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NON-LINEAR PHARMACOKINETICS OF 5-FLUOROURACIL AS DESCRIBED BY IN VIVO BEHAVIOUR OF 5,6 DIHYDRO-5-FLUOROURACIL

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INTRODUCTION

5-Fluorouracil (FU) is widely used in the treatment of disseminated cancers, especially of the gastro-intestinal tract, breast and ovary. In fact, no other drug has proven more effective so as to replace it for gastro-intestinal tumours. The metabolism of FU has been studied extensively and it is clear that it participates for the greater part in the same pathways as uracil and its metabolites. The biochemistry of FU has been reviewed recently (1); the metabolic activation, the anabolism, and degradation, the catabolism, as presented in Fig. 1 is generally accepted now. Treatment of FU produces two major effects in cells: an inhibition of DNA synthesis by inhibition of dUMP synthetase by fluorodeoxyuridine monophosphate (FdUMP) and an alteration in the processing function of some types of RNA because of extensive incorporation of FU instead of uracil (2).

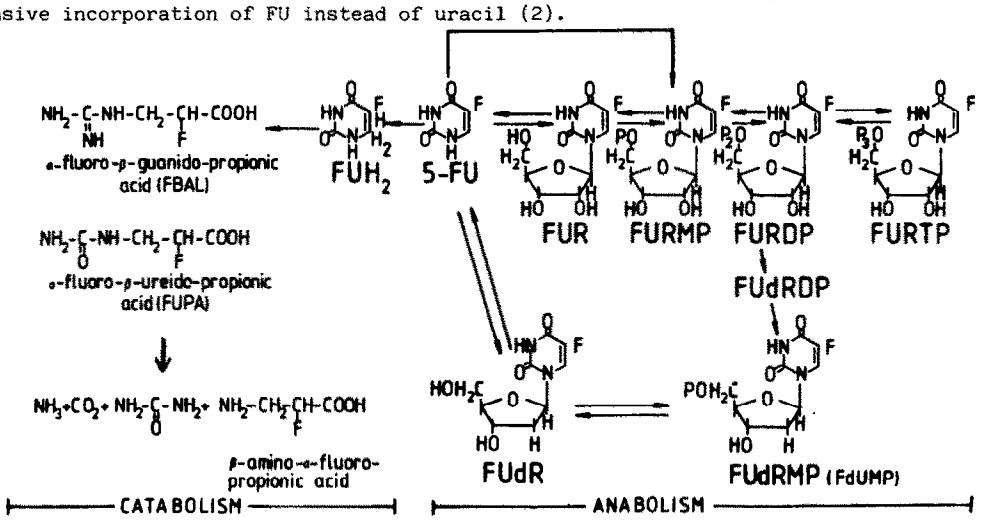

Fig. 1. The metabolic activation (anabolism) as 5-FU (FUR, FURMP, FURDP, FURTP, FUdRDP, FUdRMP/FdUMP, FUdR) and the degradation (catabolism) of the antimetabolite (FUH_2, FUDA, FBAL, β-amino-α-fluoropropionic acid).

The major part of the dose administered is converted into 5,6 dihydro-5-fluorouracil (FUH_2) by liver metabolism, a compound earlier assumed to lack antitumour activity (2). Recently, it has been demonstrated that FUH_2 could produce inhibition of thymidilate synthetase activity in Ehrlich ascites tumour cells (3).

Despite a large number of efforts put into studies of FU metabolism, only a few reports are available dealing with in vivo catabolism of FU (4–12). Studies of possible dose-dependent FU pharmacokinetics related to the in vivo behaviour of FUH_2 are lacking until now.

In this report we describe the pharmacokinetics of FU and its catabolic conversion to FUH_2 in tumour-bearing WAG/Rij rats following i.v. administration of four different doses of FU.

MATERIALS AND METHODS

Drugs: FU and FUH_2 were kindly supplied by Hoffmann-La Roche (Basle, Switzerland) and appeared to be free from contaminations with FUH_2 and FU, respectively, as was demonstrated by HPLC and Mass Spectrometry.

Animals and drug administration: Female WAG/Rij rats with two implanted tumours of about 1 cm^3 in both flanks and weighing 200 ± 10 g, were cannulated in the carotid artery and the jugular vein under light ether anaesthesia one day before treatment with FU. From cannulation till the end of the kinetic experiments the rats were fasted, while water was supplied ad libitum. During the experiments the rats were kept in cages in which free movements were possible despite the cannulation. Each cage contained one rat; the cages were placed in a temperature-controlled room at 20°C, illuminated from 7.00 till 19.00 h. Drug treatment started at 9.00 a.m.. FU was injected to at least 6 animals per dose; the 4 doses administered were 10 mg/kg, 20 mg/kg, 40 mg/kg and 80 mg/kg. The maximal number of rats that could be treated once was 4; the four different doses were equally spread over this number.

Collection of blood samples: Via the cannula in the carotid artery 400 µl of blood was collected in polythene tubes at 1, 5, 15, 30, 45, 60, 75, 90, 120, 150, 180, 240, 300 and 360 min. Blood samples were centrifuged immediately at 1000g for 10 minutes and plasma was stored at -35°C until analysis.

Assay of FU and FUH_2: FU plasma concentrations were determined with high performance liquid chromatography (12), FUH_2 was determined with gaschromatography (10,13). The amount of plasma available for the assays was 100 µl each.

a. High performance liquid chromatographic assay of FU.
After addition of the internal standard 2-thiouracil to 100 µl plasma, the mixture was adjusted to pH 6 with a 5 mM Tris buffer and was extracted with 1.4 ml ethyl acetate. The evaporation of the organic phase occured at room temperature under a gentle stream of nitrogen. The remaining fraction was dissolved in 200 µl 0.05 M Tris buffer (pH 8), after which 20 µl was injected on to a 100 mm MOS-Hypersil 5 µm column of a Shimadzu liquid chromatograph (LC4A) with a variable wavelength detector (SPD 2 AS), fixed at 269 nm, and a chromatograph terminal (BD 8/CR IB). Elution was carried out isocratically at 2 ml/min with water/0.05 mM Trix/0.005 mM Cetrimide. The retention times of FU and 2-thiouracil were 4.1 and 6.5 min, respectively. The limit of determination, based on a signal to noise ratio of 3 : 1, was 0.1 µM, the coefficient of variation for three successive determinations was 3%, and the recovery of FU extracted from plasma was 70%. The method showed good linearity in plasma concentrations from 0.1 mM to at least 0.1 M FU.

b. Gas chromatographic assay of FUH_2.
Gas chromatographic determination of FUH_2 was carried out on support-coated open tubular columns (10, 12,13). Before plasma pretreatment started, 5-Chlorouracil was added as internal standard; 100 µl plasma was extracted first with chloroform and then with ethyl acetate. The ethyl acetate layer was removed and dried under a gentle stream of nitrogen. Diphenylsuccinimide, dissolved in 200 µl ethyl acetate, was added to the residue as standard for the gas chromatogrpahic conditions, i.e. the external standard. A Packard Becker gas chromatograph (420, Packard Becker, Delft, The Netherlands) combined with a Hewlett-Packard NPSD (Hewlett-Packard, Avondale, PA, USA) and/or a Hewlett-Packard ECD was used for plasma measurements. A ball valve sample injector was applied and the inlet and detector temperature were set at 245 °C and 300 °C, respectively; the oven temperature was 195 °C. Helium was used for both carrier gas (12 ml/min) and make-up gas (30 ml/min). The limit of detection for FUH_2 was 0.5 ng, resulting in a limit of determination of 0.2 µM for NPSD and 0.05 µM for ECD. Both limits were calculated as described for the high performance liquid chromatographic assay. The coefficient of variation for the assay ranged from 6.6% at a level of 100 ng/ml to 1.0% at 5 µg/ml (n = 3). The recovery of FUH_2 extracted from plasma amounted to about 60%, the assay showed a good linearity in the concentration range of FUH_2 expected to occur in rats: 0.2 µM to 50 µM. It has to be stressed that FUH_2 in blood and plasma with pH 5-7 is unstable at room temperature owing to degradation probably by NADPH + H^+ and dihydropyrimidine dehydrogenase*. Preservation of FUH_2 can be gained by treatment of samples by low temperature and/or adjusting pH at 3.5. Since 5-FU has to be determined also and can be extracted easily at a plasma pH 6, extraction of FUH_2 on ice is preferred.

Pharmacokinetic analysis: Pharmacokinetic calculations of FU and FUH_2 were carried out by non-compartmental analysis, the elimination half-live ($t_{½,z}$) was calculated by least-squares regression analysis after log transformation. The area under the curve (AUC) was calculated by the trapezoidal rule with extrapolation to infinity. The CL was calculated by Dose/AUC; the maximal concentration (C_{max}) of FUH_2 at a specific time (t_{max}) was determined as the highest measured concentration of FUH_2 in plasma of a rat.

RESULTS

Following i.v. administration of the four doses FU concentrations declined rapidly within the first 15 min after drug administration. The subsequent plasma concentration decay could be fitted to a straight line on semi-logarithmic scale. In all rats the $t_{½,z}$ of FU was determined between 45 min and the last measured concentration of FU. The mean $t_{½,z}$ are summarized in table I. The data of the three pharmacokinetic parameters, $t_{½,z}$, AUC and CL, as depicted in table I show non-linear kinetics of FU in rats.

* Data presented at the Fifth International Symposium on Capillary Chromatography, Riva del Garda, Italy, April 26-28, 1983. Also: The stability of FUH_2 in absence and presence of FU as elucidated by Mass-Spectrometry and NMR-Spectroscopy, submitted for publication.

TABLE I

PHARMACOKINETIC DATA OF FU AND FUH_2 AFTER I.V. ADMINISTRATION OF 10 MG/KG (I), 20 MG/KG (II), 40 MG/KG (III) AND 80 MG/KG (IV)

		FU			FUH_2			
		$t_{½,z}$ (min)	AUC (ug/mlxmin)	CL (ml/min)	$t_{½,z}$ (min)	AUC (ug/mlxmin)	C_{max} (ug/ml)	t_{max} (min)
I	$\bar{x}$	10.5	281.0	7.2	19.0	13.5	0.292	41.3
(n=6)	s.d.	1.0	43.5	1.0	2.2	2.1	0.072	7.5
II	$\bar{x}$	14.0	510.0	7.8	28.0	108.8	1.381	41.3
(n=7)	s.d.	2.5	72.1	1.3	4.2	36.2	0.146	7.6
III	$\bar{x}$	23.3	1610.3	5.0	52.4	199.3	2.335	37.5
(n=7)	s.d.	2.2	239.8	0.7	11.9	23.0	0.338	8.7
IV	$\bar{x}$	34.5	4285.5	3.8	60.0	268.8	2.788	45.0
(n=7)	s.d.	2.1	640.0	0.6	4.0	48.7	0.518	12.2

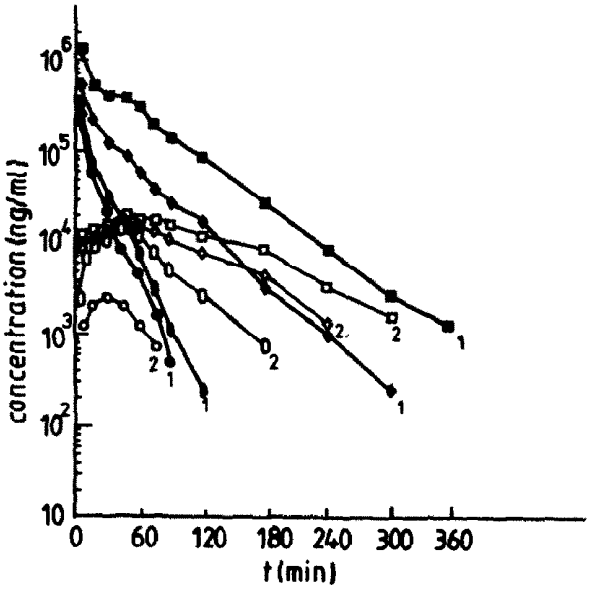

Fig. 2. Plasma concentration time curves of FU (1 st) and FUH_2 (2nd) after 10 mg/kg ●,○, 20 mg/kg (◆,◇) and 40 mg/kg (■,□) and 80 mg/kg (■,□).

In Fig. 2 the plasma concentration-time curves of FUH_2 are also presented; in all rats this compound was measured within 5 min after drug administration. In most cases the C_{max} was observed at t = 45 min, while the concentrations never exceeded 3.5 µg/ml. The elimination of FUH_2 appeared to be also dose-dependent: the $t_{½,z}$ increased with the dose of FU from 19.0 to 60.0 min (Table I). Surprisingly, the AUC did not increase linearly with the dose; doubling of the dose from 10 mg/kg to 20 mg/kg resulted in a large increase of AUC and C_{max}. The ratio of AUC_{FU}/AUC_{FUH2} versus the dose, as presented in Fig. 3, reveals a curve with a minimum at D = 20 mg/kg.

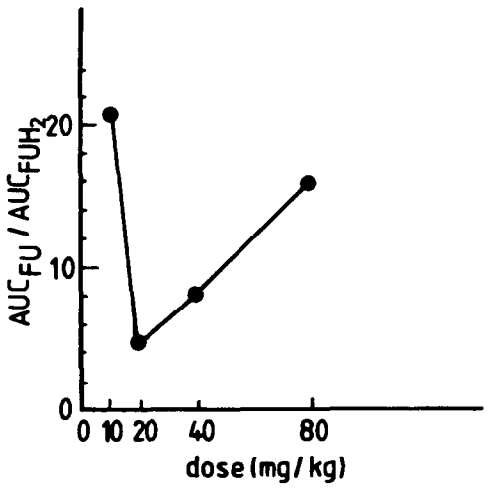

Fig. 3. Relationship between the ratio of AUC_{FU}/AUC_{FUH_2} versus the dose of FU.

DISCUSSION

Since the synthesis of FU 25 years ago, there have been extensive reports on FU metabolism. The development of selective and sensitive analytical techniques which are routinely applicable has permitted a reevaluation of FU catabolism. FUH_2 has been demonstrated to be a quantitatively important fluoropyrimidine catabolite in in vitro and in vivo studies and not an insignificant transient metabolite as was implied in the early days of FU. In view of this renewed interest in FUH_2 it is of importance to be aware of FUH_2 instability in biological fluids as plasma and ascites and tissue culture media as we have been elucidated by Mass -Spectrometry and NMR-spectroscopy*. This might explain the absence of FUH_2 in plasma samples of several patients as reported earlier (9,10). Therefore, plasma samples have to be adjusted to low pH or treated at 4°C as soon as possible following blood centrifugation.

This study shows that in rats treated with FU non-linear pharmacokinetics of both FU and the quantitative most important metabolite FUH_2 occur. Non-linear pharmacokinetics of FU was reported before (9,14,15), no data about non-linear kinetics of FUH_2 are available at this moment as far as we know. The plasma concentration-time curves of FUH_2 suggest that FUH_2 is rapidly formed and released by liver tissue. An explanation for the differences between data of FUH_2 obtained after administration of 10 mg/kg and 20 mg/kg FU can not be given. The capacity of liver tissue to convert FUH_2 to further catabolites FUPA and subsequently FBAL (Fig. 1), might be saturated at D 20 mg/kg, resulting in a plateau of FUH_2 concentrations between 1-3 µg/ml. This is stressed by the elongation of the $t_{½,z}$ of FUH_2 after increasing the dose to 80 mg/kg, while C_{max} was relatively stable. Thus, in addition to a dose-dependent conversion of FU to FUH_2, there may be also a dose-dependent conversion of FUH_2 to FUPA and FBAL (Fig. 1). This could also explain the AUC_{FU}/AUC_{FUH2} versus FU dose relationship as presented in Fig. 3. A modulation of in vitro catabolism of FU has been described recently (16). The implications of the findings of that study are now being studied in the described animal model to reveal possible ways of alteration of in vivo catabolism of FU and FUH_2. Furthermore, the tumour-bearing WAG/Rij rats allow a careful analysis of the antitumour effects and side-effects caused by FU and metabolites. The relationship between the pharmacokinetics, metabolism and effects of FU is now further under evaluation and will be published separately.

The present findings are suggestive for a key role of FUH_2 degradation in the non-linear pharmacokinetics of FU in rats. A dose-dependent relationship for the ratio of AUC_{FU} and AUC_{FUH2} was found in a range of 20-80 mg/kg.

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