Cancer Chemother. Pharmacol. 3, 61–66 (1979)

Cancer Chemotherapy and Pharmacology

Ftorafur: A Self-Limiting Source of 5-Fluorouracil?

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Summary. *Studies of the cell killing effectiveness of ftorafur were carried out against 5-fluorouracil (5-FU). It appeared that in divided doses ftorafur is both relatively less toxic in terms of LD_{50} and bone marrow stem cell killing and less effective against L1210 and a mouse osteosarcoma than 5-FU. Pharmacokinetic analysis revealed that upon repeated administration of 5-FU there is an increase in late drug serum levels and a decreased clearance of 5-FU from the serum. Repeated administration of ftorafur is not associated with significant differences in serum levels of 5-FU or ftorafur.*

It is concluded that the relatively good tolerance to divided doses of ftorafur in comparison with divided doses of 5-FU may be a consequence of the pharmacokinetic differences.

Introduction

Ftorafur (furanidyl-5-fluorouracil) is a cytostatic drug originally synthesised in Latvia [9]. It has been studied extensively in the Soviet Union, both experimentally and clinically [1, 10]. Recently, interest has focused on this drug because on the basis of preliminary results, it was suggested that it might possess a lower toxicity for the haematological system than 5-FU [1, 15, 18].

Results from studies in Japan [4] have indicated that ftorafur is not directly active, but must be activated in vivo. Among its activation products 5-FU, 5-FUdR and other transformation products of 5-FU have been identified, indicating that the major pathway of activation is through hydrolysis of ftorafur to 5-FU. Apparently this activation may take place in the liver [5]. Evidence has been presented [2, 3, 12] that, like to other antitumor drugs [6], both 5-FU and ftorafur can affect the activity of a number of enzyme systems in the liver. Since the liver is the organ where hydrolysis of ftorafur to 5-FU has been reported to occur [5], we investigated the possibility that the transformation of ftorafur to 5-FU might also be affected by these drugs. If this hypothesis is correct, a decreased effectiveness of ftorafur upon continuous administration might be expected, as a consequence of a decreased transformation to 5-FU.

In studies comparing the toxicity and effectiveness of 5-FU and ftorafur, Johnson et al. [7, 11] found no evidence for such a reduced transformation of ftorafur upon its repeated administration. Nevertheless, the present studies indicate a difference in relative blood levels between single and repeated administration of 5-FU and ftorafur, which might explain the much better tolerance to repeated administration of ftorafur.

Materials and Methods

Animal Survival

For both drugs the 50% lethal dose at 30 days ($LD_{50/30}$) after treatment of normal mice was determined.

Effect on Normal Haemopoietic Stem Cells

Cell survival was used to compare the relative effectiveness of 5-FU and ftorafur. The survival of normal spleen colony-forming haemopoietic stem cells of the mouse was assayed in the total femur's content of bone marrow of mice treated with different doses of 5-FU or ftorafur given SC and in untreated control mice. Assays were performed 16 h after single doses of the drugs or 6 h after the last of five equal doses given at 24-h intervals. In addition to studies of the stem cells in normal mouse bone marrow, studies with single doses of the drugs were also performed on rapidly proliferating stem cells in mice in which the haemopoietic system was repopulating after irradiation. The methods of assay for haemopoietic stem cells have been described earlier [19]. The mice used were 10–12 week old male (C57BL/Rij × CBA/Rij)F_1 hybrids.

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0344-5704/79/0003/0061/$ 01.20

Antitumour Effects

The increase in lifespan after treatment of mice carrying L1210 leukaemia was determined for a number of dosage groups for each drug. CD2F1 mice were inoculated IV with 10^5 L1210 cells and received the first drug dose SC 24 h later. The median survival time was used as the end point. Again, the effects of different single doses and of five daily doses of both drugs were studied. By comparing the results with a simultaneously constructed calibration curve indicating the median survival time of groups of 10 mice inoculated with different numbers of L1210 leukaemia cells, the increase in median lifespan could be expressed as a fractional cell kill [17] and dose-survival curves could be made.

Reduction of volume of mouse osteosarcoma C22LR. This osteosarcoma, transplantable in the (C57BL/Rij × CBA/Rij)F_1 hybrid in which it originated, was also used for comparing the relative effects of the drugs after single and repeated administration. The system has also been described earlier [19]. Tumour volume was measured at different intervals after single and fractionated doses of either drug. This permitted us to estimate what doses of the two agents had a similar effectiveness on tumour volume reduction and delay of regrowth.

Determination of Ftorafur and 5-FU in a Biological Specimen

Determination of ftorafur and 5-FU in serum was performed as previously described [14]. Briefly, ftorafur, 5-FU and 2-methyl-4-hydroxy-6-chloromethylpyrimidine, used as the internal standard, were extracted twice with ethylacetate at pH = 4.5; the samples were then evaporated to dryness and the chemicals converted to their chloromethyldimethylsilyl derivatives. Drug contents were measured by gas liquid chromatography, either an electron capture or a flame ionisation detection system being used, depending on the expected ranges of 5-FU and ftorafur concentration. In the conditions employed, the sensitivity limit for both compounds was 0.05 $\mu g \cdot ml^{-1}$ and the curves were linear up to 500 $\mu g \cdot ml^{-1}$. The coefficient of variation both 'within the day' and 'day to day' never exceeded 7.5%. In parallel, some of the samples were checked for specificity by mass fragmentography after a permethylation reaction [13]. Data shown are the means (± SE) of at least four sera per time per group, and pharmacokinetic parameters were calculated by the feathering approach described elsewhere [16].

Drugs

Both drugs were diluted with Hanks' BSS and injected rapidly into the tail vein in a volume of 0.02 ml/g body wt. Control mice were injected IV with the same volume of Hanks' BSS.

Results

Survival curves for haemopoietic stem cells and for L1210 leukaemia cells after a single administration of different doses of 5-FU and ftorafur are presented in Figure 1. It can be seen that proliferating normal haemopoietic stem cells are more sensitive (by a factor of 4.0) to 5-FU than resting cells; this was also true for ftorafur when given in single doses, although the difference in sensitivity between these two populations was less marked than for 5-FU. In contrast to this higher effectiveness against proliferating cells, leukaemia L1210 cells were approximately as sensitive as resting normal stem cells. To quantitate the relative effectiveness, the slopes of the curves were expressed in their reciprocals (D_0), which for both drugs are listed in Table 1A. To eliminate the difference in molecular

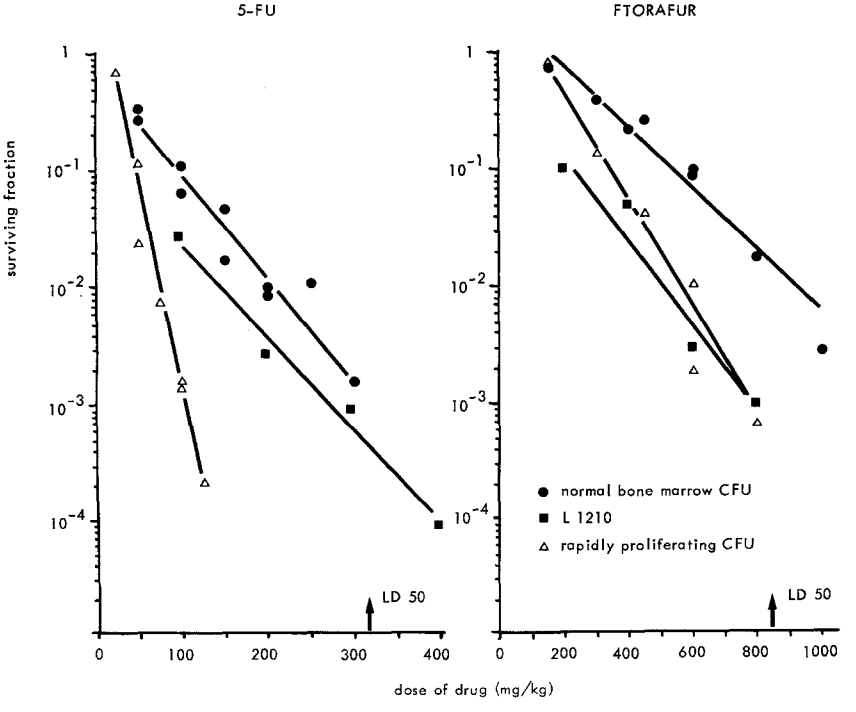

Fig. 1. Survival of haemopoietic stem cells and L1210 leukaemia cells after single SC injections of 5-FU and ftorafur. Assays for haemopoietic stem cells were carried out 16 h after drug administration. The results are expressed as fractions of the cells found per femur or spleen in untreated control mice. The assay for leukaemia L1210 cell survival was based on mouse survival time. Mice were injected IP with 10^5 L1210 cells. Twenty-four hours later they were treated with single SC doses of 5-FU and ftorafur. Increase in median lifespan was used to calculate fractional cell kill

Table 1. Effectiveness of 5-FU and ftorafur on different end points when given in single and fractionated doses

	5-FU[a] ($\mu mol \cdot kg^{-1}$)	Ftorafur[a] ($\mu mol \cdot kg^{-1}$)	Dose ratio
A. Single doses			
Mouse survival ($LD_{50/30}$)	2,421.2	4,250.7	1.8
Normal haemopoietic stem cells (D_0)	376.6	824.2	2.2
Rapidly proliferating haemopoietic stem cells (D_0)	94.5	477.0	5.0
L1210 leukaemia cells (D_0)	422.8	599.4	1.4
Osteosarcoma (volume reduction by factor 3)	2,690.2	4,495.5	1.7
B. Five daily doses			
Mouse survival ($LD_{50/30}$)	2,459.6	6,243.8	2.5
Normal haemopoietic stem cells (D_0)	169.1	739.3	4.4
L1210 leukaemia cells (D_0)	89.2	1,928.1	21.6
Osteosarcoma (volume reduction by factor 3)	1,844.7	6,618.4	3.6

[a] Molecular weights of 5-FU and ftorafur: 130.1 and 200.2, respectively

weight of 5-FU and ftorafur, the results in Table 1 are expressed on a molar basis. Also listed in Table 1 are the $LD_{50/30}$ data and the drug doses causing a comparable growth delay in the mouse osteosarcoma.

Dose-survival curves after treatment with fractionated doses of 5-FU and ftorafur are presented in Figure 2 and the D_0 values are listed in Table 1B. When the same total amount of both drugs was fractionated over a 5-day period, changes in their relative effectiveness on normal and neoplastic cells were observed. The effectiveness ratios for fractionated administration are uniformly higher than for single doses of both drugs. When searching for an explanation for this phenomenon, a difference in pharmacokinetics was considered. Ftorafur treatment has in fact been reported [2, 3, 12] to affect the activity of a number of enzyme systems in the liver, where its hydrolysis to 5-FU occurs [5]. It could thus be hypothesised that changes in enzymes, activating ftorafur, could also occur during repeated administration of this drug. For this reason, a comparison was made of serum levels of the drugs upon first administration as compared with the drug levels after the last of three daily doses. Serum levels of 5-FU after single and repeated IV injections are presented in Figure 3. It can be

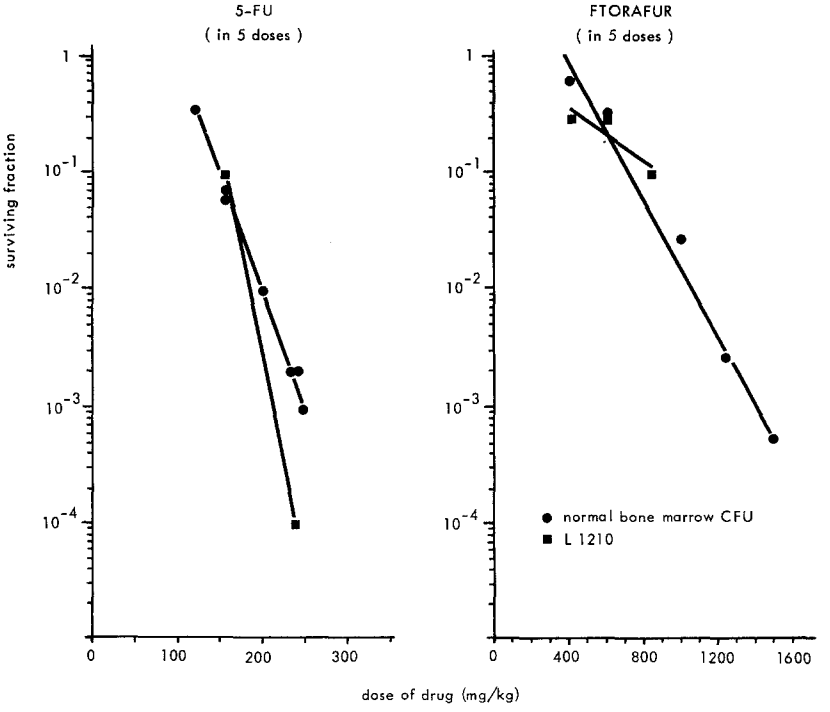

Fig. 2. Survival of haemopoietic stem cells and L1210 leukaemia cells 6 h after the last of five daily SC injections of 5-FU or ftorafur. The drug doses refer to total amounts divided in five daily fractions. Surviving fractions for haemopoietic stem cells were obtained by comparing surviving cell numbers with those obtained in untreated controls. Leukaemia L1210 cell survival was calculated from the increase in lifespan of leukaemic mice treated for 5 days, starting 24 h after IP injection of 10^5 L1210 cells

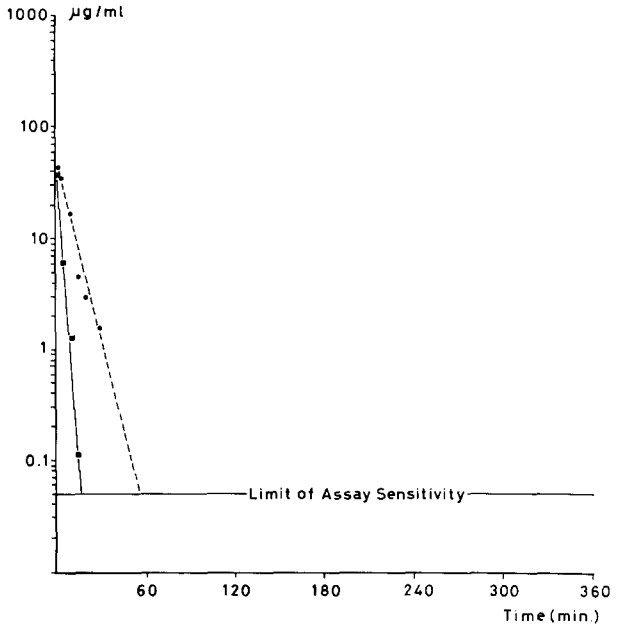

Fig. 3. Serum levels of 5-FU in mice following single (40 mg · kg^{-1}) and repeated (3 × 40 mg · kg^{-1}) IV administration of 5-FU. ■——■, 5-FU serum levels after single administration of 5-FU; ●– – –●, 5-FU serum levels after repeated administration of 5-FU

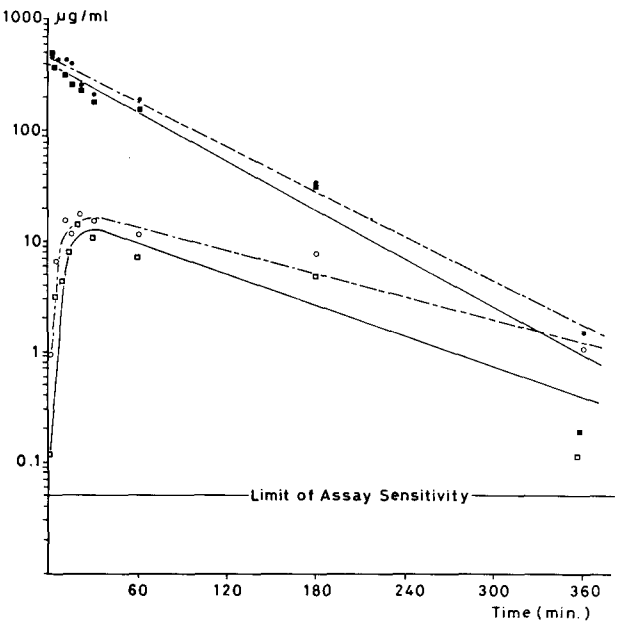

Fig. 4. Serum levels of ftorafur and 5-FU in mice following single (200 mg · kg^{-1}) and repeated (3 × 200 mg · kg^{-1}) IV administration of ftorafur. ■——■, ftorafur serum levels after single administration of ftorafur; ●– – –●, ftorafur serum levels after repeated administration of ftorafur; □——□, 5-FU serum levels after single administration of ftorafur; ○– – –○, 5-FU serum levels after repeated administration of ftorafur

seen that after both types of treatment drug levels decrease exponentially and very rapidly; it can also be observed that 5-FU levels are markedly (and highly significantly) higher, after repeated administration, this difference being detectable from the fifth minute after the injection onwards. Ftorafur and 5-FU kinetics in the serum of mice given single or repeated doses of ftorafur are shown in Figure 4. An apparently monophasic disappearance of ftorafur from the circulation was seen for both types of treatment. After ftorafur injection, 5-FU is detectable rapidly, with a peak of approximately 15 µg · ml^{-1} reached at 20 min, the levels slowly decreasing thereafter so that they are superimposable on those of ftorafur at 6 h. It can also be noted that no significant differences in the levels of either ftorafur or 5-FU were observed when single or repeated doses of ftorafur were employed. The kinetic parameters for these drugs are summarised in Table 2.

Table 2. Kinetic parameters for 5-FU, and for ftorafur and 5-FU after injection of 5-FU and ftorafur, respectively[a]

Treatment	Parameters for drug	AUC[b] (µg · ml^{-1} × min)	$T_{1/2}$ (min)	Kel[c] (min^{-1})	C_0[d] (µg · ml^{-1})
40 mg · kg^{-1} 5-FU	5-FU	109	1.71	0.34	54.95
3 × 40 mg · kg^{-1} 5-FU	5-FU	383	5.50	0.11	49.72
200 mg · kg^{-1} ftorafur	Ftorafur	26,598	34.26	0.01	423.04
3 × 200 mg · kg^{-1} ftorafur	Ftorafur	32,685	44.92	0.01	431.00
200 mg · kg^{-1} ftorafur	5-FU	2,046	52.52	0.008	26.64
3 × 200 mg · kg^{-1} ftorafur	5-FU	2,761	89.83	0.006	20.82

[a] Serum levels of ftorafur and of 5-FU were determined after single and repeated doses of both drugs. Values for the different kinetic parameters were calculated from values shown in Figures 3 and 4

[b] Area under curve

[c] Elimination constant

[d] Concentration at time zero

Discussion

In comparison of cell survival after in vivo exposure to 5-FU and ftorafur, two findings deserve attention. In the first place it is notable that upon fractionated administration, ftorafur is relatively less effective in killing bone marrow stem cells and L1210 cells, as well as in causing growth delay of an osteosarcoma and mortality of mice. In the second place, 5-FU is relatively more effective than ftorafur in killing rapidly proliferating normal bone marrow stem cells. It does not seem possible to find a common mechanism for both findings. If rapidly proliferating cells are more sensitive to 5-FU, one could explain the higher ratio for the proliferating stem cells and conclude that divided doses of the drugs would act upon cells recruited into the cell cycle. This explanation does not take into account that higher ratios were also found if L1210 leukaemia and osteosarcoma C22LR were exposed to divided doses of the drug. In these tumours all cells are in cycle and recruitment cannot explain the higher ratios seen after divided drug doses. In consequence, two separate mechanisms must be involved in causing the observed difference.

When the cell survival data reported in this paper were presented in an abstract [20] it was speculated that the decreased sensitivity of normal and malignant cells in vivo to fractionated administration of ftorafur might be a consequence of a diminished enzymatic release of 5-FU from ftorafur. Support for this hypothesis was found in observations that 5-FU [12] and ftorafur [2, 3] affect enzyme levels, which may be involved in drug metabolism. The blocking of RNA synthesis apparently affects the levels of a large number of proteins. Levels of acid phosphatase, aniline hydroxylase, and ethyl morphine *N*-demethylase were found to be decreased [12]. It was considered that if the release of 5-FU from ftorafur is an enzymatic process, this release might be decreased by previous exposure to the drug.

The data of Johnson et al. [11] seemed to argue against this hypothesis, since they showed that the 5-FU serum levels in their mice were not different upon the first or repeated administration of ftorafur. Our results confirm this finding, but have indicated another phenomenon that may be relevant: after repeated 5-FU administration the disappearance of 5-FU from the serum is slower than upon first administration. This might be important in explaining the poor tolerance for repeated 5-FU administration. In contrast, the repeated injection of ftorafur does not lead to significantly increased drug levels, and on the basis of this difference the better tolerance to chronic administration of ftorafur might still be based on pharmacokinetic differences.

These observations point to an important aspect in the evaluation of ftorafur. A major difficulty in comparing this drug to 5-FU both in model studies and in patients has been the variable tolerance ratio depending on the administration schedule. Ideally, a new drug should be compared with a well-known drug at an equitoxic level. This will permit a direct comparison of antitumour efficacy. Most of the studies with ftorafur, however, have been carried out at drug levels that were stated to be much better tolerated than conventionally used treatment with 5-FU [1, 15]. Under these circumstances a lower effectiveness of ftorafur than of conventional 5-FU is considered acceptable. However, this approach does not permit a direct comparison of the efficacy of the drugs. Higher drug doses were studied only recently and the results suggest that under those conditions GI-tract and CNS toxicity are more important than the bone marrow toxicity seen after 5-FU [8, 18].

Another aspect of these findings, the increasing serum level of 5-FU upon repeated administration, deserves further study. It is not clear whether this increased level is a consequence of, or associated with, decreased enzyme levels, as discussed earlier, or with high levels of precursor pools due to decreased DNA synthesis as a result of inhibition of RNA synthesis. In either case the increased serum level would be a consequence rather than a cause of the toxicity, and at this stage of our knowledge it does not seem justified to conclude how the increased serum levels upon repeated administration are causally related to the relatively high toxicity of repeated 5-FU administration. Nevertheless, it seems highly probable that the absence of increased 5-FU levels after repeated administration of ftorafur is the cause of the lower toxicity and efficacy of this drug when given in divided doses.

Acknowledgements: This work was performed within the programme of the EORTC Screening and Pharmacology Group. Ftorafur was made available by the Division of Cancer Treatment, NCI, Bethesda, USA.

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Received September 7, 1978/Accepted April 4, 1979