Relationship between protein binding and extravascular drug concentrations of a water-soluble drug, cytosine arabinoside[1]

M L Slevin MB MRCP
R C Woollard
T A Lister MD FRCP
A Johnston BSC
E M Piall BSC
P Turner MD FRCP
Imperial Cancer Research Fund Department of Medical Oncology and Department of Clinical Pharmacology, St Bartholomew's Hospital, London EC1A 7BE; and Division of Clinical Biochemistry University of Surrey, Guildford, Surrey

Summary: The degree of binding of a drug to plasma proteins has a marked effect on its distribution, elimination, and pharmacological effect. Since only the unbound fraction is available for distribution into extravascular space, the ratio of drug in cerebrospinal fluid (CSF) or saliva to that in plasma is often regarded as a physiological measure of the free fraction of a drug. CSF : plasma and saliva : plasma ratios of cytosine arabinoside (araC) have been measured in patients with acute leukaemia and found to be 0.1–0.28, implying a binding of 72–90%.

The protein binding of araC was measured by equilibrium dialysis in the plasma of patients with acute leukaemia at presentation. The mean binding ratio was 2.3 ± 6.8, implying that there was little or no protein binding. There was no correlation between alpha − 1 acid glycoprotein (AAG) levels and protein binding.

The low CSF and saliva : plasma araC ratios found, suggest that drugs such as araC which have low lipid solubility do not pass freely into extravascular space. Thus the CSF or saliva : plasma ratio cannot be considered a good physiological measure of protein binding for drugs with poor lipid solubility.

Introduction

Cytosine arabinoside (araC) remains one of the most effective drugs for the treatment of acute myelogenous leukaemia (AML). It is used in patients with central nervous system leukaemia by injection directly into the cerebrospinal fluid (CSF) via an Ommaya reservoir or lumbar puncture (Wang & Pratt 1970, Band *et al.* 1973). In addition, significant quantities of araC cross into the CSF when it is given by intravenous infusion in conventional doses (Ho & Frei 1971). Recently, massive doses of araC (30–60 times the conventional dose) have been used to treat relapsed and resistant patients with acute leukaemia. The extent to which araC crosses the blood-brain barrier during such high-dose therapy has been investigated (Slevin *et al.* 1982) and it has been shown that at equilibrium 10–26% of plasma araC concentrations are achieved in the CSF.

The extent to which a drug crosses into the CSF and saliva has been suggested as a physiological measure of the free fraction of the drug in plasma (Bertilsson *et al.* 1979, Piafsky 1980). The protein binding of araC has previously been measured by ultracentrifugation and ultrafiltration and found to be only 13% (van Prooijen *et al.* 1977). However, this study was performed on plasma from healthy volunteers, and might not be representative of patients with acute leukaemia. As araC is a weak base, it could bind principally to alpha − 1 acid glycoprotein (AAG) (Piafsky 1980). This is an acute phase protein, often increased in patients who are acutely ill and infected, and which might be raised in patients with acute leukaemia at presentation.

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The protein binding of araC was therefore investigated by equilibrium dialysis in plasma from healthy volunteers and patients with acute leukaemia at presentation. These results were then compared with the extent to which araC crosses into the extravascular spaces such as CSF and saliva.

Materials and methods

Patients

Six patients with AML undergoing remission induction or consolidation with chemotherapy, including continuous infusions of araC, were studied during therapy. Simultaneous saliva (2 ml) and blood (4 ml) samples were obtained on 3 occasions on the second or subsequent days of the araC infusion in 5 of these patients. In one patient simultaneous saliva and plasma samples were obtained at repeated intervals during the first 12 hours of the infusion to determine the time to equilibrium. Serum was obtained from 18 patients with AML at presentation and prior to therapy, and from 6 normal volunteers for the protein binding studies.

Sampling and assay

Saliva was collected by asking the patients to rinse their mouths with water and then stimulate saliva production by chewing a bland inert plastic material (parafilm). Blood and saliva samples for araC assay were taken into pre-cooled tubes containing tetrahydrouridine (10^{-5} mol/l) and 50 units of heparin. The samples were separated by centrifugation and stored at $-20°C$. Plasma and saliva araC concentrations were measured by radioimmunoassay (Piall *et al.* 1979). Serum samples for the protein binding studies were also separated by centrifugation and stored at $-20°C$.

Plasma protein binding

Tritiated araC (specific activity 1.54 GBq/mg) was supplied by the Radiochemical Centre, Amersham. Serum was placed in 1 ml acrylic cells and dialysed at 37°C against phosphate buffered saline at pH 7.3 (Dulbecco 'A' with mineral salt solutions: Oxoid Limited). The cells were separated by an 11.5 μm Cuprophan membrane and rotated at 45 r/min to ensure constant mixing. Each experiment was performed in duplicate. The araC concentrations in plasma and buffer dialysates were measured using a Packard Instrument PRIAS PLD scintillation counter with automatic quench correction by external standard. The quench curve was established using a standard concentration of araC (2 μl, 24 mg/ml; 74 MBq) in the scintillant mixture (4 ml Picofluor Packard Instrument) and varying concentrations (0–10% v/v) of picric acid to produce colour quenching.

The time to equilibrium and the influence of concentration on protein binding were determined in the serum from the normal volunteers. Thirty-six cells were used, 18 spiked with 200 ng araC (containing 10 ng C^{14} araC) on the buffer side and 18 on the serum side. Four cells were removed from the oven at the following times: 15, 35 and 45 minutes and 1, 2, 3, 4, 7 and 10 hours. The time to equilibrium was established by plotting buffer disintegrations per minute (DPM) over plasma DPM against time. The influence of araC concentration was studied using 9 concentrations: 20, 50, 100, 200 and 500 ng/ml and 1, 2, 5 and 10 μg/ml.

The serum obtained from AML patients at presentation and prior to any drug therapy was dialysed against 200 ng/ml of araC so that at equilibrium the plasma araC concentration would be in the conventional therapeutic range (100 ng/ml). Estimations were done in duplicate.

Total protein was estimated colorimetrically using Biuret reagent. Plasma albumin was measured by dyebinding (bromo-cresol green) and AAG was measured using M-Partigen immunodiffusion plates.

Results

Relationship between plasma and salivary araC

The saliva : plasma araC ratio in the 5 patients who had simultaneous samples taken during intravenous infusions of araC ranged from 0.05 to 0.45 with a mean value of 0.22 ± 0.14. In the single patient who had simultaneous saliva and plasma samples taken at repeated intervals throughout the first 12 hours of the intravenous infusion, equilibrium between plasma and salivary araC was established within 2.0 hours, and the mean saliva: plasma ratio during the infusion was 0.28.

Protein binding studies

The *in vitro* equilibrium dialysis studies in the serum taken from the 6 normal volunteers demonstrated rapid equilibration between serum and buffer araC (2.5 hours), and no effect of concentration on protein binding was seen over a range of 20 ng to 10 μg/ml. In serum taken from 18 patients with AML at presentation, protein binding ranged from −9.6 to 15.0 with a mean of 2.3 ± 6.8, demonstrating that protein binding of araC is negligible in patients with AML.

Serum protein concentrations in patients with AML

In the majority of patients alpha − 1 acid glycoprotein levels fell within the normal range (0.1–1.4 g/l), with a range of 0.43–1.92 g/l and a mean value of 1.05 ± 0.43 g/l. Five patients had values above 1.4 g/l. Albumin and total protein were, however, below the normal range in many of the patients, with a mean of 33.5 ± 6.8 and 61 ± 17.1 g/l respectively. There was no correlation between araC protein binding and the level of AAG in individual patients.

Discussion

This *in vitro* assessment of protein binding using equilibrium dialysis confirms that there is little or no binding of araC to proteins in patients with AML. Although some subjects did have abnormally-high levels of AAG, no correlation was found between AAG levels and protein binding.

AraC is a weak base and has a pKa of 4.3. Thus at physiological pH it will be almost entirely non-ionized, which should aid its ability to cross lipid membranes. It is also a relatively small molecule with a molecular weight of 243. The theoretical equilibrium across lipid membranes for weak bases such as araC is described by equation 1 (Matin *et al.* 1974, Taylor *et al.* 1981).

$$R = \frac{1 + 10^{(pKa - pHCSF)}}{1 + 10^{(pKa - pHp)}} \times \frac{fp}{fCSF}$$

where R = concentration ratio between CSF or saliva and plasma; pKa = pKa of drug; pHp = pH of plasma (assumed to be 7.40) or saliva (assumed to be 6.5); pHCSF = pH of CSF (7.32 to 7.37); fp = fraction of drug unbound to plasma proteins; fCSF = fraction of drug unbound to CSF proteins (assumed to be 1.0).

If this equation is applied to araC, both CSF : plasma and saliva : plasma ratios should approximate 1. However, it has previously been shown that at equilibrium a mean CSF : plasma araC ratio of 0.12 was achieved (Slevin *et al.* 1982) and the mean saliva : plasma ratio presented here is only 0.22.

There are two possible explanations for the low concentrations of araC measured in the extravascular fluids compared to those predicted by this equation. Firstly, equilibrium may not have been achieved. There is little data on the time taken to reach equilibrium between the CSF and plasma during continuous infusions of araC. However, the data from Ho & Frei (1971) and Slevin *et al.* (1982) suggested that when a short infusion was preceded by a loading dose, equilibrium was rapidly achieved. Furthermore, the simultaneous plasma and saliva samples were taken from patients who had received continuous infusions of araC for at least 24 hours.

Secondly, araC is a highly water-soluble drug with a very low oil-water partition coefficient, and this equation does not take lipid solubility into account. A study by Taylor *et al.* (1981) examined the predicted and measured CSF : plasma ratios for three betablockers. For propranolol, a highly lipid-soluble betablocker, and pindolol, of intermediate lipid solubility, the measured CSF : plasma ratios were very close to those predicted. However, for atenolol, which has a very low oil-water partition coefficient, the measured values were only 20% of those predicted. It seems likely, therefore, that the poor lipid solubility of araC is the major factor restricting its entry into extravascular fluids.

The extravascular concentrations of drugs may well represent a physiological measure of the non-protein bound fraction of lipid-soluble drugs in plasma. However, the large discrepancy between the measured extravascular concentrations of water-soluble drugs and those predicted from a knowledge of the free fraction in plasma, suggests that these ratios are not a reliable measure of the free fraction of lipid-insoluble drugs.

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