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Note

In-vivo evaluation of sustained release microspheres of 5-FU in rabbits

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Abstract

The in-vivo absorption characteristics of 5-fluorouracil (5-FU) encapsulated within ethylcellulose microspheres, prepared by an evaporation/extraction method, were evaluated in rabbits. 5-FU was administered as a solution both intravenously ($n = 5$, 12 mg/kg) and orally as a single dose ($n = 6$, 20 mg/kg), and orally as microspheres ($n = 5$, 20 mg/kg). Statistically significant differences were found between the two oral treatments. The mean residence time and mean absorption time were both considerably increased following microsphere administration compared with the solution. The absolute bioavailabilities of 5-FU from the solution and the microspheres were 5 and 17%, respectively.
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5-Fluorouracil (5-FU) is an antimetabolite of the pyrimidine analog class which is widely used alone or in combination chemotherapy regimens. After intravenous injection of 5-FU, it is rapidly distributed and eliminated with an apparent terminal half-life of 8–20 min (Diasio and Harris, 1989). 5-FU is poorly absorbed after oral administration with extremely variable bioavailability (Diasio and Harris, 1989). These disadvantages make it an appropriate candidate for microencapsulation. Indeed, microencapsulation using biodegradable and non biodegradable polymers has already been employed to achieve sustained release of anticancer drugs such as 5-FU (Ciftci et al., 1994; Boisdron-Celle et al., 1995). Furthermore, there is no controlled-release dosage form of 5-FU for oral delivery, although this would be particularly useful in cancer therapy.

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In a previous paper, we described the preparation and the characterization of microspheres prepared from ethylcellulose and loaded with 5-FU (Zinutti et al., 1994). We have shown that the drug release rate could be controlled principally by varying the drug/polymer ratio and the nature of the casting solvent (Zinutti et al., 1996). To our knowledge, there are only a few reports of in-vivo evaluation of microencapsulated 5-FU. However, in these studies, the administration was limited to the intravenous route, using very small 5-FU-loaded microparticles (2–5 μm) in order to achieve both higher drug levels in the liver and lower systemic toxicity (Ciftci et al., 1996). The objective of our work was to evaluate the in-vivo absorption characteristics of 5-FU sustained-release ethylcellulose microspheres.

Heparin (Heparine Choay®) was kindly supplied by Choay (Paris, France). The commercial injectable solution of 5-FU (Fluoro-uracil Roche®, 250 mg/5 ml) was used as the reference dosage form for intravenous and oral administration. All other reagents and solvents were of analytical grade.

Ethylcellulose microspheres containing 5-FU were prepared according to the method previously described (Zinutti et al., 1994). A drug/polymer ratio of 1:1 was selected for in-vivo studies.

Experiments were carried out on male rabbits (New Zealand breed) with an average weight of 2.58 ± 0.14 kg. The animals were fasted for 24 h before the experiment, but were allowed free access to water. During the experimental period, each rabbit was placed in a restraining stand. The marginal ear vein was cannulated using a Jelco catheter (Critikon, Chatenay-Malabry, France). After an intravenous injection of heparin (2000 IU/kg), a 5-FU solution at a dose of 12 mg/kg was rapidly injected (bolus) into the ear vein. Based on preliminary experiments, the oral dose for 5-FU was fixed at 20 mg/kg for the solution and an equivalent dose of 20 mg of 5-FU per kg for the microspheres. The microspheres were previously filled into hard gelatin capsules (size 1) to facilitate administration. Five rabbits received each dosage form.

Blood samples (1 ml) were collected before treatment and over a period of 3 h after administration of the solution by intravenous and oral routes, and over a period of 24 h after oral administration of 5-FU microspheres. After immediate centrifugation, the serum was separated and stored at -20°C until analysis.

5-FU plasma concentrations were measured using a sensitive and validated high-performance liquid chromatography assay (Barberi-Heyob et al., 1992). The procedure included liquid–liquid extraction using ethyl acetate-methanol (95:5, v/v) and preparative column chromatography to separate 5-FU from blood components. Reversed-phase high-performance liquid chromatography was performed on a C18 column with a mobile phase of water–methanol (95:5, v/v). The flow rate was 0.5 ml/min and the detector wavelength was set at 268 nm. The determination limit of the assay for 5-FU was 10 ng/ml. Concentrations of 5-FU between 10 and 500 ng/ml were measured in plasma with a relative standard deviation of 6.8%.

Pharmacokinetic parameters, assessed using a MicroPharm program (Loginserm, Creteil, France) were determined from the plasma concentration-time data. Plasma 5-FU concentrations following intravenous administration were analysed by a two-compartment pharmacokinetic model with elimination from the central compartment. The area under the plasma concentration-time curve (AUC) and the area under the first moment curve (AUMC) were estimated by the linear trapezoidal rule and extrapolated to infinity using standard techniques (Gibaldi, 1984). The mean residence time (MRT) of the drug in the body was obtained from the ratio of AUMC to AUC. The rate of absorption of 5-FU after oral administration was estimated by the mean absorption time (MAT) based on differences in MRT after oral and intravenous administration (Gibaldi, 1984).

The following parameters (AUC, MAT, MRT, absolute and relative bioavailabilities) were tested for statistical significance using the ANOVA test. Differences were considered to be significant when $p < 0.05$.

After intravenous administration of a 12 mg/kg dose of 5-FU, the plasma concentrations declined biexponentially according to a two-compartment model. The results confirmed that the distribution

and the elimination of 5-FU were very fast. Indeed, the elimination half-life was only 27 ± 7 min. This observation is similar to that of Del Nozal et al. (1994) after an intravenous administration of a 30 mg/kg dose of 5-FU in rabbits. Such a short half-life, which is also observed in humans (Diasio and Harris, 1989), necessitates the infusion of this drug in the clinical situation.

The mean plasma levels as a function of time after oral administration of a single 20 mg/kg dose of the solution, and the experimental formulation are shown in Fig. 1. The standard deviations are not represented in the figure, but they indicate a large variation in the plasma concentrations of 5-FU between different rabbits. After oral administration of the 5-FU solution, the drug was detected rapidly in plasma. The maximum concentration of 5-FU was ~ 600 ng/ml after 20 min. Thereafter, the plasma concentration decreased and the drug was undetectable as soon as 2 h after administration. The elimination half-life was 22 ± 10 min. As far as microspheres were concerned, the plasma concentration curve included two secondary peaks and it was impossible to determine an elimination half-life. Concentrations measured at 12 h were above the quantification limit of the analytical technique (Barberi-Heyob et al., 1992) in all the animals, but at 24 h, a low concentration of 5-FU was observed in only one rabbit.

The pharmacokinetic parameters of 5-FU following oral administration of the solution and the microspheres are summarized in Table 1. The MRT, MAT and absolute bioavailability of 5-FU after administration as microspheres were significantly different from those for the aqueous solution. The solution had low bioavailability. Generally, the aqueous solutions of drugs used as references for oral absorption display higher bioavailability than other formulations because the drug is present as a molecular species. In this case, the aqueous reference solution was the commercial dosage form. The lower bioavailability of 5-FU administered as a solution can be explained by its pH-dependent solubility. Indeed, this commercial preparation of 5-FU has a pH of 8.6 and 5-FU precipitates at acidic pH. Therefore, when the aqueous 5-FU solution is in the stomach, precipitation of the drug occurs and consequently decreases the overall amount of 5-FU absorbed.

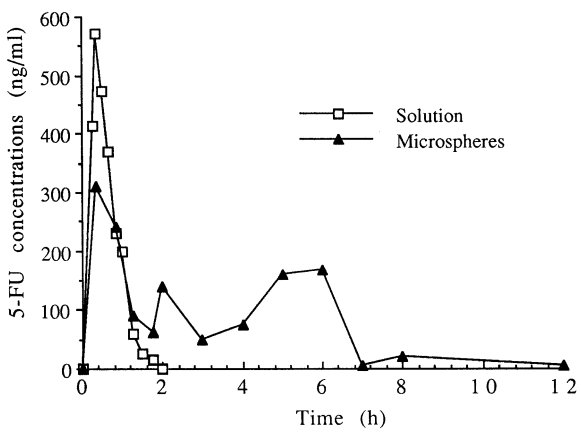

Fig. 1. Mean plasma concentrations of 5-FU after oral administration as a solution or microspheres.

Table 1
Pharmacokinetic parameters of 5-FU following oral administration to rabbits as a solution or microspheres

Parameter	Solution	Microspheres
MRT (min)	34 ± 4	304 ± 49[a]
MAT (min)	9 ± 4	279 ± 49[a]
Absolute bioavailability (%)	5.1 ± 1.8	17.5 ± 3.0[a]
Relative bioavailability (%)	100	333 ± 58[a]

[a] Statistical difference vs. aqueous solution.

The sustained-release characteristics of the microspheres were also reflected in the MRT and the MAT of 5-FU in the body. Both these parameters were considerably increased following oral administration of the microspheres compared with the solution. The MRT values for the solution and the microspheres were 34 and 304 min, respectively, while the MAT values were 9 and 279 min for the solution and the microspheres, respectively. The first and most important peak, observed at 30 min, corresponds to a rapid absorption of 5-FU. This is correlated with the burst effect observed in the in-vitro dissolution profile of microencapsulated 5-FU and the possible presence of drug crystals at the surface of the microspheres (Zinutti et al., 1994).

The secondary peaks could be attributed to the presence of enzymes able to digest cellulose in food which could also degrade the ethylcellulose

matrix. Therefore, the release mechanism of the drug could be a combination of diffusion and degradation of the polymer.

The dramatic increase in bioavailability was probably a consequence of the slow diffusion of the drug, which, in addition, may be protected from the surrounding intestinal medium.

Non compartmental pharmacokinetic parameters such as MRT, MAT or bioavailability show the potential of microencapsulated 5-FU as a complement to infusion of the drug in clinical therapy. In addition, it should be noted that after the administration of the solution by the oral route, three out of six rabbits were found dead a few hours after the experiment. Death was never observed after administration of microencapsulated 5-FU. Although we did not carry out any toxicological studies, it is clear that the decrease of the toxic effects, probably as a result of the prolonged release, is another advantage of a microencapsulated dosage form of 5-FU.

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