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Journal of Chromatography B, 759 (2001) 51–61

JOURNAL OF CHROMATOGRAPHY B

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Simultaneous determination of F-β-alanine and β-alanine in plasma and urine with dual-column reversed-phase high-performance liquid chromatography

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Received 7 June 2000; received in revised form 15 March 2001; accepted 4 April 2001

Abstract

F-β-Alanine and β-alanine were detected in plasma and urine samples with fluorescence detection of orthophthaldialdehyde derivatives of F-β-alanine and β-alanine after separation with dual-column reversed-phase HPLC. The detection limits of F-β-alanine and β-alanine in the HPLC system were approximately 0.3 and 0.7 pmol, respectively. The procedure proved to be very reproducible with intra-assay RSDs and inter-assay RSDs being less than 8%. The usefulness of the method was demonstrated by the analysis of the F-β-alanine and β-alanine concentrations in plasma and urine samples from tumor patients treated with S-1 (Tegafur, 5-chloro-2,4-dihydroxypyridine and potassium oxonate in a molar ratio of 1:0.4:1).

Keywords: Alanine; Dihydropyrimidine dehydrogenase; 5-Fluorouracil

1. Introduction

5-Fluorouracil (5FU) is one of the few drugs that shows some antitumor activity against carcinomas of the gastrointestinal tract and breast cancer. To improve the clinical response of 5FU, optimal administration schedules as well as the combination of 5FU with other drugs which should increase its antitumor activity or decrease the host toxicity have been investigated. In this respect, it has been shown that long-term continuous infusion of 5FU resulted in a higher response rate when compared to bolus administration of 5FU in patients suffering from metastatic colorectal cancers [1]. Furthermore, it has been demonstrated that a relationship exists between the 5FU systemic exposure and the tumor response and patient survival [2]. During continuous infusion of 5FU the dose-limiting factor proved to be gastrointestinal toxicity and not myelosuppression [3].

Recently, a new antitumor agent S-1 has been developed consisting of tegafur, oxonic acid and

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0378-4347/01/$ – see front matter
PII: S0378-4347(01)00203-1

5-chloro-2,4-dihydroxypyridine (CDHP). Tegafur, a prodrug of 5FU, is gradually converted to 5FU by the liver after oral administration. In order to exert its cytotoxic effect against cancer 5FU must first be anabolized to the nucleotide level. Oxonic acid proved to be an inhibitor of orotate phosphoribosyltransferase which is involved in the phosphorylation and thus activation of 5FU. It has been shown that oxonic acid markedly inhibited the conversion of 5FU to 5-fluorouridine 5′-monophosphate in tissues of the gastrointestinal tract and thus provided protection against 5FU induced gastrointestinal toxicity [4,5]. Since the vast majority of 5FU is rapidly degraded by dihydropyrimidine dehydrogenase (DPD), inhibitors of this enzyme such as CDHP have been shown to potentiate the effect of 5FU in vitro and in vivo [5–7]. The important role of DPD in the chemotherapy with 5FU has also been shown in cancer patients with a complete or near-complete deficiency of this enzyme. These patients suffered from severe (neuro)toxicity including death, following 5FU chemotherapy. So far, a number of patients have been reported with a low activity of DPD experiencing severe toxicity following 5FU chemotherapy who proved to be heterozygous for a mutant DPD allele [8–12].

Fluoro-β-alanine is by far the major catabolite of 5FU and can be detected in plasma, bile urine and tissues. Recently, it has been demonstrated that the administration of 5FU or F-β-alanine to rats resulted in the formation of the highly cardiotoxic and neurotoxic fluoroacetate [13]. In addition, it has been shown that F-β-alanine and fluoroacetate directly injured myelinated fibers in tissue culture [14]. It has been postulated, therefore, that F-β-alanine and/or its metabolites underlie the neurotoxicity and cardiotoxicity which occur in the minority of patients treated with 5FU [14–19]. Although the precise mechanism for the formation of fluoroacetate is unknown, it has been suggested that the enzymes responsible for the metabolism of β-alanine are also responsible for the metabolism of F-β-alanine [13,20]. β-Alanine might thus not only compete with the degradation of F-β-alanine, the uptake of β-alanine itself via a specific GABA transporter has been shown to be inhibited by F-β-alanine [21].

So far, F-β-alanine concentrations have been measured in plasma and urine samples using radiolabeled FU followed by separation by reversed-phase high-performance liquid chromatography (RP-HPLC) with off-line detection of radioactivity [22], by gas chromatography–mass spectrometry (GC–MS) [23] and by ^{19}F nuclear magnetic resonance (NMR) spectroscopy [20,24]. Up to now, there are no procedures available for the simultaneous detection of β-alanine and F-β-alanine. We developed, therefore, a novel method which is based on the detection of fluorescent derivatives of β-alanine and F-β-alanine after separation with dual-column RP-HPLC. The usefulness of the method was demonstrated by the analysis of the F-β-alanine and β-alanine concentrations in plasma and urine samples from tumor patients treated with S-1.

2. Materials and methods

2.1. Chemicals

Lithium citrate buffer was obtained from Pharmacia Biotech. (Cambridge, UK). Standard amino acid mixture was obtained from Sigma (St. Louis, MO, USA). Potassium borate solution was obtained from Pierce (Rockford, IL, USA). Fluoro-β-alanine was obtained from Taiho Pharmaceutical (Tokyo, Japan). All other chemicals were of analytical grade.

2.2. Patients

Samples were collected from control patients and patients participating in a phase I study to determine the safety and pharmacokinetics of oral administration of S-1 in patients with a solid tumor on a daily schedule. Preceding the start of the first course, S-1 was administered as a single dose to be able to study the pharmacokinetics during the first 24 h. During this period heparin anticoagulated blood specimens were collected at various time points (0, 0.5, 1, 2, 4, 8 and 24 h) for isolation of plasma which were subsequently stored at −80°C. In addition, urine was collected during 12 h before administration of S-1 and during the periods 0–6, 6–12, 12–18 and 18–24 h after administration. Samples of 10 ml were frozen and stored at −80°C until further analysis.

2.3. Sample preparation

Calibration standards for the analysis of amino acids in plasma (5 μ*M*) or urine (125 μ*M*) were prepared by appropriate dilution of a standard amino acid mixture (500 μ*M*) with 0.1 *M* HCl. Fluoro-β-alanine was added to these plasma and urine calibration standards to final concentrations of 6.2 μ*M* and 123 μ*M*, respectively. Plasma samples (200 μl) were deproteinized by thoroughly mixing with 20 μl of 35% (w/v) aqueous sulfosalicylic acid and storing the samples at 4°C for 30 min. After centrifugation (11000 *g*, 10 min) 100 μl of the supernatants was collected and mixed with 100 μl of 0.3 *M* lithium citrate (pH 3.0). Urine samples (200 μl) were deproteinized by thoroughly mixing with 20 μl of 12% (w/v) aqueous sulfosalicylic acid and storing the samples at 4°C for 30 min. After centrifugation (11000 *g*, 10 min) 20 μl of the supernatants was collected and mixed with 380 μl of 0.3 *M* lithium citrate (pH 3.0). In case increased sensitivity was required, 100 μl of the supernatant was mixed with 100 μl of 0.3 *M* lithium citrate (pH 3.0).

The orthophthaldialdehyde (OPA) derivatization reagent was prepared by dissolving 26 mg OPA in 0.5 ml methanol followed by addition of 5 ml 0.6 *M* potassium borate and 30 μl of 14.2 *M* β-mercaptoethanol. Derivatization of the amino acids occurred prior to the injection into the HPLC apparatus and was performed automatically in a Gilson 231 XL autosampler and a Gilson 401 dilutor (Gilson Medical Electronics, Villiers Le Bel, France) by mixing 20 μl OPA derivatizing reagent with 40 μl sample. After an incubation period of 30 s, 20 μl of the reaction mixture was injected into the HPLC apparatus.

2.4. HPLC configuration

The configuration of the HPLC system is depicted in Fig. 1. The HPLC system consisted of a Perkin-Elmer Series 200 pump (Norwalk, CT, USA), a Gilson 231 XL autosampler and a Gilson 832 sampler holder operating at 21°C with a Gilson 401 dilutor, a Waters 510 HPLC pump (Milford, MA, USA) and an RF-10A fluorescence detector (Shimadzu, Kyoto, Japan). Separation of the derivatized amino acids was performed on an OPA-HR

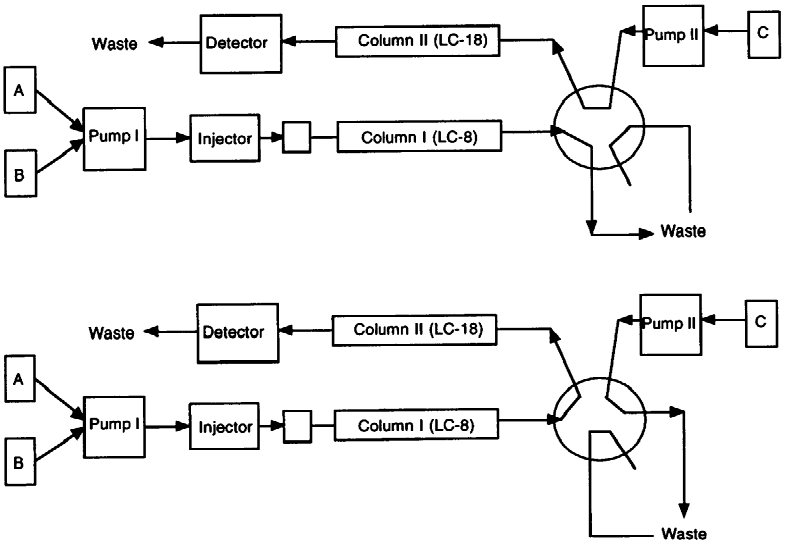

Fig. 1. Schematic diagram of the dual column HPLC system used for the separation of F-β-alanine and β-alanine. The upper panel shows the initial column configuration in which F-β-alanine and β-alanine are roughly separated from other amino acids on column I. The lower panel shows the configuration after switching the valve to allow transfer of the compounds of interest eluting from column I to column II. Afterwards, the switching valve is returned to its original position (upper panel) with subsequent separation of F-β-alanine and β-alanine on column II. A and B represent the solvents used for chromatography on column I whereas C represents the buffer used for chromatography on column II.

LC-8 analytical column (5 μm particle size, 150×4.6 mm I.D., Alltech, Deerfield, IL, USA) with a guard column (type Pelliguard, 40 μm particle size, 20×4.6 mm I.D., Supelco, Bellefonte, PA, USA) and an Alltima C_{18} analytical column (5 μm particle size, 250×4.6 mm I.D., Alltech). Switching between both analytical columns occurred via a six-position automated Vici switching valve (Valco Instruments, Houston, TX, USA). Both columns were thermostated at 21°C using a Hubner minichiller cooling waterbath (New Brunswick Scientific, Nijmegen, The Netherlands).

2.5. HPLC analysis

Solvent A used for chromatography on column I (LC-8) consisted of 70 m*M* sodium acetate and 9% (v/v) methanol (pH 5.75). Solvent B consisted of 90% (v/v) methanol and 1.8% (v/v) isopropanol. Solvents A and B were filtered before use through a 0.45-μm filter. Prior to the injection of the sample into the HPLC apparatus column I was equilibrated with 90% solvent A at a flow-rate of 0.5 ml/min. Elution was performed by applying a linear gradient from 90% solvent A to 60% solvent A in 15 min at a flow-rate of 1 ml/min. Subsequently, solvent B at a final concentration of 80% was used for removal of more hydrophobic components.

The compounds of interest eluting from column I between 15 and 18 min were introduced by means of column switching to column II (LC-18) and elution was performed isocratically with a buffer containing 100 m*M* sodium acetate and 50% (v/v) methanol, pH 5.0 at a flow-rate of 1 ml/min. Because of slight day-to-day variation in elution times, the retention times for β-alanine (17.0±0.7, $n=10$) and F-β-alanine (16.2±0.6, $n=10$) were determined daily and column switching times were adjusted accordingly. Fluorescence detection was performed using an excitation wavelength of 330 nm and an emission wavelength of 450 nm. Quantification of the amounts of β-alanine and F-β-alanine was performed by comparison with external plasma and urine calibration standards. The integration of the chromatographic peaks was performed with a Nelson 900 Series Interface in combination with Nelson Analytical Model 2600 chromatography software (Perkin-Elmer Nelson, Cupertina, CA, USA).

2.6. Assay validation

Calibration curves of F-β-alanine and β-alanine were prepared by treating standards of F-β-alanine (0.48–62 μ*M*) and β-alanine (0.85–26 μ*M*), dissolved in 0.1 *M* HCl, according to the plasma protocol and subsequent analysis by HPLC, as described above. Calibration curves for the analysis of F-β-alanine in urine were prepared by treating standards of F-β-alanine (3.8–1230 μ*M*) and β-alanine (3.3–1030 μ*M*), dissolved in 0.1 *M* HCl, according to the urine protocol and subsequent analysis by HPLC, as described above.

To determine the recovery of F-β-alanine and β-alanine from plasma and urine samples, F-β-alanine and β-alanine were added to 10 different plasma samples to final concentrations of 2.2 μ*M* and 1.9 μ*M*, respectively. Analogously, F-β-alanine and β-alanine were added to 10 different urine samples to final concentrations of 62 and 52 μ*M*, respectively. Blanks were always included to correct for the presence of endogenous amounts of β-alanine. Subsequently, the plasma and urine samples were deproteinized by sulfosalicylic acid and analyzed by HPLC as described above.

The intra-assay variation of the procedure was assessed by determination of the F-β-alanine and β-alanine concentrations in seven replicates of a plasma sample (enriched with 2.4 μ*M* F-β-alanine and 2.1 μ*M* β-alanine) and 10 replicates of a urine sample enriched with F-β-alanine (62 μ*M*) and β-alanine (52 μ*M*). The inter-assay (between-day) variation of the procedure was determined by analyzing a plasma sample and urine sample on 7 and 5 different days, respectively. The reproducibility of the assay is expressed as the relative standard deviation (RSD).

3. Results

3.1. HPLC procedure

Initial attempts to separate OPA derivatives of F-β-alanine and β-alanine from interfering amino acids by RP-HPLC using a single column were unsuccessful. For that reason, we have developed a dual-column system in which the compounds of

interest eluting in a particular fraction from column I were introduced on-line on column II (Fig. 1). A typical elution pattern of OPA derivatized amino acids on column I is shown in Fig. 2A. Under these conditions, F-β-alanine coeluted with taurine and alanine coeluted with β-alanine. However, a complete baseline separation was obtained for F-β-alanine and β-alanine and the interfering amino acids after introducing the effluent containing the fraction between 15 and 18 min from column I to column II (Fig. 2B). Note that under these slightly more acidic conditions tyrosine eluted before alanine and β-alanine on column II whereas the reverse situation occurred on column I.

In plasma samples the concentrations of interfering amino acids are much higher than those of β-alanine and F-β-alanine. Nevertheless, the analysis of a plasma sample of a tumor patient obtained prior to the start of the treatment with S-1 showed that β-alanine was nicely separated from alanine (Fig. 3A). In addition, no substantial amounts of interfering compounds at the position of F-β-alanine were detected in plasma (0.87 ± 0.26 μ*M*, $n=18$). Nevertheless, blank values were always obtained. The amount of F-β-alanine and β-alanine present in a plasma sample of the same patient during the treatment with S-1 is shown in Fig. 3B and the identification of both compounds was confirmed by spiking the sample with F-β-alanine and β-alanine (Fig. 3C). Due to small variations in elution times tyrosine was not always completely present in the fraction of column I which was further separated on column II thereby explaining the large variation in the amount of tyrosine encountered after chromatography of such a fraction on column II. Analysis of a urine sample of the same patient obtained prior to the start of the treatment with S-1 showed that only β-alanine was present with hardly any detectable interfering substances at the position of F-β-alanine (Fig. 4A). In a urine sample collected between 12 and 18 h after the administration of S-1, F-β-alanine was clearly present (Fig. 4B). Fig. 4C shows the same urine sample spiked with F-β-alanine and β-alanine to confirm the identity of the peaks.

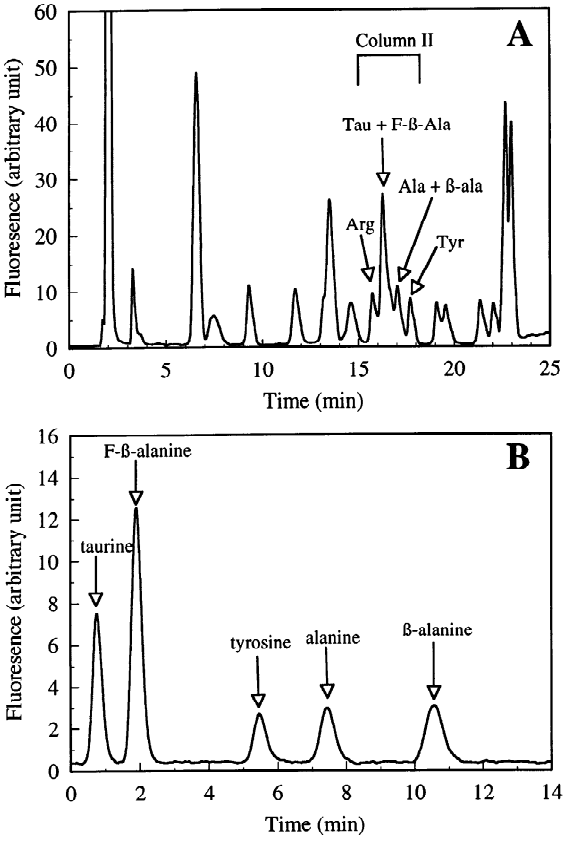

Fig. 2. Elution profile of amino acid standards. Panel A shows the elution profile of a standard of amino acids including β-alanine (5 μ*M*) and F-β-alanine (6.2 μ*M*) on column I. Panel B shows the elution profile of the amino acids eluting between 15 and 18 min from column I on column II.

3.2. Validation

To investigate the range of β-alanine and F-β-alanine concentrations that can be accurately determined in plasma, β-alanine and F-β-alanine standards were extracted according to the plasma protocol and analyzed by HPLC, as described above. Linear correlations exists between concentration and peak area, up to 26 μ*M* for β-alanine and up to 62 μ*M* for F-β-alanine (Fig. 5). The limits of quantification for β-alanine and F-β-alanine in plasma (200 μl, 1:1 diluted) are 0.1 and 0.05 μ*M*, respectively. In case β-alanine and F-β-alanine standards were extracted according to the urine protocol linear correlations between concentration and peak area were observed for β-alanine up to 1 m*M* and for F-β-alanine up to 1.2 m*M* (Fig. 6). The limits of quantification of β-alanine and F-β-alanine in urine

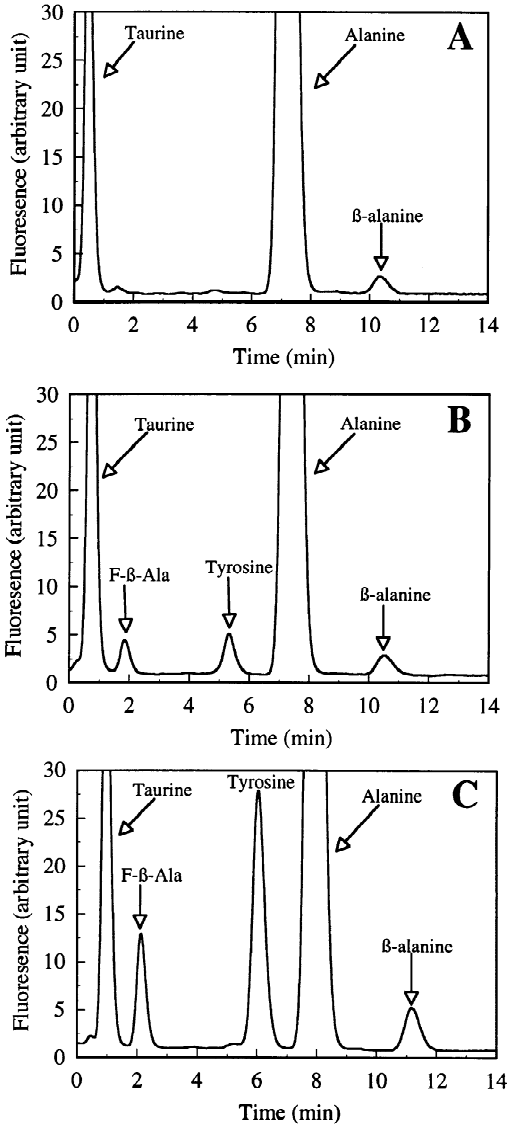

Fig. 3. Elution profile of F-β-alanine and β-alanine in plasma samples. Panel A shows the amount of β-alanine (2.9 μ*M*) before treatment. Panel B shows the amount of F-β-alanine (1.7 μ*M*) and β-alanine (3.3 μ*M*) during treatment with S-1. Panel C shows the identification of the F-β-alanine and β-alanine peaks by spiking the sample with 4.8 μ*M* F-β-alanine and 4 μ*M* β-alanine.

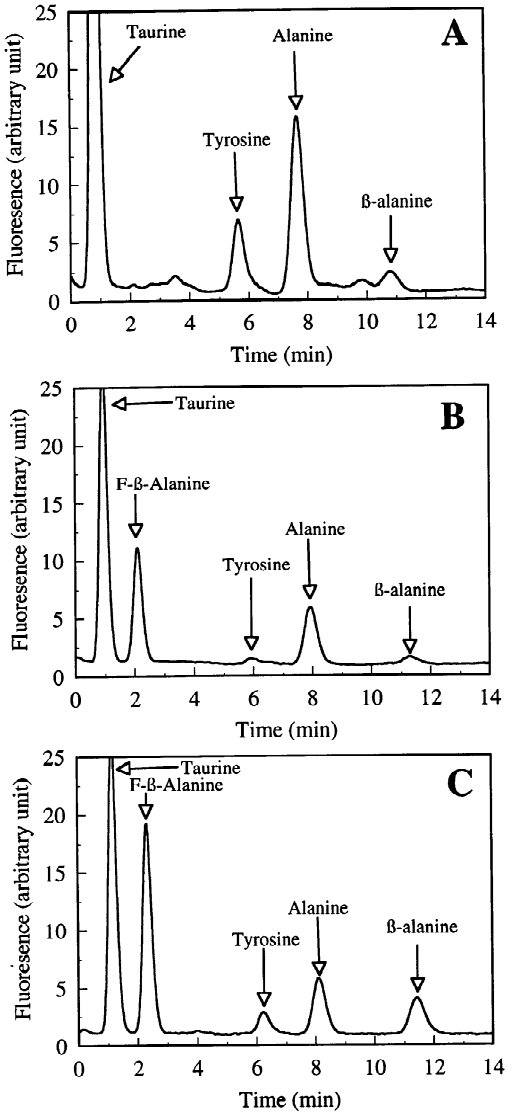

Fig. 4. Elution profile of F-β-alanine and β-alanine in urine samples. Panel A shows the amount of β-alanine (44 μ*M*) before treatment. Panel B shows the amount of F-β-alanine (62 μ*M*) and β-alanine (13 μ*M*) during treatment with S-1. Panel C shows the identification of the F-β-alanine and β-alanine peaks by spiking the sample with F-β-alanine (56 μ*M*) and β-alanine (47 μ*M*).

(200 μl, 1:1 diluted) were 0.1 and 0.05 μ*M*, respectively. Hardly any interfering compounds were detected at the position of F-β-alanine in 10 control urine samples. The detection limits of F-β-alanine and β-alanine in the HPLC system, defined as three times the value of the baseline noise were approximately 0.3 and 0.7 pmol, respectively.

The recoveries of β-alanine (1.9 μ*M*) and F-β-alanine (2.2 μ*M*) from plasma samples were 103±6.7 and 87.3±6.8%, respectively. The re-

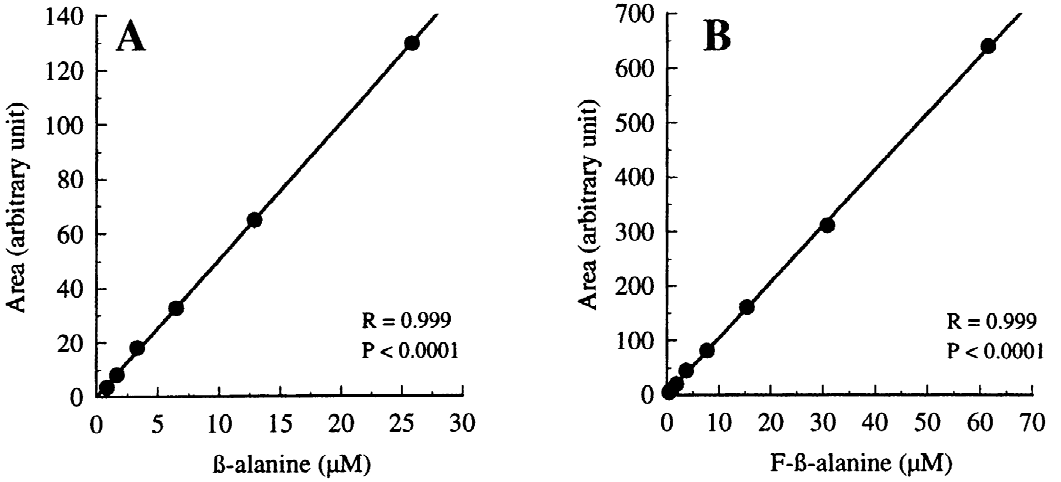

Fig. 5. Relationship between the concentration of β-alanine (panel A), F-β-alanine (panel B) and the peak area. Standards of β-alanine and F-β-alanine were extracted according to the plasma protocol and analyzed by HPLC. The correlation between the concentration of β-alanine, F-β-alanine and the peak area was studied by determination of the Pearsons correlation coefficients and linear regression (area=5.0×[β-alanine]+0.13; area=10.3×[F-β-alanine]+1.1).

coveries of β-alanine (47 µ*M*) and F-β-alanine (56 µ*M*) from urine samples were 99.2±5.1 and 99.6±4.7%, respectively. The intra-assay RSDs and inter-assay RSDs of the entire procedure to determine the concentrations in plasma were 4.2 and 8.0% (*n*=7), respectively, for β-alanine and 3.5 and 3.0% (*n*=7), respectively, for F-β-alanine. For urine the intra-assay RSDs and inter-assay RSDs were

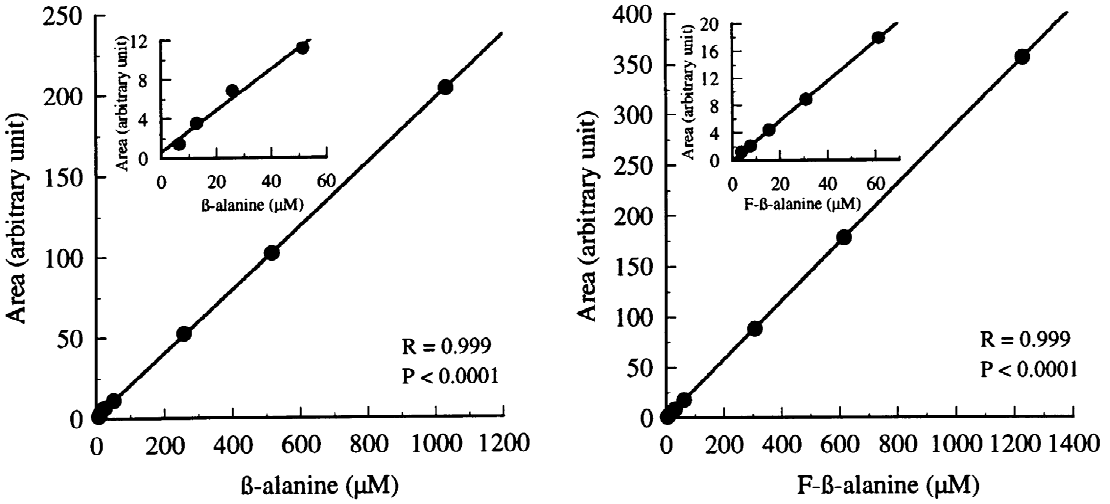

Fig. 6. Relationship between the concentration of β-alanine, F-β-alanine and the peak area. Standards of β-alanine and F-β-alanine were extracted according to the urine protocol and analyzed by HPLC. The inserts show the data points obtained for β-alanine and F-β-alanine concentrations between 3.8 and 62 µ*M*. The correlation between the concentration of β-alanine, F-β-alanine and the peak area was studied by determination of the Pearsons correlation coefficients and linear regression. (area=0.20×[β-alanine]+1.1; area=0.29×[F-β-alanine]−0.16).

5.8% ($n=10$) and 4.0% ($n=5$), respectively, for β-alanine and 4.2% ($n=10$) and 4.7% ($n=5$), respectively, for F-β-alanine. These low RSD values demonstrate the high reproducibility of the assay.

3.3. Pharmacokietics of patients treated with S-1

The usefulness of the method was demonstrated by determination of the β-alanine and F-β-alanine concentrations in plasma and urine samples of four patients receiving S-1 as a single dose. Fig. 7 shows that β-alanine was clearly detectable in all plasma samples obtained at various time points. Surprisingly, all four patients showed a profound increase in β-alanine concentration 4 h after the administration of the drug which subsequently decreased to levels more or less comparable to those observed prior to the start of the treatment with S-1. The concentration of F-β-alanine in plasma was very low (0.1–1.5 μ*M*) and maximum levels of F-β-alanine were observed 4 or 8 h after the start of the treatment with S-1. In contrast, analysis of the corresponding urine samples demonstrated the presence of large amounts of F-β-alanine whereas only low amounts of β-alanine were present (Fig. 8). Maximum concentrations of F-β-alanine, ranging from 253 to 724 μ*M*, were detected in urine samples collected between 6 and 12 h after the start of the treatment with S-1. When expressed per mol of creatinine maximum clearance of F-β-alanine was observed in three patients between 6 and 12 h while comparable levels of F-β-alanine were present in urine samples collected between 0 and 6 and 6 and 12 h of patient D. The total amount of F-β-alanine excreted during 24 h

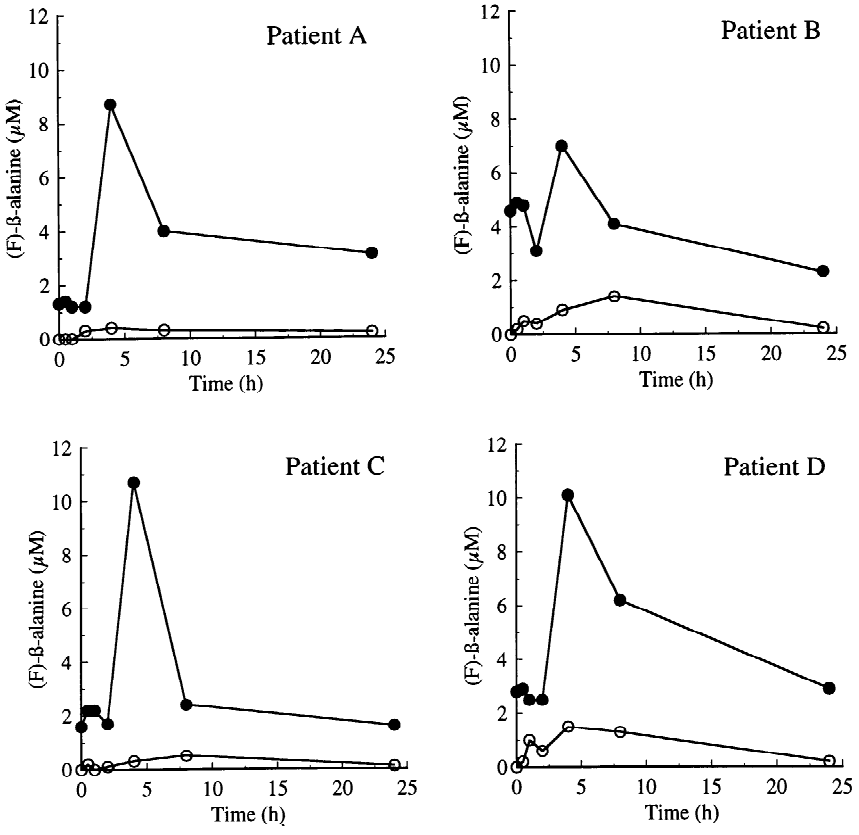

Fig. 7. Plasma levels of β-alanine and F-β-alanine in patients treated with S-1. The concentrations of β-alanine (-●-) and F-β-alanine (-○-) were determined in plasma samples obtained at various time points after the administration of S-1 at a dose of 35 mg/m^2 (patients A and B) and at a dose of 40 mg/m^2 (patients C and D).

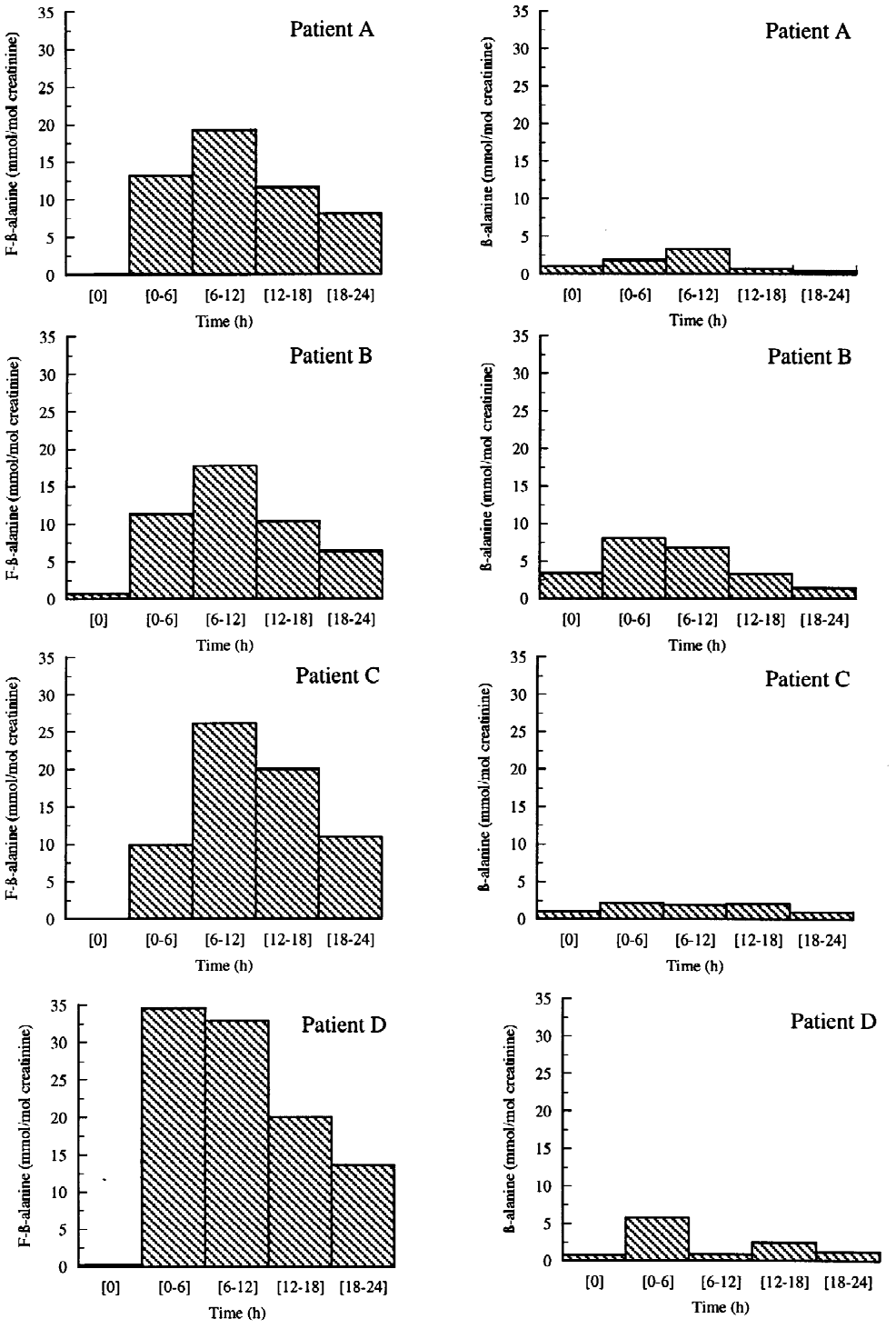

Fig. 8. Urine levels of β-alanine and F-β-alanine in patients treated with S-1. The concentration of β-alanine and F-β-alanine was determined in urine samples obtained between various time points after the administration of S-1 at a dose of 35 mg/m^2 (patients A and B) and at a dose of 40 mg/m^2 (patients C and D). In patient B a very small interfering compound was present in the urine sample obtained prior to the start of the S-1 treatment.

was higher in patient C (67 mmol/mol creatinine) and patient D (101 mmol/mol creatinine) who received a single dose of 40 mg S-1/m^2 when compared to patient A (52 mmol/mol creatinine) and patient B (46 mmol/mol creatinine) who received a dose of 35 mg S-1/m^2.

4. Discussion

Numerous methods have been published regarding the analysis of amino acids in body fluids although specific methods for the determination of β-alanine are scarce [25,26]. The most frequently used procedures are those in which the amino acids are derivatized followed by separation on an RP-HPLC column combined with fluorescent detection [25,26]. These methods are rather sensitive but time consuming with analysis time up to 1.5–4 h. In contrast, a number of different techniques are being used for the determination of F-β-alanine with the GC–MS procedure being the most sensitive one [23]. Thus, the absence of a rapid and sensitive method for the simultaneous determination of β-alanine and F-β-alanine prompted us to develop a novel procedure in which both compounds can be measured simultaneously and at very low concentrations with the same HPLC configuration thus saving time and costs.

In this study we developed a method for plasma and urine samples which is based on the fluorescence detection of OPA derivatives of F-β-alanine and β-alanine after their separation with dual-column RP-HPLC. A complete baseline separation was obtained for F-β-alanine and β-alanine and the interfering amino acids with a detection limit of less than 0.7 pmol. Even for F-β-alanine, our procedure is considerably more sensitive and faster than ^{19}F-NMR spectroscopy [20,24,27,28]. This latter technique has often been used for the non-invasive detection of 5FU and its metabolites in vivo [28]. However, detection thresholds of fluorinated compounds using ^{19}F-NMR spectroscopy have been estimated to be 3–10 μ*M* [20,24,27,28]. Thus, the application of ^{19}F-NMR spectroscopy to study the pharmacokinetics of 5FU and its degradation products is seriously hampered by its low intrinsic sensitivity and long data acquisition times (2–24 h) [20,24,27]. Our procedure to detect β-alanine is considerably faster than previously published HPLC procedures using precolumn derivatization with comparable sensitivity [25,26].

Recently, it has been shown that the therapeutic index of S-1 was four- to fivefold higher compared to that of either tegafur or 5FU in rats bearing advanced colorectal cancer and response rates of 49% were obtained in patients with advanced gastric cancer [7]. The superior therapeutic index of S-1 compared to other treatment modalities might partly be attributed to the inhibition of the degradation of 5FU. In the absence of an inhibitor of DPD, 5FU is rapidly degraded via (*R*)-5-fluoro-5,6-dihydrouracil and α-fluoro-β-ureidopropionate to F-β-alanine [22,24]. It has been shown that (*R*)-5-fluoro-5,6-dihydrouracil or further catabolites of this compound attenuated the antitumor activity of 5FU and slightly increased the toxicity of 5FU [29]. Furthermore, the occurrence of neurotoxicity and cardiotoxicity in a minority of patients receiving 5FU has been ascribed to the formation of F-β-alanine and/or its metabolites [14,19].

In four patients receiving a single dose of S-1 we could detect only very low levels of F-β-alanine ($<$1.5 μ*M*) in plasma samples obtained during the first 24 h with maximum levels occurring 4 or 8 h after the administration of the drug. The profound increase in the concentration of β-alanine 4 h after the administration of S-1 remains enigmatic. It is tempting to speculate that the metabolism of β-alanine might be influenced by that of F-β-alanine. β-Alanine can be further metabolized into malonic acid semi-aldehyde by transaminases such as β-alanine-pyruvate transaminase, β-alanine-α-ketoglutarate transaminase and D-3-aminoisobutyrate-pyruvate aminotransferase [30,31]. The latter enzyme has been shown to be inhibited by 5FU and F-β-alanine [32]. Furthermore, F-β-alanine has been shown to inhibit the uptake of β-alanine by rat hepatocytes via a specific GABA transporter [21]. Thus, F-β-alanine might not only compete with the degradation of β-alanine but also the uptake of β-alanine and GABA. However, one should bear in mind that β-alanine is not only synthesized via the three-step degradation of uracil but it can also be derived from dietary sources such as carnosine, anserine and balenine.

Whereas the concentration of β-alanine in plasma of patients treated with S-1 exceeded that of F-β-alanine the reverse was true for urine. The maximum

concentration of F-β-alanine in urine proved to be 2.6–20-fold higher than that of β-alanine. Up to now, no sound evidence has been presented whether F-β-alanine itself is further degraded in man. In rat, the administration of 5FU as well as that of F-β-alanine resulted in the formation of very small amounts of fluoroacetate [13]. Thus, the relative inertness of F-β-alanine might provide an explanation for the observation that F-β-alanine is the major catabolite of 5FU which is excreted in urine [22,24,33]. The presence of F-β-alanine in urine (and in plasma) samples collected from patients treated with a single dose of S-1 also demonstrates that DPD is not yet fully inhibited. Our results are in line with the observation that the DPD activity in liver of tumor-bearing rats decreased to approximately 20% of control values 1–2 h after the administration of CDHP [6].

5. Notation

5FU, 5-fluorouracil
CDHP, 5-chloro-2,4-dihydroxypyridine
DPD, dihydropyrimidine dehydrogenase
OPA, orthophthaldialdehyde
S-1, 1-(2-tetrahydrofuryl)-5-fluorouracil (Tegafur), 5-chloro-2,4-dihydroxypyridine and potassium oxonate in a molar ratio of 1:0.4:1

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