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5-Fluorouracil Kinetics in the Interstitial Tumor Space: Clinical Response in Breast Cancer Patients

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Abstract

Several anticancer drugs fail to exhibit sufficient activity against solid tumors in vivo despite effective inhibition of tumor cell growth in vitro. This may be due to impaired drug transfer from plasma into solid tumors. The present study, therefore, aimed at measuring interstitial tumor 5-fluorouracil (5-FU) pharmacokinetics and 5-FU transfer rates from plasma into the tumor interstitium in breast cancer patients.

Microdialysis probes were inserted into the primary tumor and the periumbilical s.c. adipose layer of 10 breast cancer patients (8 females and 2 males) scheduled to receive neoadjuvant chemotherapy due to locally advanced breast cancer. Thereafter, patients received 5-FU (600 mg/m², i.v.). 5-FU kinetics were followed in plasma and tumor and s.c. interstitial fluid.

Mean interstitial 5-FU load, expressed as area under curve (AUC), in breast tumors was 61 ± 11% (means ± SE) of the mean plasma 5-FU load. 5-FU displayed similar kinetics in the interstitial space of s.c. adipose tissue and tumor tissue. A high interstitial tumor AUC was associated with increased tumor response. There was no association with tumor response for s.c. or plasma AUC of 5-FU.

Measurement of Intestinal drug concentrations in breast tumors by in vivo microdialysis may predict response to chemotherapy. This information may explain drug resistance in some patients and help to optimize dosing and administration schedules. In the future, selection of novel cytotoxic compounds with favorable tumor penetration characteristics may become possible.

Introduction

To date, most research on anticancer drug resistance focused on the molecular biology of tumor cells, with little attention paid to the environment that these cells exist in. However, before reaching an intracellular target molecule, a cancer drug encounters several transport processes across compartment barriers (1). Impairment of each of these transport processes may contribute to in vivo resistance. This is underlined by the fact that several anticancer drugs that effectively inhibit tumor cell growth in vitro fail to exhibit a corresponding activity against solid tumors in vivo (2, 3).

Studies on drug transfer from the blood compartment into solid tumor compartments are scarce and are only available from MRS (4, 5) or biopsy studies (6). Recent MRS studies showed a significant association between a long half-life of 19F above different tumor regions and clinical response after 5-FU administration (4). An important limitation of these studies was the lack in homogeneity of tumor type, the amount of previous chemotherapy, and the type of chemotherapy administered. Furthermore, it is not possible by MRS or biopsy studies to discriminate transcapsular from transcellular transport processes.

Among the various transport processes, the drug transfer from the blood compartment into the interstitial space in particular is considered a critical step in clinical resistance of tumors (1, 4). The interstitial fluid represents the compartment of immediate vicinity of tumor cells, and the dose intensity of the free drug in the interstitial fluid, rather than in the plasma, is thus comparable to the in vitro dose intensity in cell culture. These theoretical considerations notwithstanding, most data on dose intensity relate to plasma rather than to tissue concentrations. Although information on drug penetration into tumor interstitial fluid is regarded to be of high importance, (1, 4) there were, until recently, no data available.

Recently, microdialysis, a technique based on the diffusion of analytes from the interstitial compartment through a semipermeable membrane, has been described for in vivo measurement of drug pharmacokinetics in the interstitium of human tumors (7). Microdialysis measurements allow for the determination of relevant pharmacokinetic parameters of antineoplastic agents (7, 8) and thus allow for comparison of tumor versus systemic exposure.

The present study aimed at measuring free interstitial tumor 5-FU pharmacokinetics, i.e., tumor dose intensity/cycle, in breast cancer patients receiving a preoperative chemotherapy with a regimen including 5-FU. We hypothesized that interstitial tumor dose intensity, i.e., the product of 5-FU concentration and the time of cell exposure, would be more predictive of clinical tumor response than would plasma dose intensity.

Patients and Methods

The study was approved by the local ethics committee. All patients were given a detailed description of the study, and their written consent was obtained. The study was performed in accordance with the Declaration of Helsinki and the Good Clinical Practice Guidelines of the European Commission.

Patients. The study population included 10 patients (8 females and 2 males) with a histologically confirmed diagnosis of primary breast cancer stage T3/4, N±, M0 [age, 53 ± 9 years; body surface area, 1.9 ± 0.4 m² (means ± SD); WHO performance status (ECOG scale), 0, 1, or 2; life expectancy of at least 3 months] who were scheduled to receive preoperative chemotherapy [either 600 mg/m² cyclophosphamide, 40 mg/m² methotrexate, and 600 mg/m² 5-FU (n = 2) or the FEC regimen (600 mg/m² 5-FU, 60 mg/m² epirubicin, and 600 mg/m² cyclophosphamide; n = 8)].

Admission of patients to the study was limited to the first treatment cycle; patients were restudied whenever possible if the patients agreed to participate again. Results from restudied patients were analyzed separately.

Study Protocol. In the morning of the study day, patients were admitted to the clinical research ward. The patients were in a supine position throughout the study period. A plastic cannula (Venflon) was inserted into an antecubital vein to monitor 5-FU plasma concentrations at 15-min intervals. The skin at...
the site of microdialysis probe insertion was cleaned and disinfected. Commercially available microdialysis probes (CMA 10; CMA, Stockholm, Sweden; molecular cutoff, 20 kDa; outer diameter, 500 μm; membrane length, 16 mm) were inserted into the primary tumor and into peri-umbilical s.c. adipose tissue without local anesthesia using a previously described procedure (7, 9). Subsequently, the microdialysis system was connected and perfused with Ringer’s solution at a flow rate of 1.5 μl/min by a microinfusion pump (Precidor; Infors-AG, Basel, Switzerland). The position of each probe was established by two-dimensional ultrasound scanning. A representative print is shown in Fig. 1. After a 30-min baseline sampling period, in vivo probe calibration was performed as described previously for a period of 30 min. Before the administration of 5-FU, the perfusate was changed to Ringer’s solution, and the system was flushed for 30 min. Thereafter, 5-FU was administered as described above, and sampling was continued at 15-min intervals for up to 5 h. Dialysates were collected by means of a microfraction collector (CMA 120; CMA) and stored at −20°C until analysis.

**Microdialysis.** The principles of microdialysis for clinical studies have been described in detail previously (9, 10). Briefly, microdialysis is based on a sampling of analytes from the interstitial space by means of a semipermeable membrane at the tip of a microdialysis probe. The probe is constantly perfused with a physiological solution (perfusate) at a flow rate of 1.5 μl/min. Once the probe is implanted into the tissue, substances present in the interstitial fluid at a certain concentration (c_{tissue}) are filtered by diffusion out of the interstitial fluid into the probe, resulting in a concentration (c_{dialysate}) in the perfusion medium. Samples are collected and analyzed. For most analytes, equilibrium between interstitial tissue fluid and the perfusion medium is incomplete; therefore: c_{tissue} > c_{dialysate}. The factor by which the concentrations are interrelated is termed recovery. To obtain absolute interstitial concentrations from dialysate concentrations, each microdialysis probe was calibrated in each experiment for in vivo recovery according to a retrodialysis method (7, 9).

**Study Drugs.** Patients received 5-FU (Roche, Basel, Switzerland) as a single i.v. dose of 600 mg/m² over 15 min (mean dose, 1162 ± 265 mg). Concomitant medication included 8 mg of ondansetron, 4 mg of dexamethasone, and 50 mg of ranitidine.

**Assessment of Tumor Response.** The responses of tumors to chemotherapy were assessed radiologically by the standard criteria as suggested by the WHO: a complete response was defined as complete disappearance of all measurable tumor for at least 4 weeks; and a partial response was defined as a reduction in tumor size of 50% or more as studied by either mammography, mammosonography, or direct clinical measurement of the tumor diameters. Progressive disease was defined as any increase of more than 25% in tumor diameters or as the appearance of a new lesion, and stable disease was defined as a change in tumor size of less than ±25% to −50%, as compared to pretherapy conditions. This evaluation was performed by an experienced independent radiologist. A patient was defined evaluable for tumor response after at least two courses of 5-FU-containing chemotherapy.

**Chemical Analyses.** 5-FU concentrations in plasma and dialysates were measured by capillary electrophoresis (3D CE; Hewlett Packard, Waldbronn, Germany). Microdialysates were analyzed directly without sample clean-up in 30 mM sodium tetraborate buffer (pH = 9.0) on a capillary with 56-cm effective length (inside diameter = 50 μm). Applying voltages of 30 kV led to the elution of 5-FU from the capillary after approximately 6 min. To assess free 5-FU in blood, 50–100 μl of plasma from EDTA blood were centrifuged through Ultrafree-MC filters units with a nominal cutoff of M₅ 10,000 (Millipore Corporation, Molsheim, France). Ultrafiltrates were collected at 20,000 × g at 4°C and injected onto the capillary electrophoresis system. The inter- and intraassay CVs were <5%. The detection limit was 1 μg/ml.

**Pharmacokinetic Calculations.** Data were fitted according to a two-compartment model to the following equation for serum values (11):

\[ C(t) = B e^{-K_{a}t} + A e^{-K_{1}t}, \]

where \( K_{a} \) is the elimination rate constant for serum, \( K_{1} \) is the elimination rate constant for the second compartment, \( B \) is the back-extrapolated intercept with the ordinate for the \( \alpha \) phase, and \( A \) is the back-extrapolated intercept with the ordinate for the second compartment. Data for tumor or s.c. tissue values were fitted according to a one-compartment model to the following equation:

\[ C(t) = A e^{-K_{1}t} - C_{e} e^{-K_{\alpha}t}, \]

where \( K_{\alpha} \) is the absorption rate constant. Subsequently, the transfer rate constant from the central to the peripheral compartment (\( k_{12} \)) was calculated according to \( K_{c} + K_{1} = k_{12} + k_{13} \), where \( K_{c} \times K_{1} = k_{12} \times k_{13} \) in which \( k_{12} \) is the rate constant from the peripheral to the central compartment, and \( k_{13} \) is the rate constant of excretion from the central compartment. The following pharmacokinetic key parameters were calculated: peak concentration (\( C_{\text{max}} \)), time to reach peak concentration (\( t_{\text{max}} \)), area under the time versus concentration curve (AUC), terminal half-life of elimination (\( t_{1/2\beta} \)), absorption half-life (\( t_{1/2a} \)), and transfer rate constant from the central (plasma) compartment to the peripheral (tumor, s.c.) compartment (\( k_{12} \)). AUCs (in μg/min/ml⁻¹) were

![Fig. 1. Two-dimensional ultrasound scan of a microdialysis probe. The intratumoral position of the microdialysis probe is indicated by an arrow.](image-url)
determined for plasma and tissues according to the trapezoidal rule. Penetration ratios for tissues were determined as $AUC_{\text{tumor}}/AUC_{\text{plasma}}$.

**Results**

The results of experiments in which probes were inserted simultaneously into the tumor and into the periumbilical s.c. adipose tissue ($n=10$) are shown in Fig. 2. Pharmacokinetic parameters are summarized in Table 1. The $AUC_{\text{tumor}}/AUC_{\text{plasma}}$ ratio was $0.61 \pm 0.11$ (mean $\pm$ SE; CV = 57%). $k_{12}$ was $2.5 \pm 0.8$ for the tumor compartment and $2.4 \pm 0.8$ for the s.c. adipose tissue compartment.

Of the 10 patients, 8 patients responded partially to preoperative chemotherapy [2 patients receiving cyclophosphamide (600 mg/m$^2$), methotrexate (40 mg/m$^2$), and 5-FU (600 mg/m$^2$) and 6 patients receiving FEC] and 2 patients, both receiving FEC, had a stable disease. The association between tumor or plasma AUCs and clinical tumor response for the experiments in Fig. 2 is depicted in Fig. 3.

For experiments performed during the first and the second cycle in the same patients ($n=3$), the ratio of $AUC_{\text{cycle 1}}/AUC_{\text{cycle 2}}$ was $1.08 \pm 0.33$ (CV = 53%) for interstitial tumor fluid and $1.19 \pm 0.34$ (CV = 49%) for plasma.

**Discussion**

Previous attempts to regard plasma levels of cytotoxic drugs as a predictor of tumor response to therapy have failed. It emerged that the plasma concentration-time profile is not necessarily a measure of the concentration-time profile at the target site, i.e., in the vicinity of the tumor cells. Hence, it was claimed that the transfer from the plasma compartment into the interstitial tumor compartment is critical for cytotoxic efficacy in vivo (1, 4). Lack of accessibility of cytotoxic drugs may thus play an important role in the clinical resistance of tumors to antineoplastic chemotherapy (1, 4).

The present study, therefore, aimed at measuring free interstitial tumor 5-FU kinetics in a well-defined and relatively homogeneous group of breast cancer patients by means of microdialysis, an established in vivo sampling technique. A neoadjuvant regimen seemed particularly appropriate for our study, because selection of resistant cell clones was expected to be minimal at that time (12, 13). Thus, an association between cytotoxic tumor load and clinical response is more likely to reflect resistance at a pharmacokinetic level rather than resistance at a cellular level. Interstitial AUC of free intratumoral 5-FU was defined as the main outcome variable, because the interstitial dose intensity of the free unbound drug in the tumor is a direct

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Table 1. Pharmacokinetic parameters for plasma and tumor and s.c. adipose tissue after administration of a single i.v. dose of 600 mg/m$^2$ 5-FU over 15 min ($n=10$) in breast cancer patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tumor</th>
<th>Subcutis</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (µg/ml)</td>
<td>16.4 ± 6.7</td>
<td>20.6 ± 8.2</td>
<td>27.3 ± 4.1</td>
</tr>
<tr>
<td>$t_{\text{max}}$ (min)</td>
<td>16.4 ± 3.0</td>
<td>15.1 ± 3.2</td>
<td>-</td>
</tr>
<tr>
<td>AUC (µg·min/ml)</td>
<td>374 ± 62</td>
<td>401 ± 151</td>
<td>699 ± 75</td>
</tr>
<tr>
<td>$t_{1/2\text{e}}$ (min)</td>
<td>10.1 ± 1.5</td>
<td>12.5 ± 2.2</td>
<td>12.9 ± 2.0</td>
</tr>
<tr>
<td>$t_{1/2\text{abs}}$ (min)</td>
<td>8.9 ± 1.8</td>
<td>4.8 ± 2.0</td>
<td>-</td>
</tr>
</tbody>
</table>

*a* $C_{\text{max}}$, peak concentration.

*b* $t_{\text{max}}$, time to reach peak concentration.

*c* AUC, area under the time versus concentration curve.

*d* $t_{1/2\text{e}}$, terminal half-life of elimination.

*e* $t_{1/2\text{abs}}$, absorption half-life.
measure of the pharmacologically active drug at the anatomical target site.

By relating tumor concentrations to plasma concentrations, the distribution kinetics of 5-FU into locally advanced breast tumors could be characterized much more precisely in our study than it could by estimation from plasma concentrations alone. Mean s.c. and tumor concentration time courses were not substantially different (Fig. 2). This is further supported by similar mean pharmacokinetic key parameters in the tumor and the s.c. compartment (Table 1). The mean transfer rate constant from the central (plasma) compartment to the peripheral (tumor, s.c. tissue) compartment (k12) indicates a rapid mean equilibration between the plasma concentration and free interstitial concentrations for both the tumor and the s.c. tissue. However, there was a high interindividual variability, indicating that the transfer from the plasma compartment to the interstitial space is not a fixed constant but is rather a highly individual determinant for each particular patient. Intertstitial tumor dose intensity was widely heterogeneous (Fig. 3). Surprisingly, the only two patients who did not respond to antineoplastic therapy also had the lowest interstitial 5-FU tumor load, despite a relatively high 5-FU load in plasma and s.c. tissue (Fig. 3).

Thus, a high tumor dose intensity was associated with an increased tumor response according to WHO criteria in our study. This association with tumor response was lacking for s.c. (data not shown) or plasma AUC. These results highlight the importance of drug transfer from the plasma into the interstitial tumor compartment and help explain why plasma measurements do not provide surrogate markers for clinical outcome.

Previously, several lines of evidence have indirectly supported the hypothesis that impaired transfer of cytotoxic agents from plasma into the tumor interstitium might be critical for in vivo tumor response to chemotherapy (1, 4). One pertinent observation was that the interstitial pressure in solid tumors is markedly enhanced. The intratumoral pressure gradient, which under physiological circumstances is directed across the vessel toward the interstitial space, is reversed as a tumor grows (1). This may be due to the absence of a functional lymphatic network (1) and an abnormal geometry of tumor vessels (1, 15). The tumor interstitium per se is generally very large in comparison to that of normal tissue (1) and is characterized by an abnormally high collagen and low proteoglycan and hyaluronate content (1). This may enhance tumor viscous resistance in the interstitial space. Furthermore, a decreased pressure in tumor venules has been described that may further impair transcapillary fluid and solute transport (15).

These pathophysiological and histological changes in interstitial structure and function of neoplastic tissue may thus lead to the development of functional and anatomical barriers to drug accumulation in some solid tumors. To date, however, this has never been described experimentally. To our knowledge, our study constitutes the first report of an association between interstitial tumor load and clinical tumor response.

It may be speculated that drug resistance at the cellular level may contribute to impaired clinical tumor response only if a cytotoxic drug does not encounter these early barriers. This fact may also explain, at least in part, previously described inconsistencies between tumor response to cytotoxic exposure in an in vivo and an in vitro situation (2, 3, 12).

In conclusion, measurement of interstitial drug concentrations in solid tumors by in vivo microdialysis may predict response to chemotherapy. This information may explain drug resistance in some patients and help to optimize dosing and administration schedules. In the future, selection of novel cytotoxic compounds with favorable tumor penetration characteristics may become possible.

References

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