Biochemical Pharmacology, Vol. 28, pp. 3239-3242.
Pergamon Press Ltd. 1979. Printed in Great Britain.

THE METABOLISM OF 2'-DEOXYCOFORMYCIN BY L1210 CELLS IN VITRO

Peter M. Venner and Robert I. Glazer

Applied Pharmacology Section
Laboratory of Medicinal Chemistry and Biology
National Cancer Institute, Bethesda, Maryland 20205

(Received 26 June 1979; accepted 31 July 1979)

2-Deoxycoformycin (dCF), a nucleoside analog, is a fermentation product of Streptomyces antibioticus and is the most potent inhibitor of adenosine deaminase yet described. The structure of this drug was identified in 1974 (1) as a benzodiazepine analog of inosine, the structure of which confers tight-binding properties as a transition state inhibitor of adenosine deaminase (2). This compound is of clinical interest because of its ability to potentiate the antitumor activity of adenosine analogs (3-5) and its demonstrated immunosuppressive effects as a single agent resulting from its lymphocytolytic activity in animals and man (6-9). The latter effect is believed to result from inhibition of adenosine deaminase by dCF and the subsequent accumulation of either dATP or some other cytotoxic metabolite which arrests cell growth (10-12). One process which is influenced by elevated levels of dATP produced by dCF and exogenous deoxyadenosine is DNA synthesis (11). Alternatively, it has not been ruled out that dCF is anabolized to a 2'-deoxyribonucleotide in lymphoid cells which in turn acts as an inhibitor of DNA synthesis. In this regard, coformycin, the ribonucleoside congener of dCF, is more than 50% converted to ribonucleotide metabolites in L5178Y cells in vitro (13). In vivo studies with dCF in rodents have demonstrated that 80-90% of the drug is excreted in the urine and 6% in the feces (14,15). Thin-layer chromatography of the urine failed to demonstrate any metabolites (14).

With the recent availability of [^{3}H]dCF, we were able to test whether this drug was metabolized in L1210 cells in vitro. The results of these studies form the basis for this preliminary report.

[(G)-^{3}H]dCF was purchased from the Research Triangle Institute and unlabeled dCF was provided by the Drug Synthesis and Chemistry Branch, National Cancer Institute. Alkaline phosphatase (calf intestine, grade 1, 400 units/mg) was obtained from Boehringer Mannheim. [^{3}H]dCF was purified by absorption to activated charcoal as described by Dion et al. (16). The final specific radioactivity was 33.6 Ci/mmole.

Incubations with L1210 cells were carried out in a shaking water bath at 37° in 25 ml of RPMI 1630 medium containing: 22 μCi of [^{3}H]dCF, 5×10^7 cells and varying concentrations of unlabeled dCF. Following incubation, cells were centrifuged, washed with ice-cold RPMI 1630 medium and vortexed vigorously with 1.0 ml of 1.0 N ice-cold perchloric acid (PCA). PCA extracts were neutralized with 1.0 N KOH and $KClO_4$ was removed by centrifugation at 4° at 2000 x g for 20 min. The neutralized PCA extracts were stored at -20° prior to chromatography.

Nucleotide metabolites of dCF were separated by DEAE Sephadex-urea chromatography as previously described (17). Enzymatic phosphorylysis of the PCA extract containing dCF metabolites was carried out at 37° for 2 hr in 0.6 ml of 50 mM Tris-HCl (pH 8.5) - 5 mM $MgCl_2$ containing 20 μg of alkaline phosphatase.

DEAE Sephadex chromatography of PCA extracts of L1210 cells incubated with 1×10^{-6}M [^{3}H]dCF for 0.5 to 2 hr showed two major peaks of radioactivity (Fig. 1B-D). The first peak represented unmetabolized and hence, unabsorbed [^{3}H]dCF which accounted for approximately 84-97% of the applied radioactivity. A metabolite of [^{3}H]dCF eluting with a net charge of -2 accounted for the remainder of the total radioactive material. [^{3}H]dCF that was not incubated with cells and subsequently chromatographed eluted in flow-through fractions 1-10 and not in any region of the gradient (fractions 11-50, Fig. 1A). In order to see whether the -2 charge metabolite was indeed dCF monophosphate, a neutralized PCA extract was first incubated with alkaline phosphatase before chromatography (Fig. 1C, inset). Virtually all of the monophosphate peak disappeared after enzymatic hydrolysis indicating that this fraction was a nucleoside monophosphate of dCF. Incubation in buffer without alkaline phosphatase did not reduce the amount of radioactivity eluting in the monophosphate peak.

The formation of dCF monophosphate was proportional to drug concentration in the medium (Fig. 2, left panel). Although the percentage of dCF monophosphate generated was time dependent, little difference was observed in its percentage of formation as a function of drug concentration (Fig. 2, right panel).

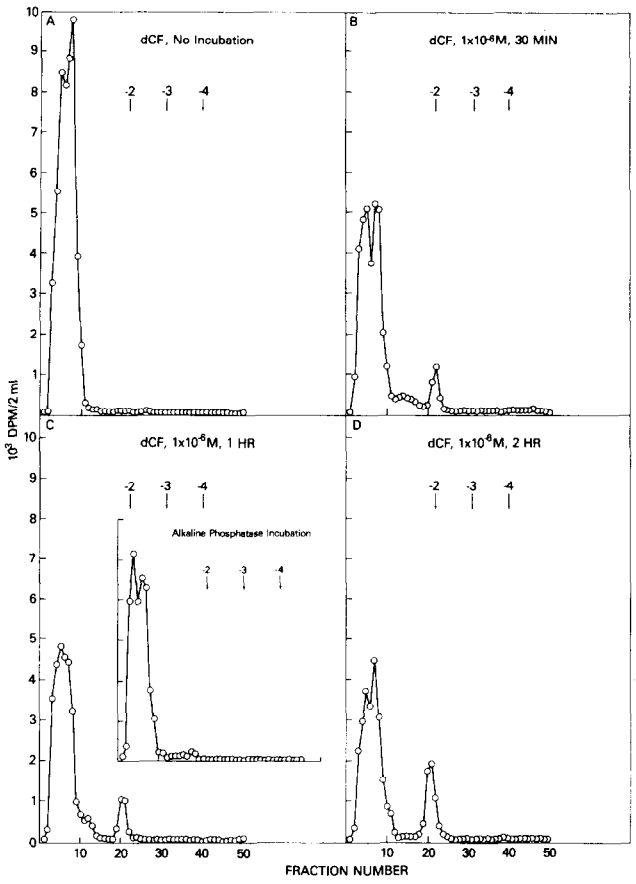

Fig. 1. DEAE Sephadex chromatography of [^{3}H]dCF before and after incubation with L1210 cells in vitro. L1210 cells were incubated with 1x10^{-6}M [^{3}H]dCF (858 mCi/mmole) for 0.5 hr (B), 1 hr (C) or 2 hr (D). Chromatography of [^{3}H]dCF without incubation with L1210 cells is shown in (A). The inset in (C) represents chromatography of neutralized PCA cell extracts after incubation with alkaline phosphatase. The designations "-2", "-3" and "-4" indicate the net charge of nucleoside mono-, di- and triphosphates, respectively.

The present studies have demonstrated that dCF is metabolized by L1210 cells only to the nucleoside monophosphate and that this metabolite accounts for 3-16% of the total intracellular concentration of drug. This anabolic process presumably occurs via deoxyadenosine kinase. In contrast, the metabolism of coformycin by L5178Y cells in vitro produced greater than a 50% conversion of the drug to nucleoside mono-, di-, and triphosphates (13). The marked differences in metabolism between dCF and coformycin probably reflect differences in the substrate specificities of deoxyadenosine kinase and adenosine kinase, respectively, for the two adenosine deaminase inhibitors.

With respect to the mechanism of the lymphocytolytic activity produced by dCF, it is unlikely that dCF monophosphate can directly account for inhibition of cell growth or impairment of DNA synthesis. Concentrations of dCF as high as 1x10^{-3}M (which is at least two orders of magnitude greater than that required for total inhibition of adenosine deaminase) failed to produce cytotoxicity in L cells or P388 cells (10,18). These results suggest that perturbation of other cellular processes as a result of impaired adenosine

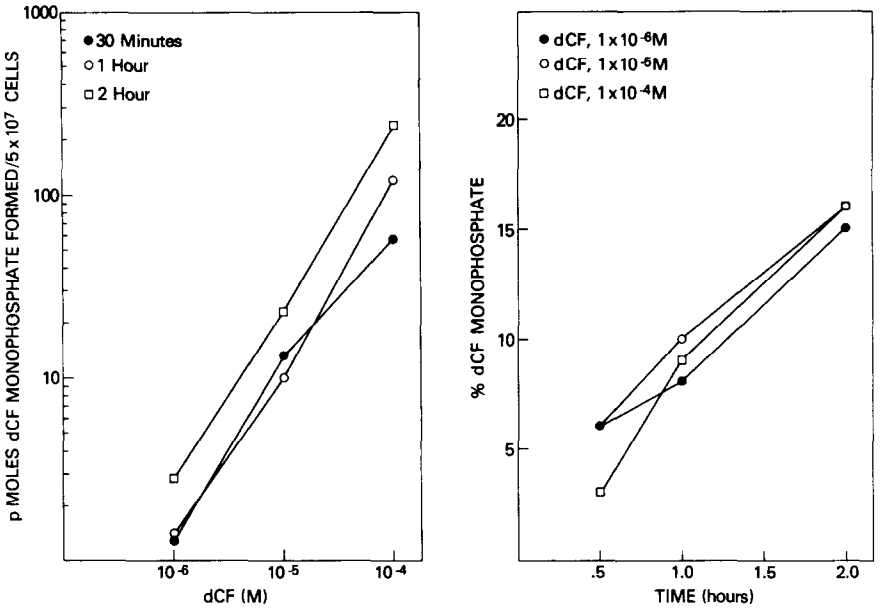

Fig. 2. Formation of dCF monophosphate by L1210 cells in vitro. L1210 cells were incubated with $1x10^{-6}$M, $1x10^{-5}$M and $1x10^{-4}$M [^{3}H]dCF (858, 88 and 8.8 mCi/mmole, respectively) for 30 min, 1 hr and 2 hr. See Fig. 1 for experimental details. Left, the concentration of dCF monophosphate formed as a function of drug concentrations at each incubation time. Right, the percentage of dCF monophosphate formed as a function of incubation time.

or deoxyadenosine metabolism is responsible for the arrest of cell growth (10,12). In this regard, greater lethality of L cells was noted in the presence of dCF and deoxyadenosine vs the combination of a less potent inhibitor of adenosine deaminase, erythro-9-(2-hydroxy-3-nonyl)adenine, and deoxyadenosine (10). Since a high concentration of dCF ($1x10^{-5}$M with $5x10^{4}$ cells) was used in these studies, it is possible that enough dCF monophosphate was generated to produce a synergism in the cytotoxic effects of deoxyadenosine by a hitherto unknown mechanism.

ACKNOWLEDGEMENT

Dr. Venner is a recipient of a McEachern Fellowship awarded by the Canadian Cancer Society. The authors appreciate the assistance of Mrs. Margaret Green in the preparation of this manuscript.

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