

METABOLISM OF 5'-DEOXY-5-FLUOROURIDINE IN HUMANS:
QUALITATIVE AND QUANTITATIVE ANALYSIS BY FLUORINE-19 NMR

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5'-deoxy-5-fluorouridine (5'dFUrd) is a recently synthesized fluoropyrimidine nucleoside reported to possess both a higher cytostatic activity against a broad spectrum of human tumors and a lower toxicity than 5-fluorouracil (5FU), 2'-deoxy-5-fluorouridine and ftorafur [1].

5'dFUrd metabolism has been investigated in human biofluids (plasma, urine) using a new method: fluorine-19 NMR spectrometry. This method allows the direct study of biological samples (with no need for separation, extraction or derivatization) and simultaneous identification and quantification of the different fluorinated metabolites without any differential treatment.

Patients evaluated in this study were treated with 5'dFUrd at 10 g/m² during a 6 hr continuous perfusion. ¹⁹F NMR spectra were recorded at 250 MHz without proton decoupling on a CAMECA 250 FT spectrometer. The concentrations of the different fluorinated metabolites were determined by comparing the areas of their respective NMR signals with those of internal references (sodium monofluoroacetate (FAC) and sodium 4-fluorobenzoate (FBEN)). The accuracy of the NMR assay is within 5-8% and the limit of sensitivity is about 10⁻⁵ M.

The first approach was a qualitative study of the different biofluids. We found, in addition to the known metabolites 5FU and 5,6-dihydro-5-fluorouracil (5FUH₂) [2], three catabolites that have not previously been reported: αfluoro β alanine (FBAL), αfluoro β ureidopropionic acid (FUPA) and fluoride ion F⁻ which probably results from FBAL defluorination (Fig. 1). We therefore proposed for 5'dFUrd the catabolic pathway represented in Fig. 2.

¹⁹F NMR is the only method which allows a complete quantitative evaluation of all the 5'dFUrd fluorinated metabolites found in body fluids. In plasma, 5'dFUrd concentration was high (3.5-7 10⁻⁴ M) from 0.5 to 6 hr after the beginning of the perfusion; it then rapidly decreased and 5'dFUrd was no more detected 3 hr after the end of the perfusion. 5FU and 5FUH₂ were only detected in some samples during the perfusion (3-4 10⁻⁵ M). FUPA and FBAL elimination curves were very similar with a maximal concentration = 3.10⁻⁴ M for FBAL and = 10⁻⁴ M for FUPA between 3 and 6 hr after the beginning of the perfusion. F⁻ concentration reached a maximum (= 4.10⁻⁵ M) between 5 and 7 hr after the beginning of the perfusion with a time shift of about 2 hr with regard to FBAL. As seen in Fig. 3, cumulative urinary excretion of 5'dFUrd and its fluorinated metabolites has shown that 67% of the injected dose was eliminated in 21 hr. The two main urinary excretion products were 5'dFUrd and FBAL (64.1% of the dose); 5'dFUrd was specially excreted during the

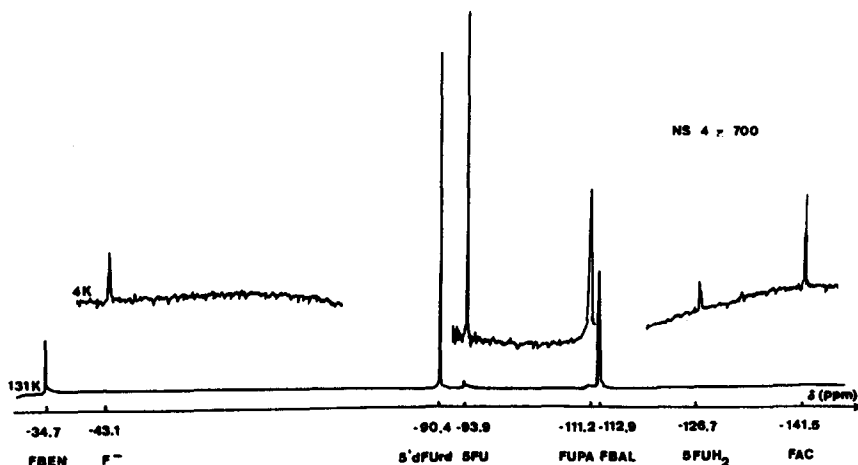


Fig. 1. ^{19}F NMR spectrum of a urine sample of a patient treated with 5'dFurd. 5'dFurd, 5FU, FUPA, FBAL, 5FUH₂ and F⁻ are 5'dFurd metabolites; FBEN and FAC are internal references. The resonance frequencies are related to CF₃COOH (0.5% aqueous solution) as an external standard.

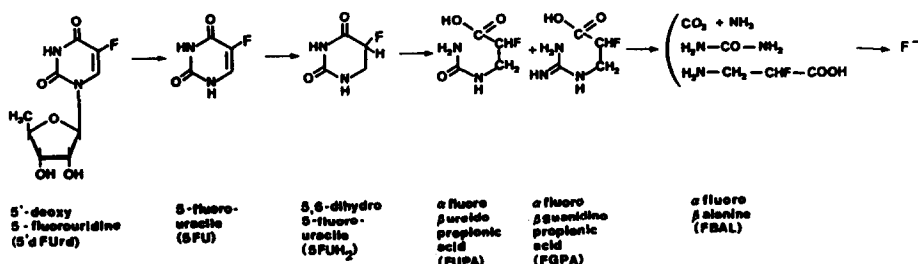


Fig. 2. Proposed catabolic pathway for 5'dFurd.

first 8 hours; FBAL cumulative excretion was about 25% during the first 7 hours and it reached 35.7% after 21 hr. Among the other metabolites (5FU, 5FUH₂, FUPA and F⁻; 2.9% of the dose), FUPA was the major compound; 5FUH₂ and 5FU were especially detected at the beginning of the perfusion whereas F⁻ increased gradually until 21 hr.

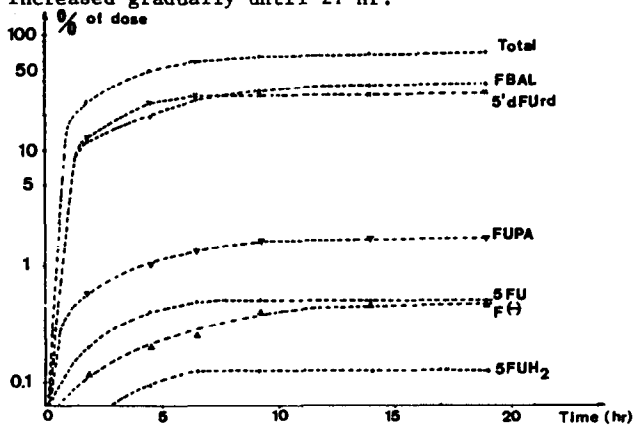


Fig. 3. Cumulative urinary excretion of 5'dFurd and metabolites following 10 g/m² 5'dFurd.

^{19}F NMR has allowed the direct, simultaneous and quantitative determination, in human biofluids, of all the fluorinated metabolites coming from 5'dFurd catabolism: unmetabolized 5'dFurd, 5FU, 5FUH₂ and 3 other metabolites that have not previously been reported: FUPA, FBAL and F⁻.

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