Fentanyl pharmacokinetics in blood of cancer patients by Gas Chromatography - Mass Spectrometry

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Abstract

In order to find a correlation between fentanyl action on pain and inter-individual variability in different cancer patients, the pharmacokinetic characterization of the drug becomes essential. Therefore, a gas chromatographic–mass spectrometric (GC–MS) in SIM mode analytical procedure has been developed and validated for the determination of fentanyl in human blood. The sample preparation consisted of a liquid-liquid extraction (LLE) from whole blood. The analysis were carried out with Agilent 7820A series gas chromatograph equipped with a 5977E series mass selective single quadrupole detector (MSD) in electron impact (EI) mode (70eV), under a temperature gradient elution. The limit of detection (LoD) and the limit of quantification (LoQ) values were found to be 5.6E-02 ± 3.5E-02ng mL^{-1} and 1.86E-01 ± 1.18E-01 ng mL^{-1} respectively. The developed method was found selective and sensitive and therefore suitable for a fast determination of fentanyl in human blood and for its pharmacokinetic characterization. Blood samples from 31 cancer patients treated with transdermal fentanyl (doses in the range of 12-100 µg h^{-1}) were collected at fixed intervals during an overall exposure time of 72 hours. The analysis of data and the pharmacokinetic parameters revealed dissimilar pharmacokinetic profiles in the patients examined. Patients were therefore grouped in three categories representing the different trends observed: fast, medium and slow

responders. These preliminary data provided significant outcomes for a correlation to clinical response.

1. Introduction

Strong opioids are the treatment of choice for chronic pain in moderate to severe cancer. Among them, fentanyl is one of the most widely used opioids thanks to its various easier administration routes, its potency approximately 75-100 times higher than morphine, its ability to strongly binding to plasma proteins, its large volume of distribution (3.5-8 L kg^{-1}) and its relatively high clearance (30/72 L h^{-1}) [1 2 3]. For what concern its administration, the most used devices are fentanyl transdermal patches which have been implemented since 1990s for the treatment of chronic pain [4]. Other products are intended for the transmucosal, buccal, sublingual or intranasal fentanyl administration [5]. The opportunity of administering this potent analgesic by non-injectable formulations pushed up its use dramatically. Transdermal fentanyl patches are indeed the most frequently prescribed strong opioid analgesic due to the easy administration and to the assessed efficacy in the relief of cancer pain [6 7]. Moreover, the development of non-injectable fentanyl formulations offers both the opportunity of prolonging the analgesic action of fentanyl as well as the possibility of generating different concentration-time profiles in patients. Certainly, the key for a rational clinical use of fentanyl, in its different formulations and dosages, is based on the overall understanding of its metabolism and pharmacokinetics [8]. In fact, fentanyl is extensively metabolized. Its renal excretion accounts 10% of the dose [9]. It is transformed into norfentanyl by piperidine N-dealkylation so it undergoes pre-systemic metabolic elimination in the liver and intestinal wall. This represents the predominant degradative pathway in humans (accounting for >99% of the metabolism) [10]. Its metabolization to despropionylfentanyl and hydroxyfentanyl, further N-dealkylated to hydroxyfentanyl, is carried out by amide hydrolysis and alkyl hydroxylation respectively [10]. All these metabolites seem to lack clinically relevant opioid agonist activity [11]. Regarding the enzymes involved in its metabolism, Cytochrome P450 (CYP) 3A4, CYP3A5 and 3A7, represent the mostly exclusively mediators [10 12 13]. Indeed, since CYP3A is the main drug-metabolising enzyme, the almost exclusive dependence of fentanyl on its metabolism has, as consequence, the possibility of being influenced by drug interactions phenomena [14]. Indeed, fentanyl metabolism can be inhibited by several CYP3A inhibitors (the list of inhibitors is included in the following website https://drug-interactions.medicine.iu.edu/MainTable). On the other hand, fentanyl itself can in turn act as an enzymatic inhibitor and reduce the clearance of co-administered drugs [15 16].

Other causes can influence fentanyl pharmacokinetic as well, that is the case of impaired liver function, difference in clearance and absorption rate[17]. The absorption rate is also subject to changes

in local conditions such as raised skin temperature, for instance during fever, with estimated increases in fentanyl plasma concentrations by approximately 33% at body temperatures of 40 °C [18], with the risk of acute opioid overdose[19]. In addition, in cancer patients the absorption was reported to be highly variable[20] and to depend on the patient's age, with lower absorption with higher age, and surprisingly also varied with the type of cancer, with breast or digestive cancer associated with higher absorption than lung cancer[21]. For all these reasons, transdermal fentanyl therapy shows high inter-individual and intra-individual variability [22, 23]. In light of these evidences, applying a similar therapy for all the patients, ignoring the physiological states and differences in the therapeutic range for each patient, often fails to treat the pain effectively[24]. The studies published so far to establish the influence of these factors on fentanyl pharmacokinetics, remain unfortunately questionable due to their heterogeneity. What is more, most of the published data describes healthy volunteers instead of cancer patients. As a consequence, the results obtained cannot be extrapolated to cancer patients to whom this therapy is addressed [22]. According to the necessity of providing an appropriate pain treatment, a study on fentanyl pharmacokinetics, carried out on cancer patients, was developed. The study was aimed to develop a fast and efficient sample preparation and GC-MS method aimed to the description of cancer patient fentanyl pharmacokinetic profile in order to personalise the pain control therapy and drug uptake, to highlight if either pharmacokinetics is equal for the patients treated with the same fentanyl dose patch or it changes within the same dose applied or among doses and if it changes which are key factors responsible for this change. In particular, the final goal of this work was indeed to develop and validate a fast GC-MS analytical procedure, with a blood sample pre-treatment, for the quantitative determination of fentanyl in the whole blood samples of cancer patients, and the description of its pharmacokinetics to perform correlation studies with several variables. The most used extraction procedures of fentanyl from biological fluids such as plasma or urine, involve solid phase extraction (SPE), liquid-liquid extraction (LLE) and protein precipitation (PP) coupled to SPE or LLE. On the other hand gas chromatography is the analytical method of choice for fentanyl determination in biological samples [25 26]. Thus, concerning fentanyl extraction, although fentanyl extraction by SPE is being widely described as purification method endowed with high selectivity and low consumption of solvents, obstruction of the column may occur, due to the complex blood matrix [26]. For these reasons, in this work a fast fentanyl LLE procedure was optimized, as an efficient blood sample preparation procedure. The developed GC-MS method was validated in terms of selectivity, linearity, sensitivity, accuracy and recovery. Then, the validated method was applied to the analysis of cancer patient blood samples to determine the pharmacokinetic parameters (AUC, Cmax and Tmax). The Istituto Romagnolo per lo studio dei tumori (IRST) provided blood samples from 31 cancer patients treated with transdermal fentanyl. The area under the concentration versus

time curve, from administration time to the last blood draw time, was calculated using the linear trapezoidal rule up to Cmax and subsequently the trapezoidal rule for the remainder of the curve. Furthermore, the inter-individual differences pointed out by this study will shed light on the opportunity to correlate the differences in distribution to several variables of studied cancer patient volunteers by a multivariate analysis. This will give the opportunity to define ad hoc individual therapies endowed with a higher level of efficacy and safety.

2. Material and methods

2.1. Materials

Fentanyl solution (100 μg mL^{-1} in methanol, ampule of 1 mL, certified reference material) and fentanyl-D_5 solution (100 μg mL^{-1} in methanol, ampule of 1 mL, certified reference material), were purchased from Cerilliant® (Paloma, TX, USA), a Sigma-Aldrich company (St. Luis, MO, USA), by the permission of the Italian 'Ministero per la salute' for research use only. Sodium carbonate ACS reagent, anhydrous, ≥ 99.5%, Sodium Bicarbonate ACS reagent, ≥ 99.7%, water HPLC Plus, diethyl ether puriss. ≥ 99.5% (GC) and methanol hypergrade for LC-MS LiChrosolv® were purchased from Sigma-Aldrich company (St. Luis, MO, USA).

2.2 Study Design

This was a biological interventional prospective, single-center study. Samples were provided by IRST institute (Istituto Romagnolo per lo Studio dei Tumori "Dino Amadori"), Meldola, FC, Italy. Blood sampling was carried out in the period from 2018 to 2020. In agreement with local ethics guidelines, 31 cancer patients were treated with the same branded transdermal patches, containing different doses of fentanyl, 12, 25, 50, 75 or 100 μg h^{-1} of drug, for chronic oncohematologic pain releasing.

Samples were collected at fixed times (0, 6, 18, 48 and 72 hours), during an overall fentanyl patch exposure time of 72 hours. The pool of 31 cancer patients were selected under specific criteria. Patients with solid tumors, patients already treated with transdermal fentanyl at any dose for chronic oncologic pain were enrolled. Patients with a history of hepatic or substance abuse and using concomitant drugs that have a contraindication with fentanyl were excluded.

Patients assuming drugs such as MAOIs, SNRI, SSRI, cancer drugs interfering with fentanyl metabolism, with an allergy to the studied drug, were not included in the study. Patients were able to write informed consent and had a life expectancy of more than 30 days. Their weight was between 50-100 kg and age range between 38 and 84 years. Pregnancy or breastfeeding were exclusion criteria.

2.3 Standard Solutions

Standard stock solutions of fentanyl (F) and fentanyl-D_5 (F-D_5), at the 100 μg mL^{-1} concentration in methanol, have been diluted to obtain 1000 ng mL^{-1} solutions. The obtained solutions have been stored at -20°C, conserved and kept from light. The solutions have been stable for more than one month after going through many freeze-thaw cycles.

2.4 Gas Chromatography-Mass Spectrometry (GC-MS) Method

The chromatographic method was optimized with an Agilent Gas-Chromatograph coupled to a single quadrupole selective mass detector (Agilent 7820A GC System, Agilent 5977E MSD) in electron ionization (EI) mode (70 eV) under a temperature gradient elution using a HP5MSUI (5%-phenyl)-methylpolysiloxane (30 m × 0.25 mm × 0.25 μm, 19091S-433UI) Agilent column. The gas carrier was helium with a flow rate of 1.5 mL min^{-1}. An aliquot of 1 μL of the pre-treated sample was injected in splittless mode. The MS source temperature was set at 250 °C, the MS quad temperature was adjusted to 150 °C, the AUX 1 temperature was fixed at 250 °C and the Front Inlet temperature at 250 °C. The GC oven temperature program started at 150 °C with hold time of 1 min. The temperature was increased to 240 °C by a linear gradient rate of 50 °C min^{-1}, then to 285 °C by a rate of 10 °C min^{-1} and hold for 2 min, finally it was increased to 300 °C by a rate of 10 °C min^{-1} and it was hold for 3 min. The analyses have been carried out in SIM mode. The 245 *m*/*Q* and 250 *m*/*Q* ions have been selected for fentanyl and fentanyl-D_5 monitoring, respectively. The total run time was of 13.58 min. Data were acquired with MassHunter GC/MS Acquisition B.07.00, 2013 and processed with MassHunter Workstation Software Qualitative Analysis B.06.00, 2012.

2.5 GC-MS method Validation

The proposed analytical method has been validated in terms of specificity, linearity, sensitivity, precision, accuracy and recovery.

Specificity. The method specificity has been determined by using three human blank blood samples and comparing the chromatograms obtained after injecting the non-spiked and spiked samples respectively. Moreover, each sample analysis has been followed by a double solvent injection. The absence of any signal at fentanyl retention time has demonstrated that there has been no carry-over effect.

Linearity. A preliminary calibration curve has been determined by analyzing ten fentanyl standard solutions diluted in methanol at the concentration range 2 to 55 ng mL^{-1}, each containing a fixed concentration of fentanyl-D_5 of 50 ng mL^{-1}. Linearity has been also determined by analysing five blood samples spiked with fentanyl and fentanyl-D_5 standard solutions (concentrations range 2 to 75 ng mL^{-1}) and 50 ng mL^{-1}, respectively. The enriched samples have then been subjected to the LLE procedure reported in par. 2.4 and subsequently analysed by GC-MS.

Sensitivity. The limit of detection (LoD=3*SE/m) and limit of quantitation (LoQ=10*SE/m) values, have been obtained by a statistical evaluation, considering the standard signal deviations[27]. In particular, LoD was calculated multiplying the standard error (SE) of the calibration curve, of spiked blood sample solutions, for a factor of three divided for the slope of the curve (LoD= 3*SE/m). The SE was obtained from a regression analysis of the calibration curve. LoQ was calculated multiplying the standard error (SE) of the same calibration curve for a factor of ten divided for the slope of the curve (LoQ= 10*SE/m).

Precision. The intra- and inter-day precision has been evaluated by analysing spiked blood sample at low (8 ng mL^{-1}) medium (30 ng mL^{-1}) and high (50 ng mL^{-1}) fentanyl concentrations, each containing fentanyl-D_5 at a fixed concentration of 50 ng mL^{-1}. Spiked blood samples were extracted twice daily. Each final solution was injected into the GC-MS five times. The same GC-MS analysis have been carried out on different days (n = 10).

Accuracy. The accuracy was determined by calculating the percentage of the deviation between the experimental concentrations of fentanyl and the nominal one.

Recovery. Recovery determination was carried out on blank blood samples from two different volunteers spiked with three incremental concentrations of fentanyl (10, 25, 50 ng mL^{-1}) and a fixed concentration of fentanyl-D_5 (50 ng mL^{-1}). The recovery values have been obtained by the following formula:

Eq. 1 % Recovery = [(Peak Area ratio of F/FD_5 pre-spiked blank blood sample solution) / (Peak Area ratio F/FD_5 of standard solution)] X 100

2.6 Sample preparation

Blood samples have been collected at five-time intervals during an overall exposure time of 72 hours. The T0 samples are related to the application time of the patch, while T1, T2, T3 and T4 correspond to 6, 18, 48 and 72 hours after the application, respectively. Samples have been stored at -80 °C. The fentanyl recovery from whole frozen blood has been carried out by LLE. The general procedure

consisted in adding 500 µL of 0.5 M carbonate buffer (pH = 11.00) and 5.0 µL of fentanyl-D_5 to 500 µL of the defrosted blood sample. The final concentration of the internal standard (IS) was equal to 50 ng mL^{-1}. 2.5 mL of diethyl ether had been added to the solution. The sample has been vortexed three times at 5 second intervals and centrifuged (Thermo Scientific CL10 centrifuge) at 1500 rpm for 5 minutes at 4 °C. The solution has been frozen at -80 °C for 2 hours. The supernatant has been collected and evaporated under a nitrogen stream. Finally, an extracted product has been dried and dissolved in 100 µL of methanol. The solution has been injected and analysed by GC-MS. Two independent LLE were performed on each blood sample of cancer patients collected at the described increasing times after the application of the transdermal patch. Fentanyl concentration in blood samples was calculated by interpolating F/F-D_5 peak area ratio in fentanyl calibration curve obtained with spiked blood samples (par 2.6).

2.7 Data analysis

Data were analysed in terms of fentanyl concentration for each blood sample, then the approximate area under the curve (AUC) of each patient was calculated following the trapezoidal rule [28] considering the formula reported below (Equation 2).

$$\text{AUC}= [(\text{concT0}+\text{concT1})* (\Delta t1)/2]+ [(\text{concT1}+\text{concT2})* (\Delta t2)/2]+ [(\text{concT2}+\text{concT3})* (\Delta t3)/2]+ [(\text{concT3}+\text{concT4})* (\Delta t4)/2]$$

Eq. 2 concT is the concentration at a determined collection time; Δt is the time difference in hours between two subsequent collection times.

The highest AUC value found among all patients was divided by three zones. The three zones obtained represent 0-33%, 33-66%, 66%-100% of the maximum AUC value. This division was carried out also on patients grouped for patch dose groups. The maximum concentration level of fentanyl (Cmax) and its interval (Tmax) were obtained. Finally, a multivariate analysis (principal component analysis, PCA) was performed with the effort of the SIMCA17, 17.0.2.34594, Sartorius Stedim Data Analytics AB software.

3. Results and Discussion

In this study we report the procedure for fentanyl LLE method from the whole blood. Since the efficiency of the LLE process can be improved by modifying organic solvents used for the extraction,

the diethyl ether was selected among the most commonly used extraction phases such as acetonitrile, acetone, ethyl acetate, hexane and toluene, usually used as a mixture of solvents or as pure. The selected conditions allowed to obtain an average recovery value of 99.02 ± 9.39E-01 %. This parameter was found to be higher than that showed for previously reported studies carried out on blood whole sample both by LLE and SPE [26] [29] [30]. Indeed, a fast and inexpensive LLE, modified from the one reported by Adamowicz et al. [26], was optimized and applied to the extraction and determination of fentanyl in human blood samples. A GC-MS method with a LLE sample pre-treatment was optimized and validated in terms of selectivity, sensitivity, linearity, precision, accuracy and recovery. The cancer patient blood samples were pre-treated and then analysed by GC-MS analysis carried out in SIM mode using fentanyl-D_5 as an internal standard. The application of LLE sample preparation and the GC-MS analysis were applied to calculate the pharmacokinetics parameters (AUC, Cmax and Tmax) of fentanyl in the whole human blood samples of 31 cancer patients.

3.1. Sample preparation and GC-MS analysis validation

Since the blood samples were provided frozen, the optimal condition to achieve the highest fentanyl recovery was found to be LLE extraction from the whole blood. In order to obtain a quick, easy and cheap extraction of fentanyl from whole blood and to prevent the possible obstruction of a SPE column, [25, 26] LLE with a binary solvents system was applied. Indeed, although SPE methods demonstrate some advantages such as low consumption of solvents, high recovery and high purified extracts, the use of whole blood could cause unwanted solid matrix clogging, impeding a smooth analysis[31] [32], then the LLE was preferred over SPE.

Various procedures, reporting many extraction solvents, were described for the liquid-liquid extraction (LLE) of fentanyl from whole blood [26] [33] often followed by additional purification [25]. In particular, LLE procedures were mainly conducted in alkaline conditions. On the other hand, acetonitrile, acetone, diethyl ether, ethyl acetate, hexane and toluene were used as pure or in mixture, in single or double phase as extraction solvent.

The efficiency of LLE was improved by increasing the fentanyl distribution coefficient, performing the extraction under basic conditions. Indeed, at basic pH values fentanyl is in uncharged form, more soluble in the organic solvent. Then its distribution in the organic solvent was improved.

Based on experimental evidence, a carbonate buffer 0.5 M at pH=11 provided efficiency, precision and repeatability. The extraction solvent was evaluated too. In terms of efficiency, fast separation and low miscibility in the aqueous layer, diethyl ether showed an higher recovery value when compared to that obtained by ethyl acetate [26]. In addition, to optimize the collection of organic phases from the aqueous layer, samples were centrifuged and then frozen at -80 °C for 2 hours. The organic phase has

been removed after a thawing cycle. This last step positively affected the efficacy of the extraction both in terms of recovery and for the opportunity to analyse samples without any further purification before GC-MS analysis.

The chromatographic conditions were optimized on Agilent GC-MS System with a HP5MSUI Agilent column. The analyses have been conducted in selected ion monitoring (SIM) mode, using fentanyl-D_5 as IS. The quantitative analysis was performed in the positive mode. The fentanyl fragmentation pattern showed a primary cleavage between the alpha and the beta site of the phenethyl fraction forming an ion of 245 mass to charge ratio (*m*/*Q*). This ion further undergoes to a second cleavage at the piperidine ring level, giving a 202 *m*/*Q* ion. Otherwise, the cleavage can occur at the level of the C-N amide bond forming a structure with a mass over charge ratio of 189 *m*/*Q*. Both these two structures undergo a third cleavage forming the same 146 *m*/*Q* ion. Fentanyl-D_5 fragmentation followed the same route, forming two ions with a mass over charge ratio of 250 *m*/*Q* and 194 *m*/*Q* respectively.

According to the signal intensity, the 245 *m*/*Q* and 250 *m*/*Q* (Figure 1) ions have been selected for fentanyl and fentanyl-D_5 monitoring, respectively. Under the described chromatographic condition, the retention time of fentanyl was found to be in the range 7.65 - 7.75 min.

The method has been validated in terms of specificity, linearity, sensitivity, precision, accuracy and recovery before starting to analyse samples collected from patients.

The method has shown a good selectivity since the absence of any coeluting interference has been proved by injecting solutions obtained after LLE of both blank and fentanyl and fentanyl-D_5 spiked blood (Figure 2).

The linearity and sensitivity of the method were also determined. The preliminary standard calibration curve, y= (1.77E-02±5.7735E-05)x + (2.83E-03±8.50E-04), was obtained with fentanyl and fentanyl-D_5 standard solutions in methanol and it was calculated in the range 2 -55 ng mL^{-1}. Linearity with good correlation coefficient (R^2=0.9998 ± 1.15E-04) was obtained.

In order to determine recovery, a calibration curve was obtained by analysing five Fentanyl and Fentanyl-D_5 spiked blank blood samples (concentration range 2-50 ng mL^{-1}), after LLE. The calibration curve, obtained by plotting analyte concentrations added to the samples versus the corresponding F/FD_5 peak area ratio, demonstrated a good correlation coefficient (y=1.78E-02 ± 1.43E-04)x (2E-16±1.10E-17), R^2= 0.9999 ± 0.001).

LoD and LoQ were found to be 5.6E-02 ± 3.5E-02ng mL^{-1} and 1.86E-01 ± 1.18E-01 ng mL^{-1} respectively. The mean recovery value, determined at three fentanyl spiked concentrations level (10, 25, 50 ng mL^{-1} of fentanyl and of a fixed concentration of 50 ng mL^{-1} of fentanyl D_5), resulted to be

99.02 ± 9.39E-01 %, confirming the higher efficiency of this extractive method when compared to those already reported [29]. The results are shown in Table SI1.

The determination of accuracy and the intra-day and inter-day precision of the method were carried out on the same samples. The obtained values are shown in Table SI2 and 3. The variation coefficient for intra- and inter-day assays demonstrated an average value of 1.95 ± 7.97E-01% and 1.20 ± 6.32E-01% respectively. Accuracy, found to be more than 99%, was determined at three different fentanyl concentration levels by calculating the percentage of the deviation between the experimental concentrations of fentanyl obtained from blood analysis and the nominal ones.

The developed chromatographic method was applied to the determination of fentanyl in human blood. The collected samples have been subjected to LLE extraction and then analyzed by GC-MS.

The unknown fentanyl concentration was calculated by fentanyl spiked blank blood samples calibration curve.

The fentanyl concentration expressed as ng mL^{-1} detected in blood samples of cancer patients, collected at increasing times, are shown in Table SI4. Except for patients **1** and **6**, all volunteers were already pre-treated with a fentanyl transdermal patch, thus justifying the presence of the drug at T0. However, at T0, not all patients showed a fentanyl measurable level, notwithstanding the pre-treatment (Table SI4).

The time course fentanyl distribution in patients, related to each collected blood sample, has been reported in the graph showed in Figure 3. Inter-individual differences in the pharmacokinetics profile resulted by determining the kinetics of fentanyl distribution among patients.

As an example, in Figure 4, fentanyl pharmacokinetic chromatograms of volunteer **14** are shown, obtained by analysing the blood samples collected at T0, T3 and T4 (T1 and T2 are not shown, because the concentration of fentanyl was below the LoD, as reported in TableSI4). The gradual increase of fentanyl blood concentration reached a maximum (C_{max}) at time T3, with the achievement of the systemic circulation level. On the other hand, fentanyl concentration decreased at time T4 supposedly due to the metabolic activity (Figure 4)**.**

The approximate area under the pharmacokinetics curve (AUC), the C_{max} and T_{max} of each patient was calculated (see par. 2.5.) and reported in Table SI5.

As an example, the AUC graph related to the patient **28,** treated with a patch releasing 75 μg h^{-1} of fentanyl, is reported in Figure 4. The integrated AUC is highlighted in grey; the maximum concentration (Cmax) was reached at 48 h. Considering an average of 4.5 L of blood in humans, the calculated AUC determined for volunteer **28**, during the total exposure of 72 h, was 396.1 ± 6.7 ng*h mL^{-1}.

Regardless of the dose, volunteers were grouped in three categories: high (below 33%), medium (33 and 66%) and low (higher than 66%) responders, considering the 0-33%, 33-66%, 66%-100% of the AUC values (Figure 5a). Hence, AUCs of volunteers were grouped according to the various administrated doses (Figure 6): 12, 25, 50, 75, 100 μg h^{-1}.

As observed from the graphs reported in Figure 6, the general trends of low, medium and high fentanyl AUC values are still present in each group of volunteers, independently to the different dosages of the transdermal patch. Therefore, we can hypothesise an inter-individual variability in fentanyl absorption and distribution. These results are in agreement with a previously study reporting a large patient to patient variations in pharmacokinetic parameters[20, 22]. It was reported that patients age is one of the factors that mainly influences fentanyl concentration in patients' serum or plasma[34] . Previously, other factors were studied, such as patch surface area as well as cytochrome P450 3A4 (CYP3A4) activity, which could cause variation in fentanyl blood concentration[5, 17, 35]. Based on these premises, we tried to find potential correlation among the obtained pharmacokinetic data and other variables (age, gender, BMI, pre-treatment dose) in a multivariate analysis. Therefore, we started from the data shown in Figure 5b, where age (black points) and gender (grey bars= female, black bars= male) related to the AUCs values for each patient did not show any correlation.

To better elucidate the fentanyl pharmacokinetic variability in cancer patients, a preliminary-principal component analysis (PCA) plot was elaborated (Figure 7). A number of 8 variables were considered: previous dose, patch dose, gender, age, body mass index (BMI), Cmax, Tmax, AUC. However, no clusters of patients were observed. The overall group of patients will be implemented to reinforce the statistics of the study and PK data. Since in a previous paper[35] it was shown that use of a cytochrome P450 3A4 (CYP3A4) inducer may cause variations in serum fentanyl concentration after fentanyl transdermal treatment, we aim to study the patients genetic profile correlation with differences in fentanyl elimination.

4. Conclusions

In this work, an original and efficient LLE procedure (recovery 99%) has been applied to the whole blood of cancer patients in order to obtain the fentanyl pharmacokinetic parameters by a validated GC-MS method. The procedure was demonstrated to be fast and efficient to determine fentanyl administered by transdermal patch in cancer patients. The validated sample preparation and GC-MS method resulted to be selective, sensitive and accurate. The high reproducibility and recovery allowed the accurate fentanyl determination at a concentration down to 1.86E-01 ± 1.18E-01 ng mL^{-1}, thus confirming the applicability of the method to the determination of fentanyl pharmacokinetic profile

in cancer patients. Cancer patients were grouped in fast, medium and slow responders, according to the obtained pharmacokinetic parameters. A multivariate analysis was carried out taking into account previous dose, patch dose, gender, age, body mass index (BMI), Cmax, Tmax, AUC, but there was not a clear correlation suggesting the inter-individual variability may depend on other factors. In light of this, the obtained results can be considered as a starting point for further investigation aimed to correlate the fentanyl pharmacokinetic inter-individual variability to other factors such as genetic profile.

Hence, these preliminary data provided significant outcomes for future correlations to clinical response as well as to individual variability, also useful for administering the drug at more appropriate doses. Further pharmacokinetics studies on cancer patients can be crucial to highlight the cause for different fentanyl plasma level profiles and understand when effective pain relief can be achieved, while avoiding adverse effects such as respiratory depression. Gaining more data on cancer patients pharmacokinetics would allow the determination of covariate associations such as the dependency of the input on skin properties or body temperature, type of cancer, genetic profiles. Finally, this study reinforces the concept that clinical monitoring is essential for determining the right dose of drug to reach the desired effect. In conclusion, in this study, the interindividual variability in pharmacokinetic parameters was high. Therefore, therapeutic drug monitoring of patients under fentanyl treatment would be particularly useful for poor responders to obtain more rapidly relevant plasma concentrations.

Local Regulations / Declaration of Helsinki

The present study was conducted following the instructions and procedures in accordance with the principles of Good Clinical Practice ICH Tripartite Guideline (January 1997) and the principles deriving from the 18th World Medical Assembly (Helsinki, 1964 and further amendments) or the laws and regulations of the Italian legislation, in order to ensure the protection of personal data.

Ethics Committee

The Protocol and any additional material provided to the patient was submitted to the Ethics Committee membership for review. The approval by the Ethics Committee took place before the start of the study.

Informed Consent

Patients were enrolled in the study after signing the informed consent form.

Author statement

I hereby certify that all authors have seen and approved the final version of the manuscript being submitted. The article is the authors' original work, has not received prior publication and is not under consideration for publication elsewhere.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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