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pH Dependence of 5-Fluorouracil Uptake Observed by in Vivo $^{31}$P and $^{19}$F Nuclear Magnetic Resonance Spectroscopy

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Abstract

Multinuclear nuclear magnetic resonance spectroscopy was used to follow the metabolism and kinetics of 5-fluorouracil (5FU) after i.v. administration at a dose of 100 mg/kg on Wistar rats. $^{31}$P spectra allow one to determine both the energetic status and the pH of the tissues under investigation, while serial $^{19}$F spectra reveal the drug clearance. Analyses of 5FU kinetics show that the half-life of 5FU elimination is about 35 min in tissue with a pH of 7.3. However, this half-life increases 2.5-fold when the local pH decreases below 6.9. Thus, acidification seems to induce a local retention of 5FU, which tends to prove the existence of active transport. This retention of the drug may have significant clinical implications for assessing and improving chemotherapy alone or in combination.

Introduction

The antineoplastic 5FU is widely used alone or in combination in the management of several common malignancies. The biochemical mechanisms of action of the fluoropyrimidines have been extensively studied (1). As an analogue of uracil, which is necessary for sustaining nucleic acid synthesis required for tumor growth, the drug must be converted to fluoronucleosides and fluoronucleotides to exert its cytotoxic activity. However, 5FU is inactivated, via the same catabolic pathways as for these sides and fluoronucleotides to exert its cytotoxic activity. How for tumor growth, the drug must be converted to fluoronucleosides and fluoronucleotides to exert its cytotoxic activity.

Materials and Methods

Experiments were performed on 17 Wistar rats [body weight, 510 ± 20 (SD) g] either on the thigh muscle (7) or on fibrosarcoma (10) induced by a s.c. injection of 7,12-dimethylbenz[a]anthracene (2 mg in 0.2 ml sterile neutral olive oil solution) in the flank of 3-month-old rats. Prior to the experiment, the rat was anesthetized by i.m. injection of ketamine (150 mg/kg body weight; Imalgene 1000; Merieux). A catheter was inserted in the femoral artery for blood pressure monitoring and blood gas analysis. A second catheter was placed in the femoral vein so that the drug could be administered after shimming and preliminary NMR tests. The rat was then laid on a thermostated support with the surface coil positioned over the area to be observed. NMR spectra were recorded on a 4.7-T, 31-cm-bore spectrometer (Biospec; Bruker) using a home-built, 18-mm-diameter, double-tuned ($^{31}$P and $^{19}$F) surface coil.

$^{31}$P NMR spectra were acquired to measure the local pH and to control the energetic status of the tissue under observation. Tissue pH was calculated from the chemical shift of the Pi peak using the Henderson-Hasselbalch equation (6). The shift of Pi is usually referenced to that of the pH-insensitive phosphocreatine peak. In the absence of phosphocreatine, the Pi chemical shift was assigned to α-nucleoside resonance, and thus 5FLU was set to —¿93.8 ppm, and the β-nucleoside resonance to that of the pH-insensitive phosphocreatine peak. In the absence of phosphocreatine, the Pi chemical shift was assigned to α-nucleoside resonance, and thus 5FLU was set to —¿93.8 ppm, and the β-nucleoside resonance to —¿7.5 ppm (7). Although many sources of error may be involved in the determination of pH, resolution is estimated to about 0.1 pH unit. Tissue pH was controlled prior to drug administration and then from time to time during the experiment. The $^{31}$P NMR acquisition parameters were a pulse length corresponding to 180° flip angle at the center of the surface coil, a spectral width of 6 kHz, a repetition time of 3 s, and an accumulation of 128 scans. An exponential multiplication (20 Hz line broadening) was applied to the FID prior to Fourier transformation.

Immediately after the i.v. administration of 5FU at 100 mg/kg body weight as a bolus, $^{19}$F spectra were acquired using a pulse duration corresponding to 180° flip angle at the center of the surface coil, a repetition time of 0.5 s, and a sweep width of 20 kHz. An accumulation of 800 FID, collected over an 8-min period, with an exponential multiplication (40 Hz line broadening) ensured a good signal-to-noise ratio for the spectra. Fluorine-19 NMR acquisitions were usually carried out for 4 h, every 15 min the first hour and then every 30 min. Chemical shifts were reported relative to trifluoroacetic acid (5% w/v aqueous solution) resonance, and thus 5FU was set to —93.8 ppm, and fluoronucleosides/nucleotides, FUPA, and FBAL were assigned to resonances at —89, —110, and —112.5 ppm, respectively (8).

Determination of the absolute metabolite concentration is not essential for pharmacokinetic studies that require only comparative measurements. Since the animal position and the experimental conditions are constant during all series of acquisitions, spectra can easily be analyzed by measuring the intensity of the peak of interest. Peak

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2. The abbreviations used are: 5FU, 5-fluorouracil; NMR, nuclear magnetic resonance; FID, free induction decay; FUPA, 2-fluoro-3-aminopropionic acid; FBAL, 2-fluoro-β-alanine; FNUCt, fluoronucleotide(s).
intensity has been estimated by height measurement from phased and baseline-corrected spectra. Data were plotted against time. The noise level determined in a peak-free region was used as an estimated error of peak height. Finally, data series have been fitted by a one-exponential model to characterize the half-life of the local drug elimination. SD level determined in a peak-free region was used as an estimated error in the area under observation could lower the normal tissue pH through microinjections of buffer bases needed, so that anesthesia via several i.m. microinjections was produced by an i.p. infusion of 10% glucose solution (10 ml/h). A local and consistent modification of pH is, however, somewhat more complicated to obtain in normal muscle tissues by such a method because of the reduced metabolism of relaxed muscle. Fortuitously, we observed that i.m. injections of anesthetics, the chemical solution of which has a pH of 4.8, decrease the local pH. Furthermore, the moderate blood flow in relaxed muscle is insufficient to supply the buffer bases needed, so that anesthesia via several i.m. microinjections in the area under observation could lower the normal tissue pH throughout the experiment.

Results and Discussion

A typical 31P NMR spectrum of fibrosarcoma is shown in Fig. 1. We observed that the phosphocreatine level in this tumoral tissue usually represents less than 10% of the total phosphorus signal. This ensures that the volume under investigation corresponds only to the tumor. Furthermore, the intensities of the other resonances, especially P, and nucleoside triphosphates, which vary slightly from one tumor to another and seem quite constant during tumor growth, prove that the tissues observed by the NMR probe do not have a significant necrotic center.

The low sensitivity of the NMR technique is a major limitation to the extensive use of this method to monitor the metabolism and kinetics of a drug. However, 5FU doses of 100 mg/kg, which are somewhat atypical for therapeutic purposes but well below the median lethal dose (300 mg/kg), represent a compromise with a sensitivity sufficient to follow the kinetics of drug elimination. Representative sequential series of 19F spectra obtained on an induced fibrosarcoma after i.v. injection of 5FU are represented in Fig. 2. The time associated with a spectrum corresponds to the period between the drug administration and the 400th FID of the running accumulation. In all types of tissues investigated, 5FU (-93.8 ppm) was immediately detected and persisted over 4 h. The highest 5FU concentration has always been obtained from either the first or the second accumulation, which indicates a rapid distribution of the drug throughout all the body tissues. This is in agreement with the published results obtained, whatever the route of administration, on either murine (2, 3, 5) or human (3, 4) tumors.

To exert antitumoral activity, 5FU must be incorporated into the tumor cells and be converted into florouronucleosides and FNUCt, which appear as a single peak (-89 ppm) in 19F spectra. This resonance has been observed within tumoral tissues but never in normal muscle. The level of FNUCt varies from one rat to another depending on the growth stage of the tumor. For instance, within small fibrosarcomas (volume <25 ml), which are usually in a fast-growing stage and thus need a fair amount of nucleic acids, FNUCt peak intensity is significantly higher than within low proliferative tumors. In fibrosarcoma, detectable amounts of FNUCt were measured about 30 min after the drug injection, which reflects the rapid incorporation of the drug into proliferating cells. The incorporation process is considered to be controlled mainly by passive and facilitated diffusion (15). However, biodispersability of 5FU in target tissues is quite low since as much as 80% of the administered compound is degraded in the liver into FUPA and FBAL. These catabolites are then spread over all tissues via the blood circulation (16), and the peak arising from these metabolites, mainly FBAL (-112.5 ppm), is usually seen about 1 h after the drug injection. FBAL is always at its maximum at the end of the observation period. In a few cases we detected a shoulder on the low-field side of the FBAL peak, which was assigned to the transient catabolic intermediate FUPA.

In addition to these peaks, we observe at about 3 h after injection a weak signal 1.2 ppm downfield from the 5FU resonance in 3 of 10 tumor-bearing animals. Such a resonance is still unassigned in spite of several attempts to characterize a particular metabolite by gas chromatography-mass spectrometry on plasma and tissue samples. An unidentified resonance in the vicinity of 5FU has already been reported by others (3, 5), who suggest the possible existence of different pools of cellular environment. Thus, variations in the local pH, which has been correlated to the chemical shift difference between 5FU and FNUCt (5, 17), could be used to distinguish between locally different concentrations of the drug. Unfortunately, accurate interpretations are made difficult by the limited sensitivity of the method and the composite aspect of some peaks (P, FNUCt) in the in vivo spectra.

While catabolite and possible anabolite resonances increase gradually, the 5FU peak diminishes as observed by others (2–5). The peak height of the free 5FU resonance was plotted versus time. Fig. 3 represents an example of a data plot obtained from tumors at different pH. A one-exponential model was used to fit the data and to determine the half-life elimination of the drug. We observed that the drop in 5FU closely fits a monoexponential decay. The half-life is then determined directly from the fit values. Thus, in Fig. 3, the t½ of 5FU elimination in a 10-ml fibrosarcoma with a pH of 7.4 is 38 ± 6 min. In a similar tumor, the pH drops to 7.0 after glucose infusion, whereas 5FU decreases more slowly (t½ ≈ 78 ± 14 min). The uneven aspect of this last decay reflects the physiological regulation of pH and the concomitant difficulties of inducing and controlling local acidosis.
All the determined half-lives of 5FU are gathered and plotted in Fig. 4 against the pH measured in situ. We observed that regression analysis of the relative intensity of the 5FU peak measured on various types of tissue at a pH between 7.0 and 7.4 gave an elimination half-life of about 30 min. This value compares well with the \( t_0 \) of 25 ± 5 min (\( n = 4 \)) measured in plasma samples by standard high-pressure liquid chromatography for a similar drug dose. This tends to prove the predominance, in a physiological range of pH, of a passive diffusion process which equilibrates the drug concentration between plasma and intracellular fluids. However, we observe in Fig. 4 that below pH 6.9 drug elimination increases 2.5-fold (\( t_0 = 95 \pm 14 \) min) regardless of the tissue. Thus the drug concentration in blood and extracellular fluids must be lower than in intracellular fluids. Therefore, a local acidification can significantly increase 5FU uptake into the cells, a phenomenon that can be described as drug trapping. Such a retention of the drug reveals the active transport process suggested by different studies on cultured cells (18, 19). Wohlhueter et al. (18) determined this process to be pH dependent by demonstrating that only the nonionized form of 5FU is a substrate for the transporter.

Different studies, on both experimental (3, 5) tumors and human (3, 4) tumors, have shown similar trends. Interestingly, a positive correlation between 5FU trapping and the response of human tumors to chemotherapy has been observed (3, 4). With increased biodispersibility of 5FU into cells, retention of the drug may have significant clinical implications for assessing and improving chemotherapy alone or in combina-

Fig. 2. Sequential series of in vivo 19F spectra obtained on an induced fibrosarcoma after i.v. injection of a 5FU bolus (100 mg/kg). Each spectrum is the result of 800 FID acquired using an 18-mm-diameter, double-tuned (19P and 19F) surface coil.

Fig. 3. Kinetics of 5FU intensity measured in situ by 19F NMR spectroscopy in induced fibrosarcoma either with pH = 7.4 (C) or after acidification (pH = 7.0) produced by a series of i.p. injection of a 10% glucose solution (B). Irregularities in this last decay reflect the difficulties in controlling an induced acidosis. Data points were fitted using a one-exponential model to determine the half-life. Error bars are defined by the noise level of a peak-free region.

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References


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