3}H]TdR incorporation was similar in the two groups.

In the second experiment, cells were cultured at serum concentrations of 0.025, 0.25, or 5%. Although cells cultured in 5% serum incorporated more than 10 times as many counts as those in 0.025% serum, addition of 5 m*M* deoxyribose inhibited [^{3}H]TdR incorporation by similar percentages in the three groups.

DNA damage and repair. Two studies have suggested that sugars can induce DNA damage (13, 14). Since DNA damage usually triggers DNA repair synthesis, a series of experiments was performed in which DNA repair synthesis was measured as hydroxyurea (HU)-resistant thymidine incorporation (15). Raji cells were first cultured for 18–24 hr with deoxyribose, then HU was added to a final concentration of 0.5 m*M* and 1 hr later the cultures were pulse-labeled with [^{3}H]TdR for 2 hr and harvested. The results of two typical experiments (Table V) show that deoxyribose but not D-arabinose caused increased HU-resistant [^{3}H]TdR incorporation.

TABLE III. EFFECTS OF CHANGING MEDIUM ON [^{3}H]TdR INCORPORATION INTO RAJI CELLS CULTURED FOR 3 DAYS WITH 5m*M* DEOXYRIBOSE

Medium changed at	± Sugar	cpm
Not changed	−	19,959 ± 1574
Not changed	+	444 ± 32
8 hr	−	20,274 ± 2301
8 hr	+	22,919 ± 330
24 hr	−	22,281 ± 1550
24 hr	+	11,641 ± 285
48 hr	−	32,236 ± 861
48 hr	+	2,773 ± 97

Note. The initial cell concentration was 0.05 × 10^6 cells/ml and deoxyribose was added at the start of culture.

Discussion. These experiments extend previous data which showed that specific sugars inhibit [^{3}H]TdR incorporation into thymocytes stimulated to divide by Con A, sodium periodate oxidation, or IL-2 (8). In that study, D-ribose did not appear to inhibit thymocyte proliferation by blocking the interaction of these mitogen with the cell. Since Raji cells grow and divide without mitogenic stimulation, the mechanism by which deoxyribose inhibits DNA synthesis and cell division may be similar in thymocytes and Raji cells. In a study published while these experiments were in progress, Marini *et al.* (10) reported that D-ribose and deoxyribose inhibited DNA, RNA, and protein synthesis in a variety of human and murine cells, both normal and neoplastic. Although the viability of Raji cells cultured with deoxyribose was slightly less than that of

TABLE IV. EFFECTS OF 5 m*M* DEOXYRIBOSE ON [^{3}H]TdR INCORPORATION INTO RAJI CELLS CULTURED FOR 3 DAYS IN EITHER SERUM-FREE OR SERUM-SUPPLEMENTED MEDIA

Medium supplemented with	± Sugar	cpm	% Viability
		Experiment 1	
10% serum	−	146,318 ± 3671	98
10% serum	+	1,356 ± 52 (−99%)	97
Fr. V + Trf.	−	25,641 ± 415	95
Fr. V + Trf.	+	1,211 ± 270 (−95%)	87
		Experiment 2	
0.025% serum	−	10,632 ± 231	—
0.025% serum	+	129 ± 24 (−99%)	—
0.25% serum	−	22,095 ± 5646	—
0.25% serum	+	339 ± 133 (−98%)	—
5.0% serum	−	115,569 ± 2303	—
5.0% serum	+	2,576 ± 147 (−98%)	—

Note. Viabilities were determined after 3 days of culture. Trf, human transferrin; Fr. V, bovine albumin fraction V; serum, heat-inactivated human AB serum.

the controls, significant loss of viability was seen only after 1–2 days of culture at concentrations of 10 and 25 m*M*. Since Raji cells go through several cycles of DNA synthesis and division during that time, the 80–90% viability suggests that the action of deoxyribose is either very slow or that a certain period of time is necessary for an inhibitor generated by the sugar to accumulate in the cells.

The experiments with HU in which cells cultured with deoxyribose showed increased DNA repair synthesis suggest that this sugar inhibits DNA synthesis and cell proliferation by damaging DNA. These results are at odds with those of Zunica *et al.* (11) who reported that D-ribose did not induce DNA repair synthesis in PHA-stimulated human lymphocytes. The discrepancy may be due to the high concentrations (25–50 m*M*) of D-ribose used by Zunica. These concentrations of D-ribose are roughly equivalent to or greater than the 10 m*M* deoxyribose which in my experiments markedly decreased Raji cell viability. 10 m*M* deoxyribose also resulted in HU-resistant thymidine incorporation that was significantly *less* than that of cells cultured without deoxyribose (data not presented). Therefore the observation of Zunica *et al.* that D-ribose did not induce DNA repair synthesis may have been due to their use of toxic concentrations of D-ribose.

TABLE V. EFFECTS OF DEOXYRIBOSE ON DNA REPAIR SYNTHESIS

Sugar	cpm
	Experiment 1
None	3267 ± 95
5 m*M* deoxyribose	5331 ± 139 (+63%)
	Experiment 2
None	3548 ± 148
2.5 m*M* deoxyribose	5490 ± 39 (+55%)
5 m*M* deoxyribose	6679 ± 434 (+88%)
7.5 m*M* deoxyribose	5387 ± 230 (+52%)
5 m*M* D-arabinose	3888 ± 177 (+10%)

Note. Cells were cultured with or without deoxyribose. Hydroxyurea (0.5 m*M*) was added after 19–21 hr and 1 hr later the cultures were pulse-labeled for 2 hr with [^{3}H]TdR and harvested.

Two studies suggest that sugars can induce DNA damage. Bucala *et al.* (13) found that glucose and glucose 6-phosphate reacted with DNA *in vitro* to produce significant structural and biological alterations. They hypothesized that accumulation of these sugars could be one mechanism for the decreased genetic viability and increased tumorigenesis observed in aged organisms. Lorenzi *et al.* (14) found that DNA from human endothelial cells exposed to high glucose concentrations showed an accelerated rate of unwinding in alkali and an increased

amount of HU-resistant thymidine incorporation.

This study was supported by a grant from the medical research service of the Veterans Administration.

1. Ades EW, Hinson A. Effector cell sensitivity to sugar moieties. II. Inhibition by monosaccharides of interferon enhanced human natural killing. J Clin Lab Immunol **11**:173–176, 1983.
2. Vose BM, Harding M, White W, Moore M, Gallagher J. Effect of simple sugars on natural killing: Evidence against the involvement of a lectin-like mechanism in target recognition. Clin Exp Immunol **51**: 517–524, 1983.
3. Ortaldo JR, Timonen TT, Heberman RB. Inhibition of activity of human NK and K cells by simple sugars: Discrimination between binding and post-binding events. Clin Immunol Immunopathol **31**:439–443, 1983.
4. Stankova J, Rola-Pleszczynaki M. α-Fucose inhibits human mixed-lymphocyte culture reactions and subsequent suppressor cell generation. Cell Immunol **83**:83–91, 1984.
5. MacDermott RP, Kienker LJ, Bertovich MJ, Muchmore AV. Inhibition of spontaneous but not antibody-dependent cell-mediated cytotoxicity by simple sugars: Evidence that endogenous lectins may mediate spontaneous cell-mediated cytotoxicity. Immunology **44**:143–152, 1981.
6. Rocklin R. Role of monosaccharides in the interaction of two lymphocyte mediators with their target cells. J Immunol **116**:816–820, 1976.
7. Amsden A, Ewan V, Yoshida T, Cohen S. Studies on cellular receptors for lymphokines. I. Interaction of chemotactic factors with monosaccharides. J Immunol **120**:542–549, 1978.
8. Ulrich F. Inhibition by specific monosaccharides of Interleukin 2-induced thymocyte proliferation. Cell Immunol **80**:241–256, 1983.
9. Pulvertaft RJV. A study of malignant tumors in Nigeria by short-term tissue culture. J Clin Pathol **18**:261–271, 1965.
10. Marini M, Zunica G, Franceschi C. Inhibition of cell proliferation by D-ribose and deoxy-D-ribose. Proc Soc Exp Biol Med **180**:246–257, 1985.
11. Zunica G, Marini M, Brunelli MA, Chiricolo M, Franceschi C. D-Ribose inhibits DNA repair synthesis in human lymphocytes. Biochem Biophys Res Commun **138**:673–678, 1986.
12. Iscove NN, Melchers F. Complete replacement of serum by albumin, transferrin, and soybean lipid in cultures of lipopolysaccharide-reactive B lymphocytes. J Exp Med **147**:923–933, 1978.
13. Bucala R, Model P, Cerami A. Modification of DNA by reducing sugars: A possible mechanism for nucleic acid aging and age-related dysfunction in gene expression. Proc Natl Acad Sci USA **81**:105–109, 1984.
14. Lorenzi M, Montisano DF, Toledo S, Barrieux A. High glucose induces DNA damage in cultured human endothelial cells. J Clin Invest **77**:322–325, 1986.
15. Brandt WN, Flamm WG, Bernheim NJ. The value of hydroxyurea in assessing repair synthesis of DNA in HeLa cells. Chem-Biol Interact **5**:327–339, 1972.

Received October 26, 1987. P.S.E.B.M. 1988, Vol. 187.
Accepted December 11, 1987.