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Research Paper

Pharmacokinetic profile of Oncofid-S after intraperitoneal and intravenous administration in the rat

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Abstract

Objectives Oncofid-S is a bio-conjugate molecule obtained from the binding of campthotecin, SN-38, to hyaluronic acid. In view of a possible clinical development for loco-regional treatment of peritoneal carcinomatosis, this study aimed to establish the pharmacokinetic profile of Oncofid-S after single intraperitoneal or intravenous administration in the rat.

Methods Single-dose intraperitoneal or intravenous administrations of Oncofid-S were performed. Groups of six rats were sacrificed at various times (up to 24 and 72 h in i.p. and i.v. study, respectively) after drug injection. Trunk blood, livers and spleens were collected for subsequent analysis. Total SN-38 was assayed by HPLC.

Key findings We found that Oncofid-S was poorly absorbed after intraperitoneal injection, the estimated AUC_{0-72} being less than2%. The drug was distributed in liver, but not spleen, and was eliminated with a terminal half-life of 16 h. After intravenous dosing, Oncofid-S was found in liver as well as in spleen.

Conclusions Here we have demonstrated that Oncofid-S administered intraperitoneally in the rat was poorly absorbed into the systemic circulation, even after the administration of an extremely high dose. This finding reinforces the rationale for developing Oncofid-S in the loco-regional intraperitoneal treatment of peritoneal carcinomatosis in man.

Introduction

Oncofid-S (OF-S) is the term adopted to describe a bio-conjugate molecule obtained from the binding of SN-38, the active metabolite of camptothecin CPT-11 (irinotecan),[1] to hyaluronic acid (HA). From a chemical point of view, OF-S is obtained through esterification between 200 kDa HA and SN-38, the latter having been pre-treated with 4-bromobutyric acid. The two components of the bio-conjugate are bound via a 4-carbon molecular bridge. OF-S is freely soluble in aqueous solutions, including glucose solution.

Previous pre-clinical evidence suggested that intraperitoneal administration of irinotecan may present significant advantages compared with the intravenous route in the treatment of some animal models of peritoneal tumour diffusion, including the intraperitoneal seeding of colon cancer cells or liver metastasis.[2,3] More recently, OF-S showed in-vitro anti-proliferative activity on a number of human cancer cells as well as on rat colon adenocarcinoma cells over-expressing CD44, the receptor for the HA moiety.[4] Moreover, OF-S also proved to possess in-vivo anti-tumour activity in two pre-clinical models mimicking peritoneal carcinomatosis.[4] Taken together, the above pre-clinical evidence provides a rationale to develop OF-S for loco-regional chemotherapy of peritoneal carcinomatosis. This, along with cytoreductive surgery, is currently thought to be the best therapeutic option in this clinical condition.[5,6]

The pharmacokinetic profile of OF-S is expected to be significantly different to that of unbound camptothecin, and therefore requires thorough investigation in animal models before moving to clinical studies. In light of its potential use in loco-regional intraperitoneal treatments, in this study we investigated the pharmacokinetic profile of OF-S focusing on the most relevant pharmacokinetic parameters, Cmax, Tmax, AUC, $t_{1/2}\alpha$, $t_{1/2}\beta$ and distribution into peripheral tissues, after a single intraperitoneal injection in the rat. These data were also compared with those obtained after single intravenous injection.

Materials and Methods

Drugs

OF-S (hyaluronic acid/SN-38 conjugate; Chemistry Research Labs, Fidia Farmaceutici SpA), batch RS172/09, was used for in-vivo experiments; batch RS009/10 was used in the analytical study to set up total SN-38 standard curves. The SN-38/OF-S ratio was 9.5%. OF-S powder was stored at 4°C. The drug was dissolved in 5% glucose solution; all solutions were freshly prepared before use and immediately administered to animals. The solubility limit of OF-S batch RS172/09 in glucose solution was 10 mg/ml; the latter was used in intravenous experiments, to keep the injection volume to a minimum (see Intravenous administration). A lower dilution, 8 mg/ml was adopted in intraperitoneal experiments, which allowed the use of relatively larger injection volumes (see Intraperitoneal administration).

Animals

Male Wistar rats, 250–350 g, aged 8–12 weeks, were used. Rats were specific pathogen free, and were obtained from the production section of the Animal House, Catholic University Medical School. The use of animals and the study protocol were approved by the Institutional Review Board of Catholic University Medical School; a licensed authorization was also provided by the Ministry of Health to Pierluigi Navarra.

The rats were kept five to a cage under controlled environmental conditions: temperature 23 ± 1.5°C, relative humidity 65 ± 2% and 12-h light–dark cycles (light on 0600–1800 h). Pelletized food (Rieper, Valdoies, Italy) and tap water were freely available. No quarantine or acclimatization period was considered necessary, because of on-site animal production.

Experimental design

Intraperitoneal administration

A fixed dose of 100 mg/kg was administered in single-dose experiments. The drug solution was 8 mg/ml and the injection volume was 1.25 ml/100 g of body weight. Groups of six rats were sacrificed by decapitation 10 min, 20 min, 30 min, 60 min, 2 h, 4 h, 6 h, 24 h, 48 h and 72 h after drug injection. Trunk blood was collected in heparinized tubes; blood samples were stored at 4°C for 10 min and then centrifuged for 30 min at 2500 rpm at 4°C. Plasma samples were collected and stored at –35°C until assayed. Moreover, six untreated rats were killed by decapitation and blood collected; plasma obtained was pooled and subsequently used for preparing standard curves, blank and hydrolysis tests.

Livers and spleens from the same rats were rapidly (within 2 min) collected, placed in a Petri dish and stored at –80°C until tissue homogenization. The latter was performed in fixed volumes (100 mg wet tissue/1 ml) of Tris-HCl 50 mM, pH 7.4, containing 0.2% bovine serum albumin (BSA) and 40 IU/ml of aprotinin. Tissues were homogenized using a Teflon glass homogenizer (DuPont Co., Wilmington, NC, USA), thereafter centrifuged at 20 000 rpm for 45 min at 4°C, and two 0.5-ml volumes of supernatant were stored at –35°C until assayed.

Intravenous administration

A fixed dose of 40 mg/kg was administered in single-dose experiments. The drug solution was 10 mg/ml and the injection volume was 400 µl/100 g of body weight. Groups of six rats were sacrificed by decapitation 5 min, 10 min, 20 min, 30 min, 45 min, 60 min, 2 h, 4 h, 6 h and 24 h after drug injection. Trunk blood and tissues were collected, processed and stored as described for intraperitoneal experiments.

Analytical study

Plasma samples obtained from time-course experiments were divided into two portions: one was used for the determination of free SN-38 plasma levels, the remainder was used to measure the levels of SN-38 obtained after the reaction of hydrolysis, the latter approach providing an indirect estimate of OF-S plasma levels. This is a standard GLP procedure in the case of conjugate molecules; however, since free SN-38 is a fraction (which we estimated to be 9.5%) of total SN-38, and is included within the estimate of total SN-38 levels, only the latter are showed in the text and referred to as SN-38.

Hydrolysis reaction

Hydrolysis reactions were carried out to cleave the ester bond between SN-38 and HA, resulting in the release of individual products. Thereafter, free SN-38 was determined in serum by HPLC technique Hydrolysis was obtained by adding 0.6 ml of NaOH 0.2 M to 0.15 ml of plasma (1 : 5 dilution). OF-S undergoes de-esterification in basic pH, releasing SN-38 in carboxylate form, a highly fluorescent yellow compound. Samples were briefly mixed on a vortex mixer and filtered through 0.45-µm filters of regenerated cellulose (Whatman Spartan syringe filters). The filtered samples were then transferred into the auto-sampler and finally injected.

Instruments and chromatographic conditions

The chromatographic system included a quaternary pump Perkin Elmer series 410 plus auto-sampler Gilson series 401. SN-38 measurements was carried out with an analytical Gemini-NX C18 column, 150 × 4.6 mm i.d., particle 5 µm (Phenomenex).The mobile phase was a mixture of KH2PO4 50 mM–acetonitrile–tethrahydrofurane (THF) (800 : 200 : 2, v/v). Detection was obtained by fluorimetric approach, using an Perkin Elmer Fluorimeter LS30 at a 380 nm wavelength

excitation and at a 550 nm wavelength emission. Elution times and areas under the peaks were analysed using a Totalchrom Perkin Elmer software.

Standard curve, detection limit of the assay and recovery after hydrolysis

Total SN-38 assay (carboxylic form) was carried out with no addition of internal standard. SN-38 was dissolved into 0.2 M NaOH to obtain 2111 ng/ml stock solution. The latter was further diluted in rat plasma to obtain graded concentrations in the range 1689–0 ng/ml. A concentration of 10 ng/ml was taken as the low limit of quantitation (LLOQ) of the total amount of SN-38 assay. OF-S (batch RS009/10) was dissolved into 5% glucose solution to obtain a 55.28 μg/ml stock solution of OF-S and 5030 ng/ml of equivalent SN-38. The latter was further diluted in rat plasma to obtain graded concentrations in the range 2340–0 ng/ml of SN-38. A concentration of 10 ng/ml was taken as the low limit of quantization (LLOQ) of the total amount of SN-38 assay from OF-S. Under the analytical conditions described here, the elution time of SN38 was 3.9 min. Recovery rate after hydrolysis was 92.4%.

Determination of SN-38 in liver and spleen homogenates

Slight modifications of the above method were required for the determination of SN-38 in liver and spleen homogenates. Briefly, standard curves were carried out diluting OF-S stock solutions in blank tissue homogenates. Samples were prepared by diluting 0.15 ml of unknown homogenate in 1 ml 5% glucose + 1 ml 0.4 M NaOH or 1 ml 5% glucose + 1 ml 1 M NaOH for liver and spleen samples, respectively. Six homogenates from the same experimental group were pooled to obtain a single sample per group; analysis of the pooled sample provided a direct estimate of average drug level for each group.

Statistical analysis

All data are expressed as the means ± 1 standard error of the mean (SEM) of (n) rats per experimental group, unless otherwise stated. Curve best-fit equations and kinetic parameters were calculated using a PRISM computer program (GraphPad, San Diego, CA, USA).

Results

In a first series of experiments, OF-S was administered intraperitoneally at a 100 mg/kg dose. Figure 1 shows that the drug was absorbed, with a Cmax of 7.88 ± 0.58 nmol of SN-38 per ml of plasma (the mean ± 1 SEM of six rats), and a corresponding Tmax 2 h after injection. The following elimination phase was best described by a 2-compartment model (inset to Figure 1), with an estimated t½α of 14 min and an estimated t½β of 16 h. The two-phase exponential decay curve fitting the experimental points (inset to Figure 1) had a $r^2 = 0.9992$. The estimated AUC_{0-72} was 94.34 nmol/ml h.

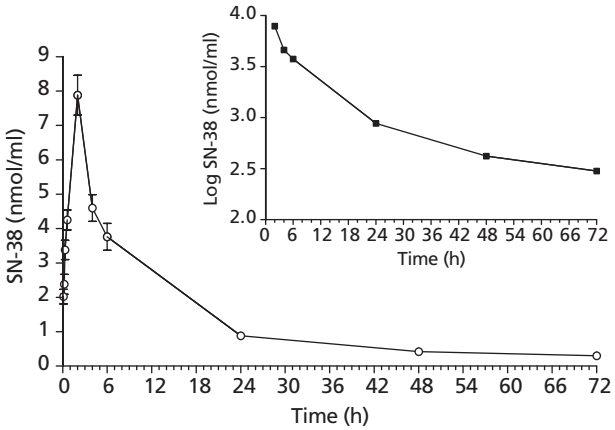

Figure 1 Plasma levels of SN-38 in rats after the administration of 100 mg/kg OF-S by the intraperitoneal route. Data are expressed as nmol/ml, the means ± 1 SEM of six rats per group. The inset shows the Log-transformed data of group means in the elimination phase, from 2 to 72 h.

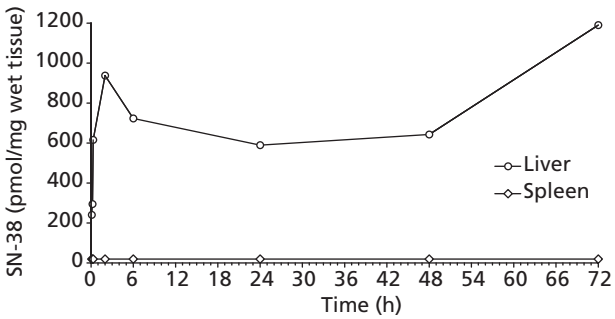

Figure 2 Levels of SN-38 found in rat liver and spleen parenchyma after the administration of 100 mg/kg OF-S by the intraperitoneal route. Data are the group means of six rats per group.

We found a rise in liver drug concentrations, with maximal levels of 938.17 pmol/mg of wet tissue reached 2 h after intraperitoneal administration (Figure 2). An elimination phase was observed during the following 24–48 h. Thereafter, a further phase of accumulation ensued (probably to be attributed to a re-distribution process), with final tissue concentrations of 1.20 nmol/mg of wet tissue reached 72 h after drug administration (Figure 2). At variance with liver, OF-S levels in spleen (taken as a paradigm of a peripheral parenchyma reached by the drug from the central compartment) were undetectable throughout 72 h of observation (Figure 2).

In experiments carried out with intravenous administration, a maximal dose of 40 mg/kg could be used, because of obvious limitations in injection volumes. Table 1 shows that a

Table 1 Circulating levels of SN-38 after the administration of 40 mg/kg OF-S by the intravenous route

	SN-38 (nmol/ml)									
Time	5 min	10 min.	20 min	30 min	45 min	1 h	2 h	4 h	6 h	24 h
Mean	239.21	817.00	890.96	398.52	784.80	178.35	336.44	137.90	29.14	3.38
σ(DS)	570.25	1196.70	829.65	492.62	515.01	340.40	344.48	181.98	64.36	4.36
SEM	232.79	488.55	338.69	201.12	210.26	138.97	140.63	74.28	2.63	1.92
C.V. %	243	146	93	123	65.62	190	102	132	220	140
n	6	6	6	6	6	6	6	6	6	6

very high intra-rat variation was observed, with % CVs ranging from a minimum of about 65% 45 min after the injection up to 243% 5 min after the injection. Peak plasma levels were observed 20 min after intravenous injection (Table 1). Despite such high variability, a linear regression analysis in the time-frame 20 min–24 h showed that the slope of drug plasma decrease with time was significantly non-zero ($F = 23.93$, $P = 0.0081$), indicating that a pattern of elimination can be estimated. Similar to intraperitoneal experiments, the elimination phase was best described by a two-compartment model, with an estimated terminal half-life of 8.7 h. The two-phase exponential decay curve fitting the experimental points had an $r^2 = 0.9995$. The estimated $AUC_{0–24}$ was 2 μmol/ml h. A proper calculation of bioavailability after intraperitoneal administration could not be carried out because of differences between the doses administered by intraperitoneal and intravenous routes (100 mg/kg vs 40 mg/kg) as well as the timing of experiments (72 h for the intraperitoneal route vs 24 h for the intravenous route). An indirect estimation could be obtained considering an intravenous AUC value 2.5-fold higher (from 2 to 5 μmol/ml h) to adjust for differences in dosage, and using a truncated intraperitoneal $AUC_{0–24}$ (from 94.34 to 70.15 nmol/ml h) to adjust for differences in timing. After normalization, the estimated bioavailability by the intraperitoneal route is 70.15/5000 nmol/ml h = 1.4%.

A peak tissue concentration of 991.8 pmol/mg of wet tissue was reached in the liver 45 min after intravenous drug injection (Figure 3). Interestingly, the maximal levels of drugs concentrating in liver parenchyma are virtually the same after intraperitoneal and intravenous administration, regardless of the huge differences in plasma drug levels attained after dosing by the respective routs. Opposite to the intraperitoneal administration, we found that drug levels in spleen are considerably higher than those in liver after intravenous drug administration, with a peak of 9.68 nmol/mg wet tissue reached 45 min after drug injection. The spleen/liver ratio of tissue concentrations ranged between 3.13 and 9.67, with an average figure of 6.82 (Figure 3).

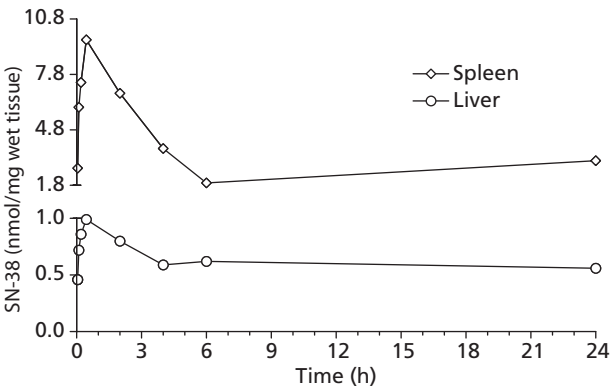

Figure 3 Levels of SN-38 found in rat liver and spleen parenchyma after the administration of 40 mg/kg OF-S by the intravenous route. Data are the group means of six rats per group.

Discussion

In this study we have investigated the pharmacokinetic profile of OF-S in the rat. In view of a possible use in the loco-regional treatment of peritoneal carcinomatosis, special emphasis was given to the kinetics of OF-S after single-dose intraperitoneal administration. We found that OF-S given intraperitoneally is absorbed in very tiny amounts. In previous experiments with different batches (RS211/07, RS131/08, RS054/09B), we estimated an $AUC_{0–72}$ of 58.16 nmol/ml h of SN-38 after the administration of 40 mg/kg intraperitoneally (Fidia, data on file). In this study we used a batch characterized by increased water solubility compared with previous formulations, and thereby we could increase the total dosage by the intraperitoneal route up to 100 mg/kg body weight. The $AUC_{0–72}$ of 94.34 nmol/ml h found under this condition showed a less-than-proportional increase in AUC with increasing doses. In any case, comparisons of those AUCs with that obtained in a 24-h time frame after the intravenous administration of 40 mg/kg allows us to estimate a putative bioavailability of less than 2% by the intraperitoneal route.

Because of anatomical and physiological reasons, the liver may be considered as a main site of drug accumulation after intraperitoneal drug administration. Actually, we could estimate a hepatic $AUC_{0–72}$ of about 53.64 nmol/mg wet tissue h of SN-38, which is less than the total amount found in the circulation in the same time frame. This is a tiny fraction of total OF-S administered. Interestingly enough, the estimated liver $AUC_{0–24}$ after intravenous and intraperitoneal

administrations were quite similar (14.94 and 16.62 nmol/mg wet tissue h, respectively), despite large differences in circulating drug levels. Taken collectively, the findings on liver OF-S accumulation might be explained assuming that a receptor for HA exists in liver parenchyma, which is saturated by pharmacological levels of HA. In fact, a family of HA receptors has been characterized in liver sinusoid endothelial cells, which is able to bind with high-affinity circulating HA of various molecular weights; these bindings sites are thought to act as scavengers of increased circulating levels of HA associated with a number of pathological conditions involving tissue repair.[7–10] The average circulating levels of HA in healthy adult subjects is about 30–40 ng/ml, and increases up to 1 μg/ml or more are observed in diseases such as rheumatoid arthritis or scleroderma;[11,12] it is therefore conceivable that levels of HA following the injection of high doses of OF-S do saturate the binding capability of the liver.

Overall, the intraperitoneal experiments showed that OF-S is poorly absorbed by the intraperitoneal route, and hepatic binding contributes in part to this poor absorption because of the first-pass effect; as a consequence, drug levels in spleen, taken as a paradigm of a peripheral compartment reached by the drug via the systemic circulation, were undetectable. These findings prompted us to conduct intravenous injection experiments, with two aims. Firstly, we attempted to obtain a quantitative measure of OF-S bioavailability. Secondly, distribution of OF-S in peripheral parenchyma (maintaining the spleen as a paradigm) was also investigated. After intravenous injection of OF-S, SN-38 levels were up to 10 times higher in the spleen compared with the liver; this apparent accumulation may be reasonably explained by pharmacokinetic mechanisms (bypass of liver first-pass effect), but high-affinity binding to lymphatic vessel endothelial HA receptor 1 (LYVE-1), an HA receptor highly expressed in spleen and lymph nodes,[13] might also account, at least in part, for our observations. A practical reason prevented us from using the same dosage as in the intraperitoneal experiments (i.e. 100 mg/kg: large injection volumes, which would have markedly increased the volume of circulating blood). Therefore, the estimate of bioavailability reported here is only indirect, although highly suggestive of scarce absorption after intraperitoneal injection. We also observed an increase in plasma drug levels in the first 20 min after injection that cannot obviously be explained by any absorption process. It is conceivable that OF-S, immediately after bolus intravenous injection, is rapidly bound to a number of different binding sites within the vascular system, and is subsequently released and made available to the subsequent distribution and elimination processes. In fact, OF-S might bind the to physiological receptor of HA, CD44, expressed on the cell membrane of vascular endothelial cells.[14] Moreover, OF-S might also bind to other putative HA receptors on endothelial surface, the receptor for HA-mediated motility (RHAMM) and the hyaluronan receptor for endocytosis (HARE), which is the vascular homologue of HA receptors expressed by liver sinusoids.[15–17] Apart from the vascular surface, OF-S might also bind to CD44 expressed by circulating cells of the erythroid and myeloid lineage, including neutrophils and platelets.[18,19]

Conclusions

In conclusion, in this study we have demonstrated that OF-S, even after the administration of a extremely high dose by the intraperitoneal route in the rat, is scarcely absorbed into the systemic circulation. Therefore, the pharmacokinetic profile of OF-S given intraperitoneally is markedly different compared with that of irinotecan administered by the same route. In fact, both irinotecan and SN-38 reach similar plasmatic levels after either intravenous or intraperitoneal administration of irinotecan in humans and rodents.[20–22] Thus, if intraperitoneal administration is indicated (e.g. in human peritoneal carcinomatosis), OF-S has a potential better risk-to-benefit ratio than irinotecan, since toxic effects related to systemic exposure are expected to be lower. These considerations lend further support to the rationale for developing OF-S in the loco-regional intraperitoneal treatment of peritoneal carcinomatosis in humans.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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