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Phase 1 study of high-dose hydroxyurea in lung cancer

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Summary. The in vitro chemosensitivity of a human lung cancer cell line to hydroxyurea (HU) was measured, and concentrations of 1 m*M* HU effected 99% inhibition of cell growth. Therefore, infusions designed to achieve serum levels of over 1 m*M* HU were assessed by escalating doses of hydroxyurea (HU) administered by continuous i.v. infusion at 3-weekly intervals in 18 patients with lung cancer. Dose increments from 24 g in 24 h to 48 g in 48 h were achieved. The dose-limiting toxicity at 48 g in 48 h was myelosuppression. Oral administration of HU did not result in sustained levels comparable to those achieved with continuous infusion. Two patients showed evidence of radiological response after three courses of treatment. Serum HU profiles were monitored after administration i.v. in 26 courses and after administration p.o. in 5 courses of treatment. A mean serum level of >1 m*M* was achieved by 6 h and then maintained during treatment. The standard error of the mean area under the curve showed an overall 5% variation. HU can be given in doses up to 48 g in 48 h 3-weekly with manageable tissue and bone marrow toxicity, and the in vivo blood levels attained are equal to those necessary for effective cell inhibition in an appropriate in vitro model. This schedule provides a basis for combination studies with other cytotoxics or for use of HU as a DNA repair inhibitor.

Introduction

Hydroxyurea is a structural analogue of urea, which has been in clinical use as a cytotoxic agent for the past 20 years [6, 12]. It has been used principally to treat chronic myeloid leukaemia [11], but has also been evaluated in a variety of other cancers [1, 10].

A phase II study of HU in lung cancer demonstrated minimal antitumour activity [14]. Creasy et al. [4] used HU in doses up to 100 mg per kg per day and found that serum levels were of the order necessary to inhibit thymidine incorporation into DNA in sensitive human leukaemia cells.

HU acts as an S-phase cell-cycle-specific agent [26] which selectively inhibits DNA synthesis by inhibition of ribonucleotide reductase [27]. This is a key enzyme in the pathway of DNA synthesis [21]. As HU is a cell-cycle-specific agent, its most effective use should be in continuous exposure of tumour cells to the drug [19]. The average cell generation time for a variety of solid tumours is 50–60 h [8].

This primary action of HU leads to secondary effects, such as impairment of DNA replication and inhibition of strand break repair by depletion of deoxyribonucleotide pools [24]. Thus, HU has been used in vitro as a DNA repair inhibitor, but the usual concentrations have been 1–10 m*M*. Additionally, HU has direct effects on DNA, leading to fragmentation of metaphase chromosomes [16].

If hydroxyurea could be given as a stustained infusion in adequate doses to inhibit DNA repair it might potentiate agents causing DNA damage, as has been shown in vitro. Earlier phase I and II trials of HU did not achieve levels required for this effect.

We have used HU in patients with lung cancer to assess the effect of increasing doses given by continuous infusion. We assessed the clinical and toxic effects of escalating doses to determine whether we could achieve levels in the serum similar to the levels required to inhibit a lung cancer cell line in culture.

Patients and methods

Eighteen patients with lung cancer (Table 1) and aged 49–79 years (median 65) were given HU as sole therapy, either by continuous i.v. infusion or as 6-g oral doses 6-hourly. Only one patient had had previous chemotherapy. The i.v. solution of HU was constituted on the day of use with 8 g HU dissolved in 1 l dextrose saline (0.5% solution). A total of 45 courses of HU were given (for details see Table 2). Patients received 24 g in 24 h or 36 g in 36 h as a first dose, and for some patients the dose was then escalated to 48 g over 48 h. Courses were repeated every 3 weeks.

Haemoglobin, white cell count and platelet estimations were assessed weekly, and serum urea, electrolytes and liver function tests at each visit. Creatinine clearance was measured prior to each course of therapy, and blood samples were taken during the infusion and immediately spun at 3000 rpm, then frozen at $-4°$ C for later estimation of HU levels.

Assay method. Levels of HU were assayed using a modified colorimetric assay described by Fabricius and Rajewsky [7]. HU was oxidised by iodine and nitrite was formed,

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Table 1. Patient characteristics

Sex	
Male	14
Female	4
Age 49–79 years (mean 60.4 years)	
Creatinine clearance 83 (±22) ml/min	
Squamous carcinoma	6
Adenocarcinoma	5
Large cell carcinoma	5
Papillary carcinoma	1
Small cell carcinoma	1
Previous chemotherapy	1 (small cell)
Previous radiotherapy	1
Previous surgery	6 (5 inoperable, 1 debulking surgery)

Table 2. Dosage regimens of hydroxyurea

Dose	Patients	No. given p.o.	No. given i.v.	Total
24 g	2	1	6	7
32 g	1	0	1	1
36 g	16	8	20	28
48 g	5	0	9	9
	24	9	36	45

Four patients each received one course; eight patients, two courses; three patients, three courses; two patients, five courses, and one patient, six courses

which diazotised sulphanilic acid. After reduction of excess iodine with sodium thiosulphate, the diazotised sulphanilic acid was coupled with *N*-(1-napthyl)-ethylene-diamine dihydrochloride to give a red colour product. Colour intensity was measured in an absorption spectrophotometer at 540 nm and compared with blank and standard solutions. The limit of detection of HU by this method was 1 μg/ml. The coefficient of variation was 8%.

Cell culture experiments. A549 lung carcinoma cells [9] were grown as a monolayer culture and were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum in a humidified 5% CO_2/95% air atmosphere at 37° C. Cells of passage 204 were seeded in replicate six-well plates at 20000 cells per 35-mm diameter well in 2 ml drug-free medium. After 24 h, this medium was replaced by graded concentrations of HU, or by fresh drug-free medium as internal control. The number of cells per well was determined after a further 72 h by means of a Coulter counter. The results were calculated by a graph of growth inhibition per concentration of HU versus percentage of control growth without the drug.

Results

Cell culture (Fig. 1)

At concentration levels of 1 m*M* HU, cell cultures of A549 cell lines showed 99% inhibition of growth. Thus, based on previous infusion data, we designed an infusion regimen to achieve a sustained HU level of > 1 m*M*.

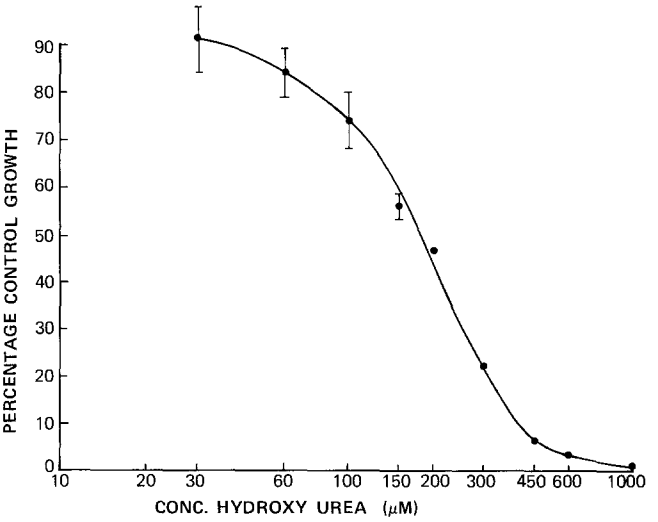

Fig. 1. Dose-response curve of A549 lung carcinoma cell lines for hydroxyurea ED_{50} = 180 μ*M* HU

Clinical results

Eighteen patients had 45 courses of HU, of which 9 were given p.o. and 36 i.v. Therapy was stopped after 1 course of HU in four patients, because of disease progression in three cases and death due to myocardial infarction in the fourth.

After three courses, two patients had an objective response and one other patient had subjective improvement with no change in extent of disease apparent on chest X-ray. One of these three patients went on to have six courses of HU and the other two patients had five courses.

Toxicity. Dose-limiting toxicity appeared at 48 g in 48 h with the development of grade 3 (WHO) leucopenia [25] in one patient who developed septicaemia but recovered. There were two episodes of grade 2 toxicity at 36 g and one of grade 1 toxicity at this dose. No other myelosuppression was encountered. Patients developed nausea and vomiting at 36 g, which was responsive to standard antiemetic drugs. Two patients developed grade 2 (WHO) alopecia at 48 g in 48 h.

Other toxicities encountered at HU doses of 48 g included mucositis in two patients, one of whom had superimposed oral candidiasis. Two patients had excess sweating during therapy. There was no biochemical evidence of hepatic or renal toxicity at the doses given.

Pharmacokinetics. Serum HU profiles were monitored in 26 courses of i.v. and 5 of p.o. treatment. With i.v. therapy, a serum level of > 1 m*M* was achieved by 6 h of infusion and maintained throughout the infusion (Fig. 2). The levels achieved were reproducible with different infusions (Fig. 3). With oral HU, there were fluctuations in HU levels throughout the course of treatment (Fig. 4).

The area under the curve was calculated for each profile by use of a graphics tablet program on an Apple IIe computer. The area under the curve showed little variation between patients and was not related to creatinine clearance.

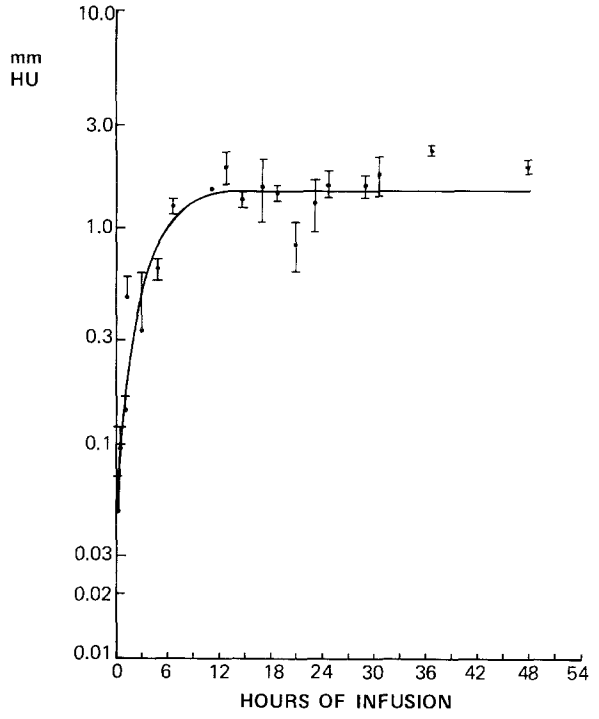

Fig. 2. Mean (SEM) plasma hydroxyurea levels achieved with continuous infusion in 17 infusions

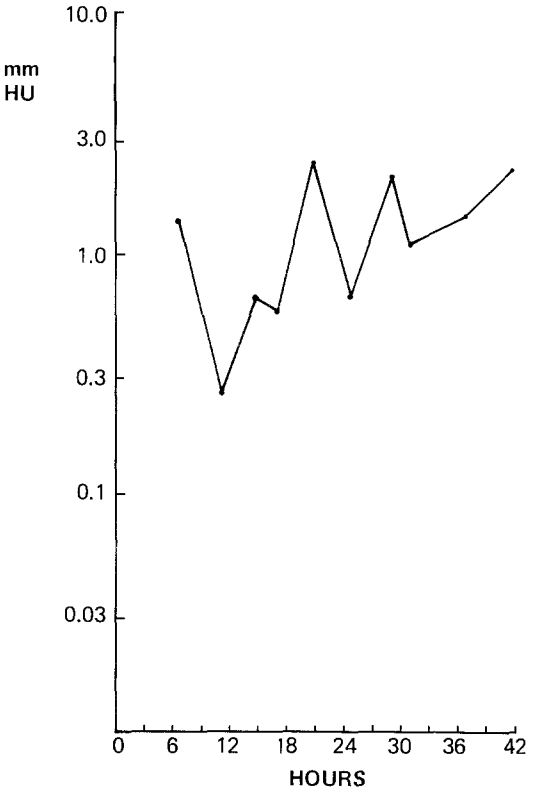

Fig. 4. Plasma levels achieved with oral hydroxyurea in five courses

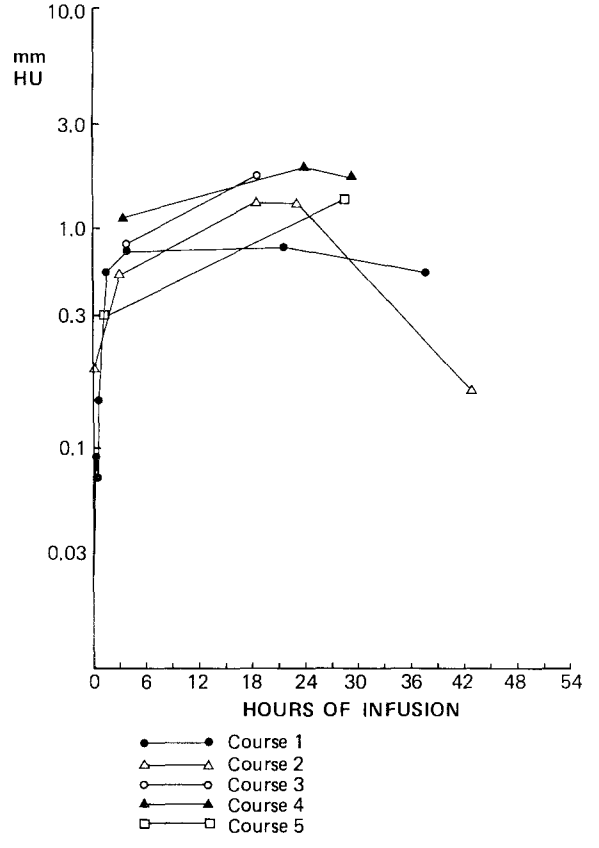

Fig. 3. Plasma HU concentrations with different infusions in one patient

Discussion

We have shown that HU can be given in higher doses than have previously been given, with manageable toxicity. HU has usually been given orally [6] but as it is a cell-cycle-specific agent [18], it is more rational to give the drug as a continuous infusion. Belt et al. [2] gave HU as an infusion of approximately 26 g in 72 h and achieved levels in the range of 0.9–1.4 m*M*. We have shown that sustained levels of 1 mM are required for 99% inhibition of A549 lung cancer cell growth in culture, and we have been able to maintain that level in patients for up to 42 h without marrow toxicity. We achieved a mean serum HU level of 1.73 mM (0.58–2.47 mM) after 24 h of infusion, which is higher than that of Belt et al. [2].

When given p.o., the dose-limiting toxicity has been bone marrow depression, with leucopaenia predominating [22]. All patients developed leucopaenia at 80 mg per kg per day in 14 days, and 70% did so at 40 mg per kg per day for 14 days [22]. Belt et al. [2] found that myelosuppression correlated well with plasma HU levels, all patients having myelosuppression at levels over 1 m*M*. Our patient who developed myelosuppression had levels up to 2 m*M*, but these levels were not excessively high compared with those in others of our patients. Myelosuppression is related to duration of exposure to HU as well as dose; it develops after continuous low-dose oral therapy and with i.v. infusion lasting up to 72 h [2].

Patients developed nausea and vomiting at the dose of 36 g in 36 h but this was controlled by antiemetics; similar effects have been reported with oral therapy [22].

We noted objective clinical responses in two patients with HU. HU has been shown to have some clinical effect

on lung cancers at lower dose schedules [10], but patients with more responsive small cell lung cancers may have been included in the data. We had one patient with small cell lung cancer who relapsed after previous combination chemotherapy but who did not respond to HU. The lack of response in our non-small cell lung cancer patients could be due to a low growth fraction in squamous and adenocarcinoma [23].

HU is primarily a ribonucleotide reductase inhibitor and is an S-phase cycle-specific drug [26]. Thus, it could be used in combination with other treatments, such as radiation therapy or DNA-damaging agents. It has been used in lung cancer in combination with radiation therapy, but showed no benefit [13, 15]. Le Par et al. [15] treated 12 patients with HU at 10 mg per kg concurrently with 6000 rads of radiation in 6 weeks and found no increase in survival compared with treatment with radiotherapy alone. However, in those studies there was no attempt to achieve any particular drug level based on biochemical or pharmacological data.

HU could be used as a DNA repair inhibitor, as its inhibition of ribonucleotide reductase leads to depletion of deoxyribonucleotide pools [20]. HU leads to accumulation of strand breaks at sites where enzyme incision is not followed by repair synthesis and ligation [3].

Wawra et al. [24] have shown that 1 m*M* HU inhibits cell replication in addition to repair, and conclude that HU interacts with steps of the replication process which are responsible for the processing of primary products, so that it could have other effects, besides ribonucleotide reductase inhibition.

HU can induce breaks in template strands of replicating DNA [3] and has been shown to lead to fragmentations, translocations and rearrangements of metaphase chromosomes [17]. These effects, together with inhibition of ribonucleotide reductase, imply that HU is a highly active cytotoxic agent. In previous studies, HU has not usually been used in an optimal manner.

HU given as a DNA repair inhibitor in continuous high-dose infusion may be useful in combination therapy with agents inducing DNA damage, such as cisplatin, which is an active agent in non-small cell lung cancer [5]. Thus, we have commenced a trial of a combination of high-dose HU and cisplatin, and this phase I study has provided a rational approach to dosing.

References

1. Arul IM (1970) Therapeutic effects of hydroxyurea: experience with 118 patients with inoperable tumours. Cancer 25: 705–714
2. Belt RJ, Hass CD, Kennedy J, Taylor S (1980) Studies of hydroxyurea administered by continuous infusion. Cancer 46: 455–462
3. Borenfreund E, Krim M, Bendlich A (1969) Chromosomal aberrations induced by hyponitrite and hydroxylamine derivatives. J Natl Cancer Inst 32: 667–680
4. Creasy WA, Capizzi RL, DeConti RC (1970) Clinical and biochemical studies of high dose intermittent therapy of solid tumours with hydroxyurea (NSC-32065). Cancer Chemother Rep 54: 191–194
5. De Jaeger R, Longeval E, Klastersky J (1980) High dose cisplatin with fluid and mannitol induced diuresis in advanced lung cancer: a phase II trial of the EORTC Lung Cancer Working Party. Cancer Treat Rep 64: 1341–1346
6. Donehower RC (1982) Hydroxyurea. In: Chubner B (ed), Pharmacological principles of cancer treatment. Saunders, Philadelphia, pp 269–275
7. Fabricius E, Rajewsky MF (1971) Determination of hydroxyurea in mammalian tissues and blood. Rev Eur Et Clin Biol 16: 679–683
8. Frindel E, Malaise E, Tubiana M (1968) Cell proliferation kinetics in five human solid tumours. Cancer 22: 611–620
9. Giard DJ, Aaronson SA, Todaro GJ, Arnstein P, Kersey JH, Dosik H, Parks WP (1973) In vitro cultivation of human tumours: establishment of cell lines derived from a series of solid tumours. J Natl Cancer Inst 51: 1417–1423
10. Kaung DT, Walsh WS, Sbor S, Patno ME (1968) Hydroxyurea in therapy for nonresectable cancer of the lung. Cancer Chemother Rep 52: 271–274
11. Kennedy BJ (1972) Hydroxyurea therapy in chronic myelogenous leukaemia. Cancer 29: 1052–1056
12. Krakoff IH (1975) Clinical and pharmacological effects of hydroxyurea. In: Sartorelli AC, Johns DG (eds) Handbuch der experimentellen Pharmakologie, vol 38/II. Springer, Berlin Heidelberg New York, pp 789–792
13. Landgren RC, Hussey DH, Barkley HT, Samuels ML (1974) Split course irradiation compared to split course irradiation plus hydroxurea in operable bronchogenic carcinoma: a randomised study of 53 patients. Cancer 34: 1598–1601
14. Lerner HJ, Beckloff GL (1965) Hydroxyurea administered intermittently. JAMA 192: 138–140
15. Le Par E, Faust DS, Brady LW, Beckloff GL (1967) Clinical evaluation of the adjunctive use of hydroxyurea in radiation therapy of carcinoma of the lung. A comparative study. Radiol Clin 36: 32–40
16. Martin RF, Radford J, Pardee M (1977) Accumulation of short DNA fragments in hydroxyurea treated mouse L cells. Biochem Biophys Res Commun 74: 9–15
17. Pearson CM (1983) In: Downes CS, Collins ARS, Johnson RT. International Workshop on DNA Repair. Mutat Res 112: 75–83
18. Sinclair WJ (1965) Hydroxyurea: differential lethal effects on cultured mammalian cells during the cell cycle. Science 150: 1729–1731
19. Skipper HE, Schabel FM Jr, Wilcox WS (1967) Experimental evaluation of potential anticancer agents: XI. Scheduling of arabinosylcytosine to take advantage of its S-phase specificity against leukaemia cells. Cancer Chemother Rep 51: 125–141
20. Synder RD (1984) Inhibitors of ribonucleotide reductase alter DNA repair in human fibroblasts through specific depletion of purine deoxynucleotide triphosphates. Cell Biol Toxicol 1: 81–93
21. Thelander L, Reichard P (1979) Reduction of ribonucleotides. Annu Rev Biochem 48: 133–158
22. Thurman WG, Bloedow C, Howe CD, Levin WC, Davis P, Lane M, Sullivan MP, Griffith KM (1963) A phase I study of hydroxyurea. Cancer Chemother Rep 29: 103–107
23. Tubiana M, Malaise EP (1976) Growth rate and cell kinetics in human tumours. In: Symington T, Carter RL (eds) Scientific foundations of oncology. Heinemann, London, pp 119–125
24. Wawra E, Wintersberger E (1983) Dose hydroxyurea inhibit DNA replication in mouse cells by more than one mechanism? Mol Cell Biol 3: 297–304
25. World Health Organization (1979) Handbook for reporting results of cancer treatment. WHO, Geneva
26. Young CW, Hodge S (1964) Hydroxyurea: inhibitory effect on DNA metabolism. Science 146: 1172–1174
27. Young CW, Schachetman G, Karnofsky DA (1967) Hydroxy urea-induced inhibition of deoxyribonucleotide synthesis: studies in intact cells. Cancer Res 27: 526–534

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