

## Metabolism of 5-fluorouracil in human liver: An *in vivo* $^{19}\text{F}$ NMR study

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*In vivo* fluorine-19 nuclear magnetic resonance ( $^{19}\text{F}$  NMR) spectroscopy was used to study the metabolism and pharmacokinetics of 5-fluorouracil (5-FU) in human liver. Nine patients received 5-FU, and additional chemotherapeutic agents (methotrexate, leucovorin, or levamisole) either prophylactically after breast cancer surgery or for colorectal cancer. The time constant for the disappearance of 5-FU from the liver *in vivo* varied from 5 to 17 min, while the time constant for the appearance of  $\alpha$ -fluoro- $\beta$ -alanine (the major catabolite of 5-FU) varied from 7 to 86 min. The modulators of 5-FU metabolism did not appear to affect the time constant for the disappearance of 5-FU from the liver or for the appearance of  $\alpha$ -fluoro- $\beta$ -alanine. Results obtained indicate that the pharmacokinetics of 5-FU and  $\alpha$ -fluoro- $\beta$ -alanine may vary substantially at different times in a given individual.

THE cytotoxicity of 5-fluorouracil (5-FU), a widely used antineoplastic agent, requires its anabolic incorporation into RNA or conversion to 5-fluoro-2-deoxyuridine monophosphate (FdUMP), a thymidylate synthase inhibitor. In the catabolic (detoxification) pathway, hydrogenation and opening of the pyrimidine ring of 5-FU result in  $\alpha$ -fluoro- $\beta$ -ureido propionic acid, and final products  $\alpha$ -fluoro- $\beta$ -alanine (FBAL), fluoride ion ( $\text{F}^-$ ), carbon dioxide, and ammonia. Since the activity of the enzyme dihydrouracil dehydrogenase is the highest in liver<sup>1,2</sup>, liver is the major site for catabolism of 5-FU.

Since the response rate of 5-FU alone is very low (~10–20%), other agents are often co-administered to modulate its efficacy. One such agent, methotrexate (4-amino,4-deoxy,*N*-10-methylpteroyl gluamic acid), binds tightly but reversibly to its target enzyme dihydrofolate reductase, leading to a cessation of *de novo* purine synthesis. This enhances 5-FU activation and cytotoxicity by increasing cellular pools of phosphoribosyl pyrophosphate (PRPP), a necessary cofactor in one enzymatic pathway to FdUMP<sup>3</sup>. In clinical studies, the administration of methotrexate 24 h prior to 5-FU has increased

tissue levels of PRPP and enhanced the antitumour efficacy of 5-FU (ref. 4). Another folate analog, leucovorin or [R, S]5-formyltetrahydrofolate, modulates 5-FU cytotoxicity by stabilizing the inhibitory ternary complex formed between its metabolite 5,10-methylenetetrahydrofolate, FdUMP, and thymidylate synthase. The adjuvant effect of levamisole, 2,3,5,6-tetrahydro-6-phenylimidazo(2,1,b)thiazole, comes from its ability to inhibit phosphofructokinase, thus decreasing ATP synthesis<sup>5</sup>.

Because of the absence of organic fluorochemicals in the human body, *in vivo* fluorine-19 nuclear magnetic resonance ( $^{19}\text{F}$  NMR) spectroscopic studies of 5-FU metabolism<sup>6–8</sup> offer a noninvasive means to assess the efficacy of the treatment regimen. The formation of fluorinated nucleosides/nucleotides was detected post-administration of 5-FU in animal models using *in vivo*  $^{19}\text{F}$  NMR studies<sup>9</sup>. A few studies in humans have also been reported<sup>10–15</sup>, but not in the liver (after high dose bolus injection) of human beings.

The half-life of 5-FU in the liver is typically short. When 5-FU is trapped in the tumour, its half-life can increase<sup>10,11,14</sup> to about an hour. These workers have found that such 5-FU trapping by tumour tissue appears to correlate with the clinical efficacy of treatment. Thus it is important to confirm independently, in different laboratories and in different treatment regimens, the difference between the 5-FU disappearance in liver and its trapping in tumours. It is also important to assess the variability in an individual. In this report, we present the results of our  $^{19}\text{F}$  NMR studies of 5-FU metabolism in the liver of patients undergoing different treatment regimens with leucovorin, methotrexate, or levamisole as modulators.

Fluorine-19 *in vivo* NMR spectra were acquired at 60.1 MHz on a GE 1.5 T Signa clinical MRI scanner, using a home-built, single-turn, distributed-capacitance surface coil (16 cm in diameter) placed above the right lobe of the patient's liver. Any 5-FU or its fluorinated metabolite detected is likely to arise mostly from liver on first-pass metabolism. Within liver, the metabolism should be relatively uniform (except perhaps in patients with tumour involvement). Hence neither the localization of the coil position by prior magnetic resonance imaging nor localized spectroscopic acquisition were deemed necessary.

Rectangular pulses of 200  $\mu\text{s}$  (tip angle = 45°) were used. The repetition time and the spectral width were 0.5 s and 6000 Hz, respectively. Each spectrum was the result of 200 acquisitions (~3.5 min/spectrum). Spectra were acquired sequentially during the administration of the drug as an intravenous bolus injection (600 mg/m<sup>2</sup> over 10 min) for a total of about 30 min. The free induction decays (FIDs) were transferred to a off-line Nicolet data station for Fourier transformation and spectral processing which involved an exponential multi-

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plication of the FID and manual phasing. In a few cases, baseline correction was applied.

The patients were receiving 5-FU and additional therapeutic agents either prophylactically after breast cancer surgery or for colorectal cancer. Voluntary informed consent was obtained from each subject. No diet restriction was enforced. Each patient was scanned similarly: in midafternoon, after a morning appointment in the outpatient clinic.

Figure 1 is a stack plot of the sequential spectra from one of the patients (#1, Table 1) with breast cancer. In this case, the initial spectrum has only a signal at about 0 ppm due to 5-FU in the first 3.5 min of drug administration. The intensity of this signal increases in the second spectrum. Concomitantly, another signal appears about -19 ppm from that of 5-FU. This signal is due to FBAL on the basis of its relative chemical shift. In subsequent spectra, the 5-FU signal intensity

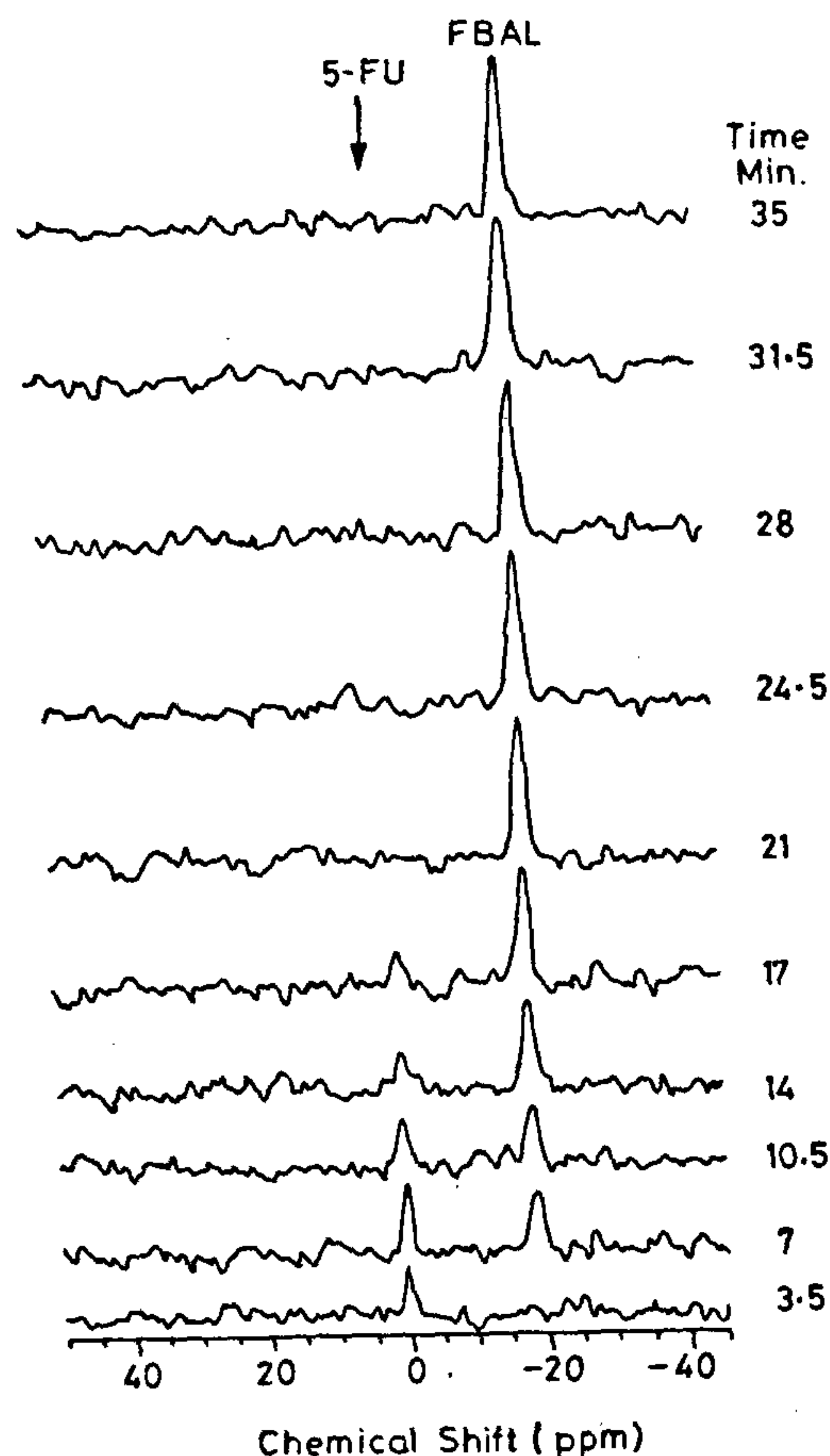


Figure 1. Stacked plot of the sequential 60.1 MHz *in vivo*  $^{19}\text{F}$  NMR spectra of the liver of patient #1 following a bolus infusion of 5-fluorouracil ( $600\text{ mg/m}^2$  over 10 min) 2 h after administering methotrexate. Each spectrum is the result of 200 acquisitions ( $\sim 3.5$  min/spectrum) using  $45^\circ$  rectangular pulses of 200  $\mu\text{s}$  duration, a repetition time of 0.5 s, and a spectral width of 6000 Hz.

decreases and FBAL intensity increases with time. At about 21 min, no 5-FU signal is visible.

The time constants ( $t$ 's) for the disappearance of 5-FU and the appearance of FBAL in liver were determined by fitting the signal intensities to single exponential decay processes. A typical fit for patient 6 is shown in Figure 2. For 5-FU, the initial point in the run was ignored since 5-FU was still increasing and we did not have sufficient time resolution to fit the data to a biexponential, absorption-elimination model. The clinical diagnosis, the treatment regimen, and the apparent time constants for 5-FU and FBAL from sixteen  $^{19}\text{F}$  NMR studies of the liver for nine patients *in vivo* are summarized in Table 1.

Patients #5-9 were on the same modulator therapy. Measurements were made on two different occasions for patient #6 and on four different occasions for patient #9. For these patients, the  $t$  measured for 5-FU on separate occasions varied within the standard error of the mean (SEM) of the individual pharmacokinetic runs. On the other hand,  $t$  for the appearance of FBAL had a relatively large variation in cases of more than one measurement (patients 1, 4, 6 and 9) on a single individual. These variations appear to be real, since in most cases the SEMs of the experimental fits are substantially smaller than the variations for an individual over time. Such intra-individual variations may arise from factors such as dietary changes. For example, 5-FU metabolism is affected by protein uptake<sup>16</sup>. Given the spectral signal-to-noise ratio and relatively small SEMs of the fits, it is unlikely that any technique deficiency alone could be responsible for such large variations.

Three patients (with breast cancer) received methotrexate in addition to 5-FU. For patient #1, the  $t$ 's of 5-FU and FBAL were measured for three different combination conditions. For patient #4, the only one studied with 5-FU-levamisole combination therapy, the  $t$ 's estimated are comparable to those listed for other combinations.

The  $t$  values for 5-FU in liver varied between 5 and 17 minutes among the 9 patients studied under different combinations. This agrees with the value for 5-FU in blood following a bolus injection, and in liver *in vivo* by  $^{19}\text{F}$  NMR<sup>12-14</sup>. On the other hand,  $t$  values for 5-FU trapped in tumours are higher, about 20-78 min (refs 10, 11). Despite increases in  $t$  values for intratumoral 5-FU, fluorinated nucleoside/nucleotide signals were not detected. It may be possible that any FdUMP formed is rapidly incorporated into DNA/RNA and the fluorine signals from these macromolecules are too broad to detect. However, it should be noted that a fluoronucleotide signal was detected in human liver in low dose continuous infusion therapy for colon cancer with alpha interferon<sup>15</sup> and as early as 30 min after intra-arterial administration of 5-FU.



Table 1. Summary of patient data, chemotherapy regimen and  $^{19}\text{F}$  NMR-estimated time constants of 5-FU and FBAL\*

Patient #	Tumour type and/or location	Treatment protocol	Time constants <sup>#</sup>		Post-treatment result
			5-Fu (min)	FBAL (min)	
1	Breast: infiltrating ductal positive nodes	5-FU, MTX	13 ± 1.7	39 ± 1.8	Did not respond and died
		MTX 1 h, 5-FU	11 ± 0.8	26 ± 2.5	
		MTX, 2 h, 5-FU	8 ± 0.6	20 ± 1.2	
2	Breast: infiltrating ductal nodes	MTX, 1 h, 5-FU	7 ± 1.0	24 ± 5.0	NED
3	Breast cancer metastasized to neck	5-FU, MTX	8 ± 1.9	41 ± 6.2	Did not respond well and died
4	Colorectal carcinoma	LEV, 5-FU	13 ± 2.5	31 ± 2.1	NED
		LEV, 6 h, 5-FU	10 ± 0.9	52 ± 2.5	\$
5	Sigmoid colon cancer: moderately differentiated adenocarcinoma with liver metastasis	LEUC, 5-FU	5 ± 0.5	14 ± 2.3	Had a recurrence of tumour and died.
6	Colon cancer with liver metastases	LEUC, 5-FU	15 ± 1.6	25 ± 2.2	Did not respond well and died
			13 ± 2.2	86 ± 5.7	
7	Skin and colon adenocarcinoma one positive node	LEUC, 5-FU	—	30 ± 11	NED \$
8	Colon, historical uterine and tube adenocarcinoma moderately differentiated	LEUC, 5-FU	7 ± 2.4	11.4 ± 4.0	NED
9	Sigmoid colon cancer, adenocarcinoma moderately differentiated	LEUC, 5-FU	17 ± 4.8	7 ± 3.2	NED
			10 ± 5.8	20 ± 3.0	
			15 ± 4.0	44 ± 8.8	
			13 ± 3.4	33 ± 5.3	

\*Abbreviations: FBAL,  $\alpha$ -fluoro- $\beta$ -alanine; LEUC, leucovorin; LEV, levamisole; MTX, methotrexate; NED, no evidence of disease.

<sup>#</sup>The time constants above are expressed as  $t \pm$  the standard error of the mean (SEM) from fitting the experimental data to a single exponential function. \$ Developed liver metastasis subsequently.

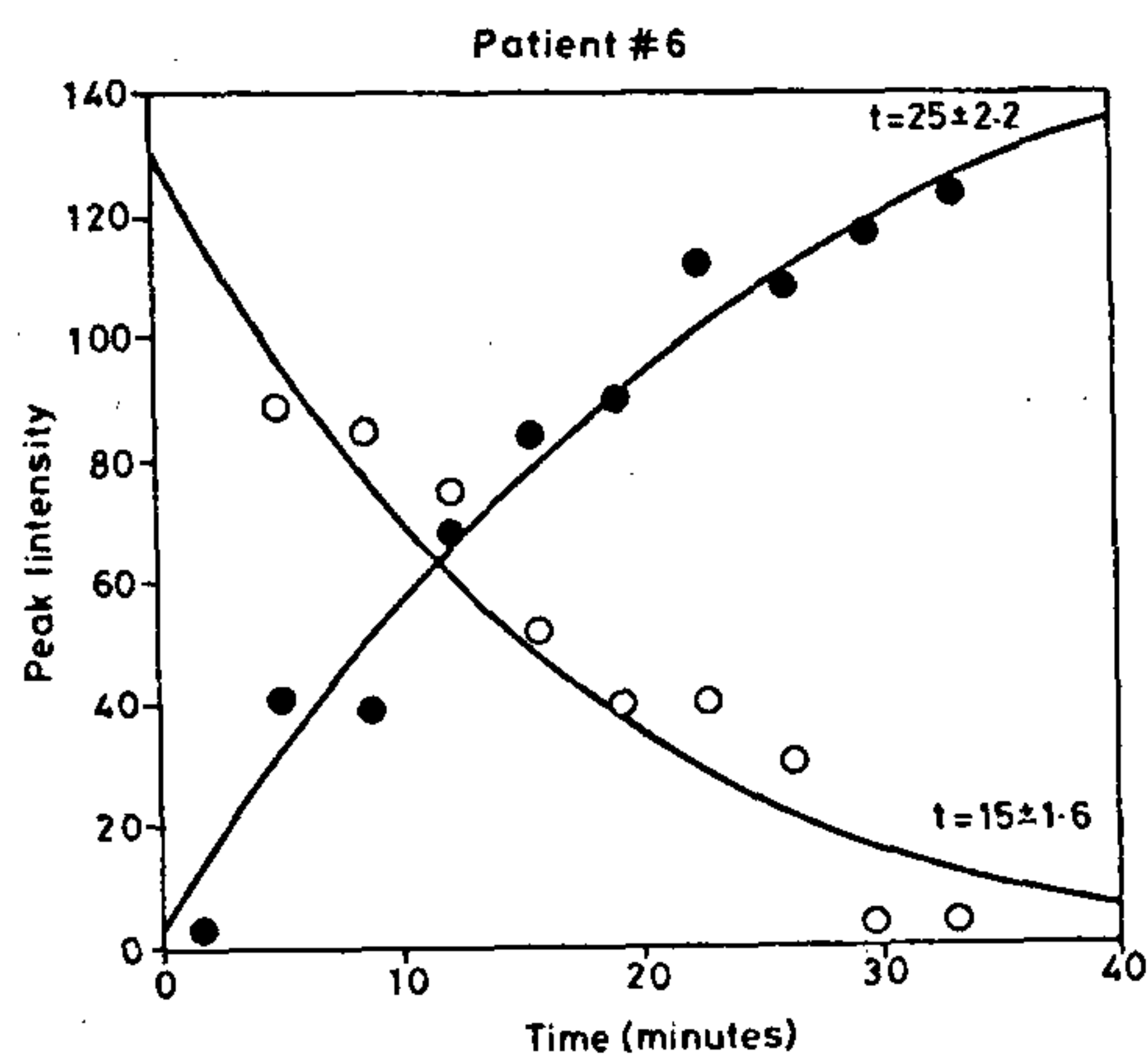


Figure 2. A typical fit of the  $^{19}\text{F}$  NMR pharmacokinetic data. The apparent time constants were determined from the spectra by fitting  $\alpha$ -fluoro- $\beta$ -alanine and 5-fluorouracil signal intensities in each examination to a single exponential decay or growth process, as appropriate.

Methotrexate enhances the production of cytotoxic fluoronucleotides in animal tumours<sup>9,18,19</sup>. Pretreatment with methotrexate, however, does not cause any change in the pattern of 5-FU metabolites in plasma or urine as measured by  $^{19}\text{F}$  NMR<sup>20</sup>. A recent study of advanced colon cancer found a 24 h interval, rather than a 1 h

interval, to be effective in producing tumour regression<sup>21</sup>. Patient # 1, who was being treated prophylactically for breast cancer, underwent three different combinations of 5-FU and methotrexate under otherwise constant conditions. From the time-constant estimates in Table 1, it appears that prior methotrexate infusion accelerates the disappearance of 5-FU and appearance of FBAL, suggesting an enhancement of the drug catabolism in the liver.

The large jump in  $t$  for FBAL for patient # 6 (see Table 1) may arise from changes in liver involvement during the intervening four weeks. Because of the difficulties in obtaining longitudinal data from the same patients in a controlled fashion, the variation in FBAL  $t$  for patient # 9 may arise from variation in diet.

The average time constants for the first three patients are  $8.56 \pm 1.90$  and  $31.11 \pm 8.83$  min, respectively for 5-FU and FBAL, when methotrexate is used as the modulator. The corresponding values for the four cases (except for patient # 7) of leucovorin modulation are  $9.84 \pm 4.62$  and  $26.73 \pm 17.50$  min. The differences in  $t$  between the two groups are not significant. There was a linear correlation ( $r = 0.67$ ) between the NMR estimates of the two time constants. Based on the results of our limited study, it is concluded that the modulators of 5-FU do not significantly affect the time constants for disappearance of 5-FU or appearance FBAL in the liver.

Small changes in pharmacokinetics of 5-FU and FBAL may result if there are differences in times of administration of 5-FU and its modulators in combination chemotherapy.

In conclusion, our present study documents that  $^{19}\text{F}$  *in vivo* NMR is a useful tool to follow the pharmacokinetics and metabolism of fluorinated drugs in patients undergoing cancer chemotherapy. Such studies allow noninvasive determinations of variations in the metabolism of drugs at various organ and tissue sites of patients having various pathophysiologies and/or undergoing selected pharmacological interventions.

1. Pinedo, H. M. and Peters, G. F. J., *J. Clin. Oncol.*, 1988, 6, 1653-1654.
2. Chabner, B. A., in *Pharmacological Principles of Cancer Treatment*, Saunders, Philadelphia, 1988, pp. 183-200.
3. Cadman, E., Heimer, R. and Davis, L., *Science*, 1979, 205, 1135-1137.
4. Kemeny, N. E., Ahmed, T., Michaelson, R. A., Harper, H. D. and Yip, L. C., *J. Clin. Oncol.*, 1981, 2, 311-315.
5. Stevenson, H. C., Green, I., Hamilton, J. M., Calabro, B. A. and Parkinson, D. R., *J. Clin. Oncol.*, 1991, 9, 2052-2066.
6. Evelhoch, J. L., *Invest. New Drugs*, 1974, 7, 5-12.
7. Maxwell, R. J., *Pharmacokinetics Canc. Chemother.*, 1993, 17, 415-423.
8. Komoroski, R. A., *Anal. Chem.*, 1994, 66, 1024A-1033A.
9. Kamm, Y. J. L., Rietjens, I. M. C. M., Vervoort, J., Heerschap, A., Rosenbusch, G., Hofs, H. P. and Wagener, D. J. T., *Cancer Res.*, 1994, 54, 4321-4326 and references therein.
10. Present, C. A., Wolf, W., Albright, M. J., Servis, K. L., Ring, III R., Alkinson, D., Ong, R. L., Wiseman, C., King, M., Blayney, D., Kennedy, P., El-Tahtawy, A., Singh, M. and Shani, J., *J. Clin. Oncol.*, 1990, 8, 1868-1873.
11. Present, C. A., Wolf, W., Waluch, V., Wiseman, C., Kennedy, P., Blayney, D. and Brechner, R. R., *Lancet*, 1994, 343, 1184-1187.
12. Semmler, W., Bachert-Baumann, P., Guckel, F., Eremark, F., Schlag, P., Lorenz, W. J. and van Kaick, G., *Radiology*, 1990, 174, 141-145.
13. Schlemmer, H-P., Bachert, P., Semmler, W., Hohenberger, P., Schlag, P., Lorenz, W. J. and van Kaick, G., *Magn. Reson. Med.*, 1994, 12, 497-511.
14. Wolf, W., Present, C. A., Servis, K. L., El-Tahtawy, A., Albright, M.J., Barker, P.B., Ring III, R., Alkinson, D., Ong, R., King, M., Singh, M., Ray, M., Wiseman, C., Blayney, D. and Shani, J., *Proc. Natl. Acad. Sci. USA*, 1990, 87, 492-496.
15. Findlay, M. P. N., Leach, M. O., Cunningham, D., Collins, D. J., Payne, G. S., Glaholm, J., Mansi and J. L. McCready, V. R., *Ann. Oncol.*, 1993, 4, 597-602.
16. Lenkinski, R. E., Shinkwin, M. A., Daly, J. M., Kressel, H. Y. and Holland, G. A., Book Abstracts of the 7th Annual Meeting of the Society of Magnetic Resonance in Medicine, 1988, pp. 450.
17. Cohen, J., Irwin, L. E., Marshall, G. J. and Darvey, H., Bateman, J. R., *Cancer Chemother. Rep.*, 1974, 58, 723-731.
18. McSheehy, P. M. J., Prior, M. J. W. and Griffiths, J. R., *Br. J. Cancer*, 1989, 60, 303-309.
19. Koutcher, J. A., Sawyer, R. C., Kornblith, A. B., Stolfi, R. L., Martin, D. S., Devitt, M. L., Cowburn, D. and Young, C. W., *Magn. Reson. Med.*, 1991, 19, 113-123.
20. Hull, H. E., Port, R. E., Herrmann, R., Britsch, B. and Kunz, W., *Cancer Res.*, 1988, 48, 1680-1688.
21. Marsh, J. C., Bertino, J. R., Katz, K. H., Davis, C. A., Durivage, H. J., Rome, L. S., Richards, F. II, Capizzi, R. L., Farber, L. R.,

Pasquale, D. N., Stuart, R., Koletsky, A. J., Makuch, R. and O'Hallaren, K., *J. Clin. Oncol.*, 1991, 9, 371-380.

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## Thermal structure and heat budget of Priyadarshini Lake, Schirmacher Oasis, East Antarctica

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**Data on thermal structure and heat budget of Priyadarshini Lake, Schirmacher Oasis, East Antarctica are presented. Available data during the summer of the sixteenth Indian Antarctic Expedition (December 1996-March 1997) reveal that the Priyadarshini Lake was weakly stratified. The lake became unstratified as the winter period approached.**

SCHIRMACHER Oasis of Antarctica is a group of low-lying hills of 50-200 m height and is interspersed with a number of freshwater glacial lakes. The available information indicates that the size of these lakes ranges from a few hectares to a few km<sup>2</sup> and the maximum depth of water in them ranges from a few meters to about 150 m. Depending upon their topographic setting, they occur as inland lakes, ice margins lakes or epishelf lakes. The Priyadarshini Lake, with a total water spread area of 0.75 sq km, is one of the largest lakes in the region and is closest to the Indian Station, Maitri. This lake, located about 255 m away from Maitri, is the lifeline of the Indian expedition. It supplies water to the station. It has been described as a proglacial lake<sup>1</sup>, formed at the edge of the ice cap during the deglaciation phase. The water and sediment input to the lake is entirely through melt water during warmer periods of spring and summer. A study of Priyadarshini Lake was taken up by IIT Kanpur during the XVI Indian Antarctic expedition (December 1996-March 1997) to determine its thermal structure and heat budget.

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