Journal of Medicinal Chemistry

Volume 21, Number 7 July 1978

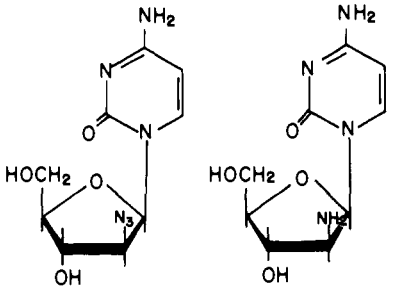
Communications to the Editor

Novel Arabinofuranosyl Derivatives of Cytosine Resistant to Enzymatic Deamination and Possessing Potent Antitumor Activity

Sir:

The antitumor efficacy of *ara*-C[1] and its various derivatives[2,3] is diminished by their susceptibility to deamination. We have now synthesized 2′-azido- and 2′-amino-2′-deoxy-β-D-arabinofuranosylcytosine (cytarazid and cytaramin) and have found them to be resistant to deamination by partially purified CR-dCR deaminases derived from the blast cells[4] of patients with acute myelocytic leukemia as well as from human liver.[5] Both compounds were markedly cytotoxic to some human and murine cell lines in vitro and were curative against L1210 cells in vivo.

In this report, a brief description of the synthesis and antitumor effects of 1-(2-azido-2-deoxy-β-D-arabinofuranosyl)cytosine (**1**) and 1-(2-amino-2-deoxy-β-D-arabinofuranosyl)cytosine (**2**) is given. Condensation of



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5-*O*-benzoyl-3-*O*-acetyl-2-azido-2-deoxy-D-arabinofuranosyl chloride[6] with the silylated cytosine furnished a mixture of blocked α and β anomers of **1** in 9.4 and 38.7% yields, respectively. After separation of the anomeric mixture on silica gel, the protecting groups of the blocked nucleosides were removed by treatment with K_2CO_3 in methanol. Compound **1** and its α anomer showed UV spectra typical of cytidine derivatives. The identification of **1** as the β anomer relies on the NMR and CD spectra. In the NMR spectrum of **1** the signal for the anomeric proton appeared at a lower field resonance[7] than that of the α anomer. The circular dichroism spectrum of **1** gave a positive Cotton effect while that of the α anomer had a negative effect, which is in agreement with the empirically determined rule.[8] For **1**: UV (MeOH) λ_{max} 273 nm; NMR (Me_2SO-d_6, Me_4Si internal standard) δ 7.76 (d, 1, $J_{5,6}$ = 7.0 Hz, C_6H), 7.20 (br s, 2, NH_2), 6.17 (d, 1, $J_{1',2'}$ = 6 Hz, C_1H), 5.75 (d, 1, $J_{5,6}$ = 7.0 Hz, C_5H), 5.86 (d, 1, $O_{3'}H$), 5.07 (t, 1, $O_{5'}H$). For the α anomer of **1**: UV (MeOH) λ_{max} 273 nm; NMR (Me_2SO-d_6) δ 7.68 (d, 1, $J_{5,6}$ = 7.5 Hz, C_6H), 7.23 (br s, 2, NH_2), 5.76 (d, 1, $J_{1',2'}$ = 5 Hz, C_1H), 5.82 (d, 1, $J_{5,6}$ = 7.5 Hz, C_5H), 4.93 (t, 1, $O_{5'}H$). Catalytical reduction of **1** using Pd or Pt as the catalyst furnished the 2′-amino-2′-deoxy derivative **2**: UV (pH 2) λ_{max} 276 nm; NMR (Me_2SO-d_6) δ 7.79 (d, 1, $J_{5,6}$ = 7.5 Hz, C_6H), 7.03 (br s, 2, cytosine NH_2), 5.99 (d, 1, $J_{1',2'}$ = 5.0 Hz, C_1H), 5.68 (d, 1, $J_{5,6}$ = 7.5 Hz, C_5H). Compounds **1** and **2** gave acceptable elemental analysis for carbon, hydrogen, and nitrogen.

Table I. Inhibitory Effects of 2′-Azido- and 2′-Aminoarabinosylcytosine on the Growth of Various Mammalian Cell Lines in Vitro[a]

cell line	concn for 50% inhibitn of growth: 2′-azido-*ara*-C	2′-amino-*ara*-C
HeLa	2×10^{-7}	3×10^{-5}
Molt 4F (T-type from lymphoblastic leukemia)	7×10^{-8}	N.T.
L1210	6×10^{-7}	4×10^{-6}

[a] The culture conditions used have been published previously.[9]

Table II. Effects of 2′-Azido- and 2′-Aminoarabinofuranosylcytosine on Leukemia L1210 in Vivo

compd	dose,[a] mg/kg	no. of mice[b]	av survival time, days
control		12	8.7
2′-azido-*ara*-C	40	24	>120
2′-amino-*ara*-C	75	24	>120

[a] DBA/2 HaDD mice were inoculated ip with 1×10^6 leukemia L1210 cells and the compounds were administered twice daily (8 h apart) for 2 days beginning 24 h after tumor inoculation. [b] In groups of six mice.

Biological. As shown in Tables I and II, the two compounds inhibit the growth of both human and murine tumor cells in vitro at concentrations ranging from 7×10^{-8} to 3×10^{-5} M. When used at 40 and 75 mg/kg, respectively, administered twice per day (8 h apart) for 2 days beginning 24 h after tumor inoculation, the compounds produced long-term survivors (>120 days at this writing). Cytarazid and cytaramin were not susceptible to enzymatic deamination under conditions which completely converted *ara*-C to the biologically inactive *ara*-U. The fact that chemically synthesized 2′-azido- and 2′-aminoarabinofuranosyluracil[6] showed no inhibitory activity against leukemia L1210 in vitro demonstrates that the resistance of cytarazid and cytaramin to deamination is a major determinant in establishing their optimal biological effects.

0022-2623/78/1821-0597$01.00/0

Deamination Assay. CR-CdR deaminase was prepared from two different sources: (1) human liver following the procedure of Wentworth and Wolfenden;[5] (2) AML blast cells,[4] by ammonium sulfate fractionation and DEAE column chromatography. The assay procedure has been published previously.[5] Under these conditions 50% of *ara*-C was deaminated in 45 min. Using the same assay conditions, no significant deamination of cytaramin or cytarazid was detected in 8 h.

Acknowledgment. This investigation was supported in part by Grants CH-55B from the American Cancer Society and CA-13038, CA-18499, and CA-12585 from the National Cancer Institute.

References and Notes

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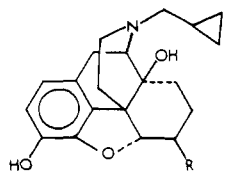
Received February 24, 1978

6β-[*N*,*N*-Bis(2-chloroethyl)amino]-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan (Chloranaltrexamine), a Potent Opioid Receptor Alkylating Agent with Ultralong Narcotic Antagonist Activity

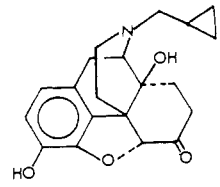
Sir:

Agents which selectively form covalent bonds with opioid receptors, both in vivo and in vitro, would find wide use as pharmacologic probes and might prove to be of considerable clinical value as ultralong-acting narcotic antagonists. For these reasons there have been a number of attempts to develop such drugs.[1-7] In this communication we report on the first example of such a compound, 6β-[*N*,*N*-bis(2-chloroethyl)amino]-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan (**1**), which we have named chlornaltrexamine (CNA).



1 (CNA), R = $N(CH_2CH_2Cl)_2$
3, R = $N(CH_2CH_2OH)_2$
4, R = NH_2

The design rationale involved modification of naltrexone (**2**),[8] a powerful narcotic antagonist, by attachment of a



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reactive moiety to C-6. This position was selected for modification because the introduction of groups at this location does not abolish antagonist activity.[9-11]

Addition of divided amounts of $NaCNBH_3$[12] over a period of 90 h to a mixture of **2**·HCl, diethanolamine, and molecular sieves in methanol[13] maintained at ambient temperature afforded **3** (44%),[14] which was purified by dry column chromatography (silica gel, 90:10:4 EtOAc–MeOH–NH_4OH) and was crystallized (MeOH–Et_2O) as **3**·2HCl: mp 205–207 °C; $[\alpha]_D$ −133.4° (*c* 0.5, MeOH). Anal. ($C_{24}H_{36}N_2O_5Cl_2$·0.5MeOH) C, H, N. An alternate synthesis of this intermediate involved the reaction of the 6β-amino compound **4**[11] with ethylene oxide.[15] The conversion of **3**·2HCl into **1**·2HCl (42%) was effected by a modification of the triphenylphosphine–CCl_4 procedure[16] using DMF as solvent. After the mixture was kept at 4 °C for 16 h, the residue (free of DMF) was purified by extraction (EtOAc) and dry column chromatography (silica gel, 98:2 Et_2O–NH_4OH). Pure **1**, EIMS *m/e* 468 (M^+), TLC R_f 0.63 (silica gel, 100:1 Et_2O–NH_4OH), was converted to the dihydrochloride salt: mp 185–195 °C; $[\alpha]_D$ −126° (*c* 0.5, MeOH). Anal. ($C_{24}H_{34}N_2O_3Cl_4$) C, H, N, Cl.

Using the tail-flick assay[17,18] CNA had no analgesic effect of its own at doses of 0.6, 1.2, 2.4, and 4.8 nmol/mouse 2 h after icv[19] injection. A dose of 4.8 nmol/mouse of CNA produced analgesia in 18% of the mice when tested 10 and 20 min after the injection. This analgesic effect was no longer apparent after 60 min. Saline controls had no effect. This same dose of CNA (4.8 nmol/mouse) also had a lethal effect in 12% of the animals injected which was manifested within 1.5 h of the injection. The only other dose with lethal effects was the 2.4 nmol/mouse dose which caused death in 2% of the animals.

Analgesia[17,18] was measured 30 min after sc morphine injection and 2 h after icv injection of either saline, naltrexone (2.4 nmol/mouse), or CNA (0.6, 1.2, or 2.4 nmol/mouse). In contrast to naltrexone which showed no residual inhibition of morphine-induced analgesia 2 h after icv injection, CNA exhibited significant dose-dependent inhibition. Doses of 0.6, 1.2, and 2.4 nmol/mouse of CNA increased the ED_{50} of morphine of 5.0 (3.5–7.2) mg/kg by 7-, 66-, and 179-fold,[20] respectively. In addition, the antagonistic property was apparent as long as 3 days after a single icv injection of the antagonist but could not be detected after 6 days (Figure 1). In preliminary studies, CNA was also an effective antagonist when administered parenterally and the effect lasted about the same duration as that after icv administration.

To show that CNA was inhibiting morphine analgesia by binding to the same receptor system, naloxone pretreatment was demonstrated to block the effect of icv injected CNA on morphine analgesia when it was tested 24 h after CNA administration. In control animals a threefold increase in morphine ED_{50} was observed 24 h after CNA treatment. Naloxone had no residual antagonistic effect at 24 h after administration. The dose required to block the effect of CNA (1.2 nmol/mouse) was 150 mg/kg divided into three injections given in a span

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