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A study in the guinea-pig of the pharmacokinetics and pharmacodynamics of cytosine arabinoside

A.L. HARRIS (introduced by D.G. GRAHAME-SMITH)

MRC Unit and University Department of Clinical Pharmacology, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE

Cytosine arabinoside (Ara-C) is a nucleoside analogue with useful antileukaemic activity in man (Armentrout & Burns, 1974). However, it has a plasma half-life of 6–20 min (Momparler, 1974), being excreted in the urine and deaminated to an inactive metabolite – uracil arabinoside. Ara-C itself is inactive, but after phosphorylation to the active metabolite cytosine arabinoside triphosphate (Ara-CTP) inhibits DNA polymerase (Furth & Cohen, 1968). I have studied the relationsip *in vivo* in the guinea-pig between plasma Ara-C concentration and bone marrow intracellular Ara-CTP, and *in vitro* the relationship between Ara-C concentration, intracellular Ara-CTP and inhibition of DNA synthesis.

Twenty male Dunklin Hartley guinea-pigs, weight 350–450 g, were injected i.v. through indwelling jugular venous cannulae with [^{3}H]-Ara-C 2 mg/kg. This dose produces bone marrow depression when given daily i.v. for seven days. Serial blood samples were taken to measure plasma Ara-C levels. At various times up to 5 h after injection the animals were killed and bone marrow Ara-CTP measured. [^{3}H]-Ara-C was measured after separation on Ag50W-1X4 200–400 mesh ion-exchange columns. [^{3}H]-Ara-CTP was measured following separation on PEI ion-exchange plates or by column chromatography on Ag 1 × 8 200–400 mesh columns.

For *in vitro* studies, guinea-pig bone marrow was suspended in Eagle's medium and incubated with 100 nM [^{3}H]-thymidine (TdR). The effect of Ara-C (10 nM to 100 μM) on the inhibition of TdR incorporation into DNA was studied. In a parallel series of incubations using [^{3}H]-Ara-C and unlabelled TdR, the [^{3}H]-Ara-CTP production was measured.

In vivo, the plasma Ara-C levels declined in a biphasic manner after the initial distribution phase. The first phase had a half-life of 27 ± 6 min and the second phase 60 ± 8 minutes. An hour after the injection of [^{3}H]-Ara-C the plasma Ara-C level had fallen to 439 ± 8.5 nM whearas the bone marrow Ara-CTP concentration had risen to 1.8 ± 0.34 μM, and then declined exponentially with a half-life of 143 ± 33 minutes. By 5 h the plasma Ara-C concentration was 62 ± 6 nM. *In vitro* this level of Ara-C produced 50 ± 3% inhibition of DNA synthesis. However, the bone marrow Ara-CTP level *in vivo* was 810 ± 90 nM and

this level *in vitro* produced 77 ± 3% inhibition of DNA synthesis. The duration of greater than 50% inhibition of DNA synthesis *in vivo* would be expected to be 5 h based on plasma Ara-C levels. However, based on intracellular Ara-CTP levels, DNA synthesis would be inhibited by more than 50% for 11 h, at which time plasma Ara-C would be expected to have no effect on DNA synthesis. It is apparent when considering dosage schedules of Ara-C in the treatment of leukaemia, the cellular pharmacokinetics and pharmacodynamics of Ara-CTP may be more important determinants than plasma Ara-C levels.

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The interaction of antibiotics with synthetic steroids in the rat

D.J. BACK, A.M. BRECKENRIDGE, M. CHALLINER, FRANCESCA E. CRAWFORD, M.L'E. ORME, P.H. ROWE & EILEEN SMITH

Department of Pharmacology & Therapeutics, University of Liverpool, Liverpool L69 3BX

Norethisterone and ethinyloestradiol are the synthetic steroids present in many combination type oral contraceptive preparations. Animal studies have established that both steroids undergo extensive biliary excretion, principally as glucuronides, and enterohepatic circulation (Steinetz, Meli, Giannina & Beach, 1967; Hanasono & Fischer, 1974; Smith, 1974). Neomycin has previously been reported to interfere with the enterohepatic circulation of radioactivity associated with the synthetic oestrogen mestranol, by affecting the viability of the gut microflora which are partly responsible for deconjugation (Brewster, Jones & Symons, 1977).

We have investigated the biliary excretion of labelled ethinyloestradiol and norethisterone both quantitatively and qualitatively. In addition, the effect of ampicillin and neomycin on the enterohepatic circulation has been studied using the 'linked rat' preparation previously described by Ladomery, Ryan & Wright (1967).

When administered intravenously (i.v.), 71.1% of a dose of [^{3}H]-ethinyloestradiol ([^{3}H]-EE_2; 10 μCi/kg; 10 μg/kg) and 76.0% of an i.v. dose of [^{3}H]-norethisterone ([^{3}H]-N; 10 μCi/kg; 125 μg/kg) were excreted in the bile of anaesthetized female rats in 4 hours. The majority of radioactivity appeared in the glucuronide fraction. Characterization (by thin layer chromatography, chemical transformation and recrystallization to a constant specific activity) of the hydrolysed glucuronide fraction obtained after giving [^{3}H]-EE_2 showed that approximately 10% of the administered dose was excreted as EE_2-glucuronide; the remainder of the glucuronide fraction contained conjugates of metabolites of EE_2. In contrast, the hydrolysed glucuronide fraction obtained after giving [^{3}H]-N contained only conjugates of metabolites of norethisterone and there was no evidence of direct conjugation of the steroid.

In the 'linked rat' preparation the bile duct cannula from a 'donor' rat is inserted into the duodenum of a 'recipient' rat. Of a dose of [^{3}H]-EE_2 administered to donor rats (i.v.), 15.4% was excreted in the bile of control recipient rats in 7 hours. When recipient rats were pretreated with either neomycin or ampicillin (100 mg body weight^{-1} day^{-1} for 5 days; orally), 5.2% and 6.0% of the dose appeared in bile respectively. With [^{3}H]-N, 13.2% of the dose was excreted in the bile of control recipient rats in 7 h and this was reduced to 3.6% in neomycin pretreated and 3.9% in ampicillin pretreated animals.

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