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Pharmacokinetics and Pharmacodynamics of Hydroxyurea

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Summary

Hydroxyurea is used in the treatment of various forms of cancer, sickle-cell anaemia and HIV infection. Oral absorption of the drug is virtually complete, the volume of distribution is equivalent to total body water and elimination is through both renal and nonrenal mechanisms. Nonrenal elimination of hydroxyurea is characterised by Michaelis-Menten kinetics.

Further studies are necessary to clarify several aspects of the pharmacokinetics and pharmacodynamics of hydroxyurea: the effect of age and disease state, concentration–effect relationship, the role of therapeutic drug monitoring, and the mechanisms of renal and nonrenal elimination. The recent development of improved assays for hydroxyurea should have benefits for future pharmacokinetic studies.

Hydroxyurea was originally synthesised in Germany in 1869 by Dressler and Stein.[1] But it was not until 1928 that it was first administered to animals (by Rosenthal et al.[2]) and observed to produce leucopenia, macrocythaemia, anaemia and death. Later, in 1963, Stearns et al.[3] found hydroxyurea

to be active against L1210 mouse leukaemia and subsequent phase I trials showed the drug to have significant activity against a number of malignancies. Its principal mechanism of cytotoxicity, the ability to inhibit DNA synthesis, was established shortly afterwards, in 1965.[4] Other mechanisms for the cytotoxicity of hydroxyurea have subsequently been proposed.[5] These include direct damage to DNA (possibly as the free radical) and inhibition of repair of spontaneous DNA lesions; however, inhibition of DNA synthesis is still regarded as the principal mechanism of cell death.

Hydroxyurea is a hydroxylated analogue of urea. It inhibits DNA synthesis by inhibiting the activity of ribonucleotide reductase[5,6] which transforms ribonucleotides into deoxyribonucleotides. This enzyme is synthesised in low amounts and is the only highly regulated enzyme involved in the conversion of ribonucleotide precursors to DNA.[7] Thus, it forms the rate-limiting step in the *de novo* synthesis of DNA. The enzyme consists of 2 protein dimers, M1 and M2. Dimer M1 contains binding sites for the ribonucleotide substrates. The M2 dimer is the catalytic subunit and includes a tyrosine free radical stabilised by a nonhaem iron complex. The stabilised tyrosine free radical functions to abstract a hydrogen atom from the ribonucleotide substrate.[8] Hydroxyurea inhibits enzyme activity by a 1-electron transfer from hydroxyurea to the enzyme-bound tyrosine radical.[9] It has also been suggested that hydroxyurea may destabilise the nonhaem iron centre, thereby inactivating the M2 catalytic subunit.[9]

It appears that the hydroxyurea-inactivated enzyme can regenerate spontaneously upon removal of hydroxyurea.[10] Unlike a number of the antineoplastic agents, the pharmacological action of hydroxyurea is, therefore, highly dependent upon the concentration-time course of the drug in the body (i.e. its pharmacokinetics). Furthermore, because of its mechanism of action, hydroxyurea principally affects cells actively synthesising DNA. In this respect, the drug is cell-cycle specific for the S-phase. Because of the highly regulated nature of ribonucleotide reductase, the short transit time of the S-phase of the cell cycle and the relatively rapid elimination of hydroxyurea, optimal drug therapy is likely to be achieved by multiple (or continuous) drug administration which maintains hydroxyurea concentrations above the concentration of drug that inhibits activity by 50% (IC_{50}) (approximately 0.5 mmol/L)[11] for ribonucleotide reductase.

1. Analytical Methodology

Most of the literature describing the pharmacokinetics of hydroxyurea has relied upon spectrophotometric or colorimetric assay methods. These have evolved since the earliest method described by Davidson and Winter.[12] The spectrophotometric assay currently used was developed by Philips et al.[13] and modified by Fabricius and Rajewsky.[14] Using this assay, hydroxyurea in plasma is reacted with iodine and the products are then coupled with a chromophore that absorbs at 540nm. The authors reported a limit of detection of 0.033 mmol/L. Similar limits of detection were reported by Tracewell et al.[15] (0.01 mmol/L) and Veale et al.[16] (0.01 mmol/L). Because the concentration of hydroxyurea required to be effective *in vivo* is much greater than the assay limit, the lack of assay sensitivity does not appear to pose a problem in determining the pharmacokinetics of hydroxyurea in humans or animals.

Perhaps more troubling is the uncertainty regarding the specificity of this assay. While urea does not interfere with the assay, a lack of knowledge regarding the metabolites of the drug raises concerns about their contribution, particularly in light of the chemical treatment of the sample.

Havard et al.[17] have reported a high performance liquid chromatography (HPLC) assay with electrochemical detection (ED) for use in pharmacokinetic studies. The reported limit of detection is similar to that of the method of Fabricius and Rajewsky.[14] Villani et al.[18] recently used this assay, with minor modifications, to study the pharmacokinetics of hydroxyurea in patients with HIV infection. The limit of detection was reported as 0.0006 mmol/L. The reason for the marked difference in the limits of detection between the 2 assays

is not apparent. Villani et al.[18] included the use of solid phase extraction columns as part of serum sample preparation, perhaps removing an interfering substrate. A recently published article also describes a method of determination for hydroxyurea using HPLC with ED.[19] The limit of quantitation in plasma was 25 μg/L (approximately 0.33 μmol/L), which confirms the sensitivity reported by Villani et al.,[18] and in peritoneal fluid was 5 μg/L (approximately 0.066 μmol/L).

El-Yazigi and Al-Rawithi[20] used capillary gas chromatography with thermionic nitrogen-phosphorus specific detection to study the stability of hydroxyurea in aqueous solution. The sensitivity of the assay (0.07 mmol/L) appears to be no greater than the existing assays.

2. Pharmacokinetics

Detailed, formal assessments of the pharmacokinetics of hydroxyurea in animals and humans have only recently appeared in the literature. The recently published articles coupled with the observations of earlier researchers provide considerable information regarding the absorption and disposition of hydroxyurea. This is discussed below.

2.1 Absorption

Hydroxyurea crosses the intestinal wall by passive diffusion.[21] Based upon the physicochemical characteristics of hydroxyurea [i.e. freely water soluble, log partition coefficient of –1.27, lack of ionisation in the gastrointestinal tract (pKa = 10.6)], it is anticipated that at therapeutic oral doses of 20 to 30 mg/kg, the drug is reasonably well absorbed. This has been shown in studies on animals and humans. Following oral administration of hydroxyurea to mice and rats, Adamson et al.[22] recovered only 0.1 and 0.8%, respectively, of the drug in faeces.

Pharmacokinetic studies in the rat gave an oral bioavailability of 73%.[23] In patients with cancer, hydroxyurea was found to be 79% available to the systemic circulation following oral administration.[15] Previous clinical studies support fairly complete bioavailability as plasma concentrations are comparable following long term intravenous and oral administration.[24]

2.2 Distribution

Hydroxyurea enters cells via passive diffusion.[25] Tissue concentrations of hydroxyurea are in rapid equilibration with those in the blood and the time-course of hydroxyurea concentrations in tissues, including transplantable solid animal tumours, parallel hydroxyurea blood concentrations.[14] Thus, blood concentrations appear to reflect tumour-tissue hydroxyurea concentrations. Hydroxyurea also rapidly diffuses into tissues such as the brain.[26]

Pharmacokinetic analysis in both rats and humans indicate that hydroxyurea has a volume of distribution approximately equal to total body water.[15,23] The extent of binding to proteins in the blood has not been published. Some insight into this interaction might be gained by considering the findings of Beckloff et al.[27] These investigators measured hydroxyurea concentrations in both ascites fluid (n = 6) and cerebrospinal fluid (n = 3) simultaneously with serum hydroxyurea concentrations in humans. Because hydroxyurea appears to rapidly equilibrate with body tissues and fluids, and ascites and cerebrospinal fluid are low in protein content, these ratios can be considered to represent ratios of unbound drug to total drug (i.e. both bound and unbound) in the serum.

The ratio of ascites fluid to serum hydroxyurea concentrations (mean ± standard deviation) was 0.25 ± 0.15 and cerebrospinal fluid to serum was 0.20 ± 0.07. This suggests that hydroxyurea is 75 to 80% bound to serum proteins. It should also be noted that the serum concentration range studied in this report varied from 12.4 to 156.8 mg/L without any indication of nonlinearity in the apparent fraction unbound. In contrast with these results, however, are those of P.R. Gwilt who studied the *in vitro* binding of hydroxyurea in human serum and found that hydroxyurea is not appreciably bound (unpublished results).

2.3 Metabolism

A significant fraction of hydroxyurea is eliminated from the body by nonrenal mechanisms. It has been assumed that this fraction primarily represents hepatic metabolism. Little work has been performed, however, to verify this or to characterise the products of metabolism. Adamson et al.[22] noted that between 30 and 50% of an intraperitoneally administered hydroxyurea dose was recovered in the urine as urea. They further demonstrated that hydroxyurea was reduced to urea in several tissues in the mouse. The most efficient biotransformation occurred in the liver and kidneys. Colvin and Bono[28] showed that the conversion of hydroxyurea to urea in mouse liver was mediated by the mono-oxygenase system located in the liver mitochondria. Andrae[29] has also established the importance of hepatic cytochrome P450 (CYP) mono-oxygenase in the metabolism of hydroxyurea.

Davidson and Winter[12] demonstrated degradation of hydroxyurea by urease, possibly producing hydroxylamine. In mammals, urease appears to be limited to the gut, and hydroxylamine has not been observed in the blood. Fishbein and Carbone[30] proposed that hydroxylamine is produced *in vivo* but is subsequently rapidly methylated by acetylcoenzyme A to produce acetohydroxamic acid. In support of this theory, these investigators were able to measure acetohydroxamic acid in the blood of 3 patients with chronic myelogenous leukaemia and to estimate that between 1 and 10% of administered hydroxyurea is converted to acetohydroxamic acid.

In view of the widespread use of this drug and the diversity of indications, it is unfortunate that a detailed analysis of the biotransformation of hydroxyurea, using modern analytical techniques, has not appeared in the literature since Colvin and Bono[28] reported their work in 1970.

2.4 Elimination

As described in section 2.3, hydroxyurea is eliminated, in part, by metabolism to urea and other products. Hydroxyurea has also been recovered unchanged in the urine. In both rats and humans, hydroxyurea renal clearance has been determined to be 75% of the glomerular filtration rate (GFR).[15,23] In the case of humans, with an average GFR of 7.2 L/h, renal clearance is about 5.4 L/h. Renal clearance, in rats at least, is independent of dose over a wide dose range.[23]

2.5 Pharmacokinetic Models and Parameters

A nonlinear relationship between plasma hydroxyurea concentrations and dose is apparent in virtually every animal and human pharmacokinetic study reported, whether the study describes single or continuous hydroxyurea administration. In many cases, linearity has been claimed, but upon closer inspection, the pharmacokinetics are seen to be more consistent with Michaelis-Menten elimination. For example, a recent study of the pharmacokinetics of hydroxyurea in mice claimed a linear relationship between hydroxyurea plasma concentration and dose.[31] However, the regression line relating concentration to dose had a markedly negative intercept on the ordinate, consistent with saturable elimination. A linear relationship would have been characterised by an intercept not different from zero.

Nonlinearity is also evident from clinical trial data (table I). Beckloff et al.[27] reported plasma hydroxyurea concentrations following both oral doses of 20 and 80 mg/kg. The area under the plasma concentration-time curves (AUC) for hydroxyurea differed by a factor of more than 6 rather than by the factor of 4 which would be predicted by linear kinetics. In the study by Belt et al.,[24] hydroxyurea was infused to steady state at rates varying from 2.0 to 3.5 mg/min/m^2. A plot of the resulting steady-state plasma concentrations versus infusion rates is curvilinear (concave up), whereas linear elimination kinetics would result in a straight line relationship.

More recently, Charache et al.[34] reported plasma concentrations in patients with sickle-cell anaemia receiving oral doses ranging from 10 to 35 mg/kg. Despite assertions by these authors of a linear

Table I. Summary of studies of the pharmacokinetics of hydroxyurea

Dose (route)	Assay[a]	n	$t_{1/2}$ (h)	Vd	C_{max} and C_{min}	CL and CL_R	F (%)	Reference
20 mg/kg (oral)	Analytical technique by Davidson and Winter[12] used	4	3.4[b]		C_{max} = 276.3 mmol/L			27
80 mg/kg (oral)		10	3.9[b]	V/F = 0.48 L/kg[b]	C_{max} = 1684 mmol/L	CL/F = 114.5 ml/min[b]		
60 mg/kg (oral)	Analytical technique by Davidson and Winter[12] used	7	3.9[b]	V/F = 1.62 L/kg[b]	C_{max} = 381.6 mmol/L	CL/F = 360.4 ml/min[b]		32
100 mg/kg (oral)		6	3.9[b]	V/F = 0.74 L/kg[b]	C_{max} = 1526.3 mmol/L	CL/F = 153.2 ml/min[b]		
100 mg/kg (oral)	Analytical technique by Fabricius and Rajewsky[14] used	1	1.9[b]	V/F = 0.75 L/kg[b]	C_{max} = 1300 mmol/L	CL/F = 316.0 ml/min[b]		14
500 mg/m^2 q4h (oral)	Analytical technique by Fabricius and Rajewsky[14] used	3			$C_{max,ss}$ = 990 mmol/L $C_{min,ss}$ = 460 mmol/L			24
800 mg/m^2 q4h (oral)		4			$C_{max,ss}$ = 2480 mmol/L $C_{min,ss}$ = 550 mmol/L			
2.0 mg/m^2/min (continuous infusion for 72h)		5	4.1		C_{ss} = 488 mmol/L	CL = 53.8 ml/min/m^2[c]		
2.25 mg/m^2/min (continuous infusion for 72h)		2	4.45		C_{ss} = 460 mmol/L	CL = 64.3 ml/min/m^2[c]		
2.5 mg/m^2/min (continuous infusion for 72h)		4	3.75		C_{ss} = 537 mmol/L	CL = 61.3 ml/min/m^2[c]		
2.75 mg/m^2/min (continuous infusion for 72h)		1	3.67		C_{ss} = 760 mmol/L	CL = 47.7 ml/min/m^2[c]		
3.0 mg/m^2/min (continuous infusion for 72h)		7	4.27		C_{ss} = 1090 mmol/L	CL = 36.2 ml/min/m^2[c]		
1000 mg/h q6h (oral)	Analytical technique by Fabricius and Rajewsky[14] used	18 (9 oral and 9 IV infusion)			$C_{max,ss}$ = 2000 mmol/L $C_{min,ss}$ = 1000 mmol/L			16
1000 mg/h (IV infusion for 48h)					$C_{ss,av}$ = 1730 mmol/L	CL = 126.8 ml/min		
1 g/m^2/day (continuous infusion for 120h) [n = 3]	Analytical technique by Fabricius and Rajewsky[14] used	18	2.8		C_{ss} = 93 μmol/L	CL_R = 2.7 L/h/m^2; CL_{NR} = 3.6 L/h/m^2		33
2 g/m^2/day (continuous infusion for 120h) [n = 9]			3.2		C_{ss} = 230 μmol/L	CL_R = 1.8 L/h/m^2; CL_{NR} = 3.0 L/h/m^2		

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Table I. Contd

Dose (route)	Assay[a]	n	$t_{1/2}$ (h)	Vd	C_{max} and C_{min}	CL and CLR	F(%)	Reference
3.2 g/m^2/day (continuous infusion for 120h) [n = 6]			3.7		C_{ss} = 302 μmol/L	CL_R = 2.6 L/h/m^2; CL_{NR} = 4.0 L/h/m^2		
20 mmol/m^2 q6h (oral)	Analytical technique by Fabricius and Rajewsky[14] used	8	dependent on plasma concentration range	Vd = 0.186 × bodyweight (kg) + 25.4L		CL_{NR}: V_{max} = 3.71 mmol/h (or 95.6 μmol/h/L) Km = 0.32 mmol/L	79	15
1.9-24.3 mmol/h (IV infusion every 24 or 48h)		46	1.6-4.2			CL_R = 90.8 ml/min		

a All assays were performed using spectrophotometric methods.

b The pharmacokinetic parameters were obtained by extracting the data from figures in the text referenced and fitting the data to a pharmacokinetic model using PCNonlin (Statistical Consultants Inc., Apex, North Carolina, USA).

c Clearance was determined by dividing the infusion rate by the steady-state hydroxyurea plasma concentration.

Abbreviations: C_{max} = maximum plasma drug concentration; $C_{max,ss}$ = maximum drug plasma concentration at steady state; C_{min} = minimum drug plasma concentration; $C_{min,ss}$ = minimum drug plasma concentration at steady state; C_{ss} = concentration at steady state; $C_{ss,av}$ = average concentration at steady state; CL = total body clearance; CL_R = renal clearance; CL_{NR} = total nonrenal drug clearance; CL/F = apparent clearance; F = bioavailability; h = hours; IV = intravenous; Km = Michaelis-Menton constant; n = number of patients; $t_{1/2}$ = half-life; Vd = volume of distribution; V/F = apparent volume of distribution.

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relationship between dose and plasma concentration, the figure used to demonstrate the relationship is markedly curvilinear, concave up. Since renal clearance is linear with dose, the nonlinear pharmacokinetics might be attributable to saturable metabolism.

Two recent studies using relatively low dosages of hydroxyurea demonstrate apparent linear pharmacokinetics. Villani et al.[18] administered oral doses of hydroxyurea 500mg every 12 hours for 4 weeks to 9 patients with HIV-1 infection. Serum hydroxyurea was measured between 1 and 4 weeks after the start of hydroxyurea therapy. A mean maximum serum concentration of 135 μmol/L hydroxyurea was obtained and the pharmacokinetics of the drug were satisfactorily described by a linear, 1-compartment model. Newman et al.[33] administered hydroxyurea by continuous infusion for 120 hours at 3 dosage levels (1.0, 2.0 and 3.2 g/m^2/day). The average steady-state hydroxyurea plasma concentrations were 93, 230 and 302 μmol/L, respectively, reflecting a linear relationship between hydroxyurea infusion rate and steady-state concentrations. Both of these studies reported hydroxyurea concentrations below the Michaelis-Menten constant value of 307 μmol/L reported by Tracewell et al.,[15] although the second study clearly approaches that value.

Based upon both animal and human data, the most appropriate pharmacokinetic model for hydroxyurea requires elimination to be described by parallel Michaelis-Menten nonrenal elimination and linear renal elimination.[15,23] Furthermore, such a model predicts that a low hydroxyurea dose, the plasma concentration-time curve would be log linear (i.e. linear on a semi-log scale) because the nonrenal elimination pathway would be below the level of saturation.[18,33] At an intermediate dose, the concentration-time curve would be nonlinear on a semi-log scale because of saturation of the nonrenal pathway.[15,24] At a high dose, the shape of the concentration-time curve would revert to linear on a semi-log scale as the contribution of the nonrenal pathway is saturated and contributes less to the overall elimination of hydroxyurea.[23] This

also suggests that renal function is particularly important in patients receiving high dosages of hydroxyurea therapy. This model was successfully used to fit plasma and urine hydroxyurea data in rats,[23] as well as plasma hydroxyurea obtained from 54 patients receiving escalating infusions and oral doses of hydroxyurea.[15] It was not successful, however, in accurately predicting plasma hydroxyurea concentrations in those patients receiving the fastest infusions (>14 mmol/h). The reason for this appeared to be because of the large variance in renal function in these patients; however, including creatinine clearance as a covariate in the model did not result in a statistically significant difference.[15]

2.6 Regional Delivery

Pharmacokinetic theory suggests that regional delivery is advantageous only when the arterial blood flow to the target tissue is much smaller than the total body clearance of the drug.[35] The maximum total body clearance of hydroxyurea in humans can be calculated to be about 18 L/h (table I). As most tissues have blood flows of this order or greater,[36] regional delivery is not likely to offer any significant therapeutic advantage.

2.7 Drug Interactions Affecting Disposition and/or Effects

No studies of pharmacokinetic interactions between hydroxyurea and other drugs appeared in the literature. However, modulation by hydroxyurea of the cytotoxic effects of other antineoplastic agents has been reported. Hydroxyurea influences the activity of other anticancer drugs by 1 of 3 mechanisms:[37]

- by depleting the cellular pool of deoxyribonucleotides, the activities of pyrimidine and purine antimetabolites are enhanced
- by inhibiting DNA repair, the activities of topoisomerase inhibitors and alkylating agents are increased
- by accelerating the loss of extrachromosomal amplified genes, hydroxyurea may reverse acquired drug resistance to several anticancer drugs.

The first interaction is represented by combination therapy with cytarabine. Through hydroxyurea depleting endogenous pools of deoxycytidine triphosphate, the uptake of cytarabine, its phosphorylation to cytosine arabinoside triphosphate (ara-CTP), the binding of ara-CTP to DNA polymerase, and subsequent incorporation into DNA was increased.[38,39] The combination of the 2 drugs in a phase II clinical trial involving patients with refractory malignant lymphoma resulted in a 43% response rate.[40]

Enhancement of the activity of fluorouracil by hydroxyurea has also been reported.[41] One mechanism by which fluorouracil causes cytotoxicity is inhibition of thymidylate synthetase. The natural substrate for this enzyme is deoxyuridine monophosphate. Fluorouracil produces a metabolite, fluorodeoxyuridine monophosphate, which competes with the natural substrate for thymidylate synthetase but forms a covalent ternary complex. Hydroxyurea reduces the pool of deoxyuridine monophosphate and thus increases the amount of complex formed, leading to greater inhibition of thymidylate synthetase and reduced DNA synthesis. This interaction is particularly effective in patients who have developed resistance to fluorouracil because of an accumulation of deoxyuridine monophosphate.[41]

The same rationale supports the combined use of hydroxyurea and antiviral agents such as zidovudine (azidothymidine) and didanosine to inhibit viral DNA synthesis in patients with HIV infection. However, the interaction of hydroxyurea with fluorouracil may be less predictable than with these other agents because 1 of the metabolic pathways from fluorouracil to fluorodeoxyuridine monophosphate requires ribonucleotide reductase,[42] which is inhibited by hydroxyurea.

The second type of interaction is thought to involve the inhibition of DNA repair by hydroxyurea.[43] In a report by Minford et al.,[44] hydroxyurea enhanced protein-associated DNA strand cleavage produced by amsacrine. In another study,[45] synergism was observed when hydroxyurea was administered with etoposide. Finally, a

combination of cytarabine and hydroxyurea with cisplatin produced persistence in DNA interstrand crosslinks, suggesting inhibition of DNA repair.[46] The mechanisms that characterise hydroxyurea modulation of other anticancer drugs suggest that the scheduling of their administration with respect to hydroxyurea is critical to realise optimal modulation.

The third type of interaction relates to accelerated loss of extrachromosomal-amplified genes upon continuous exposure to hydroxyurea. These genes are responsible for the overexpression of: (i) dihydrofolate reductase, resulting in methotrexate-resistant cells; (ii) MDR1 (a multidrug resistance gene responsible for production of *P*. glycoprotein) in vinblastin-resistant cells; and (iii) carbamylphosphate synthetase, aspartate transcarbamylase and dihydro-orotase in N-(phosphonacetyl)-L-aspartic acid-resistant cells. Thus, in resistant cells, hydroxyurea potentiates the activity of the aforementioned drugs.[47-49]

3. Pharmacodynamics

3.1 Efficacy and Toxicity

The principal therapeutic use for hydroxyurea in cancer chemotherapy is the treatment of myeloproliferative disorders such as chronic myelogenous leukaemia and polycythaemia rubra vera.[50,51] The effectiveness of hydroxyurea has also been evaluated in the treatment of solid tumours such as malignant melanoma, refractory ovarian cancer, squamous cell carcinoma of the head and neck, renal cell carcinoma, transition cell carcinoma of the urinary bladder[52,53] and advanced prostate cancer.[54] The response rates of these malignancies is low and hydroxyurea is not part of standard chemotherapy for any solid tumour.

Hydroxyurea has proved to be an effective radiation sensitiser and has been employed in this role in the treatment of head and neck cancer.[55] Hydroxyurea has also been used with some success in advanced cervical carcinoma, producing an increase in response and survival with concurrent therapy.[56,57]

A potential clinical use for hydroxyurea is in the management of drug resistance. It has been demonstrated that hydroxyurea can accelerate the elimination of extrachromasomally-amplified genes with a corresponding increase in drug sensitivity.[47-49]

Hydroxyurea is also indicated in diseases other than cancer. It is now the principal drug used to treat sickle-cell anaemia[34] (see section 4.1). Hydroxyurea is also effective in the treatment of psoriasis[58] and, recently, hydroxyurea has been found to inhibit HIV-1 replication at relatively low concentrations in patients with AIDS[59] (also discussed in section 4.2).

The dose-limiting toxicity of hydroxyurea is myelosuppression, with leucopenia being predominant.[60] Since nonhaematological toxicity is usually mild, inclusion of the drug in therapy requiring bone marrow transplantation is reasonable and is currently under investigation.[61] Patients receiving hydroxyurea may experience nausea and vomiting accompanied by either diarrhoea or constipation.[62] Large oral doses of the drug may cause ulceration and occasionally stomatitis. Long term administration of hydroxyurea also leads to dermatological changes, such as hyperpigmentation and erythemia of the hands and face.[62] Rare complications include nephrotoxicity, neurotoxicity and elevation of hepatocellular enzymes.[62] Hydroxyurea is also teratogenic.[63]

3.2 Concentration-Effect Relationships

The cytotoxic effects of hydroxyurea correlate with dose or concentration, as well as with duration of drug exposure.[64,65] A 1 mmol/L concentration of hydroxyurea will inhibit DNA synthesis in most mammalian cells.[64] Hydroxyurea concentrations measured in a transplanted mammary tumour in the rat equalled those in the blood.[14] It is likely then that monitoring blood hydroxyurea concentrations to maintain drug concentrations equal to, or above, 1 mmol/L would optimise and individualise hydroxyurea chemotherapy. Several investigators have demonstrated that drug blood concentration of 1 mmol/L can be maintained without undue ad-

verse effects using prolonged infusion.[16,24,66] Clinical trials are currently underway to study the therapeutic advantages of monitoring and adjusting hydroxyurea blood concentrations during infusion therapy to maintain a minimum concentration of 1 mmol/L hydroxyurea.

Therapeutic monitoring may also be desirable in the use of hydroxyurea to reverse drug resistance because of gene amplification, since concentrations of hydroxyurea above 0.3 mmol/L reportedly increase gene amplification.[67]

4. Hydroxyurea in Diseases Other Than Cancer

4.1 Sickle-Cell Anaemia

Indications of changes in the pharmacokinetics of hydroxyurea in individuals with altered physiological or pathological states are suggested by those investigating the use of hydroxyurea in the treatment of sickle-cell anaemia. In 1 study,[34] the mean AUC of hydroxyurea measured over 6 hours following oral ingestion (AUC_6) correlated with the ages of the patients ($r = 0.47$, $p = 0.007$), where the distribution of age was described (mean ± standard deviation) as 27.6 ± 6.3 years. While the range of ages studied was not large, a relationship between clearance and age is not unexpected for a drug that is significantly eliminated by the kidney. It was further found that the maximum tolerated dose (MTD) was significantly higher in men than women (with a mean MTD of 24.2 *vs* 17.5 mg/kg; $p = 0.01$). The toxicity measured was bone marrow depression. MTD, however, did not correlate with the AUC. In the same study, the AUC_6 did not correlate with baseline serum creatinine or creatinine clearance.

In earlier studies by the same investigators,[68] mean serum creatinine levels (with a range of 0.5 to 1.1 mg/dl during therapy for each patient correlated significantly with 6-hour plasma hydroxyurea concentrations ($r = 0.85$, $p = 0.01$). In a population pharmacokinetic study of hydroxyurea by Tracewell et al.,[15] including creatinine clearance in the model just failed to achieve a statistically significant improvement in the fit ($0.01 < p < 0.042$). Thus, despite some results to the contrary,[68] it does seem very probable that renal dysfunction will affect the pharmacokinetics of hydroxyurea, given the contribution of the kidneys to the elimination of hydroxyurea.

4.2 AIDS

A principal component of the treatment of patients infected with HIV is the administration of nucleoside analogues such as zidovudine and didanosine. However, the therapeutic benefits of these compounds are temporary and improved therapy is urgently needed.

In vitro studies have shown that hydroxyurea inhibits HIV replication.[59,69,70] This effect is greatly enhanced when hydroxyurea is used in combination with nucleoside analogues, particularly didanosine. The antiretroviral effects of hydroxyurea are because of the depletion of intracellular deoxyribonucleotides. This depletion further permits increased cellular uptake of nucleoside analogues.

Several clinical trials have been performed to evaluate the effects of hydroxyurea alone[71,72] and combined administration of hydroxyurea and nucleoside analogues in patients with HIV.[73-75] Overall, the combination therapy is well tolerated and accompanied by a significant reduction in plasma viral load that is related to hydroxyurea dosage. Monotherapy with hydroxyurea has not been shown to be beneficial in patients with HIV infection.

As part of a clinical trial, Villani et al.[18] examined the pharmacokinetics of hydroxyurea. Oral doses of 500mg every 12 hours were administered for 4 weeks to 9 patients with HIV-1, 5 of whom continued to take zidovudine (250mg every 12 hours) while the other 4 were maintained on hydroxyurea alone. The pharmacokinetics of hydroxyurea were modelled using a linear, 1-compartment model. In this case linear kinetics were appropriate because the serum concentrations were low compared with those used in cancer chemotherapy. A mean half-life of 2.5 hours and an ap-

parent clearance (CL/F) of 0.182 L/h/kg were reported. Based on *in vitro* studies[59,70,76] and clinical studies with similar hydroxyurea dosage regimens,[73-75] the hydroxyurea concentrations achieved in the study by Villani et al.[18] [showing mean minimum and maximum drug concentrations (C_{min}/C_{max}) of 0.0085/0.135 mmol/L] are likely to be adequate for the inhibition of HIV-1 *in vivo*, when combined with an appropriate nucleoside analogue.

5. Conclusions

Hydroxyurea is an old drug for which new uses have been found. Because hydroxyurea's dose-limiting toxicity in cancer chemotherapy is myelosuppression, larger doses are now possible with concommitant haematopoietic growth factor. Hydroxyurea is currently the only drug approved for the treatment of sickle-cell anaemia. The drug is also promising in the treatment of HIV infection. Despite the long history of hydroxyurea use, much is still unknown.

The effect of pathophysiological changes on the pharmacokinetics, metabolic pathway and other routes of elimination, and the precise hydroxyurea concentration range required to increase cellular response to nucleoside analogs in both cancer and HIV infection are among the unknown properties of hydroxyurea. Elucidation of these and other properties should lead to improved and more widespread use of this versatile drug.

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