# Evidence for the Conversion of Docetaxel into 7-Epidocetaxel in Patients Receiving Conventional Taxotere® Based Chemotherapy

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**Abstract:** *Purpose*: Epimerization at the C7 atom of the baccatin moiety is a common in-vitro pathway for all taxanes, including the natural precursor 10-deacetyl baccatin III and the antineoplastic drugs paclitaxel and docetaxel. To date this in-vitro epimerization of both drugs has been elucidated completely, but epimerization of docetaxel in patients during chemotherapy has not yet described. The goal of this study was to identify the epimer of docetaxel in plasma and urine of taxotere treated patients.

*Patients and Methods*: 12 patients suffering from mamma carcinoma, lung cancer or prostate cancer were treated with various docetaxel-based schedules. Blood samples were drawn before start of infusion, at the end of infusion and 20 min thereafter, urine was collected and pooled for 6 hours. Docetaxel and its epimer epidocetaxel were quantified by solid phase extraction and reversed phase HPLC.

*Results*: In 8 of 12 patients epidocetaxel could be quantified in plasma at the end of infusion (range 0.05 – 0.54 µg/ml). 20 minutes later concentrations were below LOQ due to rapid distribution of docetaxel into tissue. In urine, epidocetaxel has been found in 7 of 12 patients (range 0.1 – 0.5 µg/ml).

*Conclusion*: Epidocetaxel is a distinct docetaxel metabolite in man. So our knowledge, this is the first time that quantification of epidocetaxel in blood and urine of chemotherapy patients has been reported. This finding is important for designing of new docetaxel generic drugs and the development of new chemotherapeutic schedules using docetaxel. To date the in-vivo pharmacologic and toxic properties of the epimer remain unclear.

**Keywords:** Docetaxel, epidocetaxel, epimerization, patients, plasma, urine.

## INTRODUCTION

Docetaxel (DTX) has been successfully approved for head and neck cancer, gastric cancer, breast cancer, prostate cancer and non small lung cancer [1]. The drug undergoes predominantly hepatic metabolism (75 %) in all species including humans, a smaller amount (6 %) is excreted *via* the renal route [2]. *In vitro* metabolism studies concerning paclitaxel (PTX) and DTX in mouse, rat and dog and human liver microsomes showed metabolic profiles very similar to those of the in vivo studies. Oxidation of the tert-butyl group on the synthetic side-chain is the main metabolic pathway. In all species including humans the CYP3A family of cytochrome P450 enzymes plays the key role in the biotransformation of DTX. CYP 3A4 and 3A5 isoenzymes are the most responsible for the oxidation of DTX to the primary metabolites and for a subsequent oxidation of the latter [3]. As far as isolated and tested all metabolites resulting from oxidation showed a marked reduction in both cytotoxic and myelotoxic properties compared with the parent compound [4].

Epimerization of a drug is a common phenomenon that may occur under in-vitro and in-vivo conditions. The antineoplastic drugs epirubicin and doxorubicin represent a well known example for epimerization. The only difference between both molecules is the position of the hydroxy group at C4 atom of the amino sugar moiety (position equatorial or axial). Contrary to PTX, both drugs do not show any interconversion under in-vitro or in-vivo conditions. Once the amino sugar is splitted by glucosidases, doxorubicin and epirubicin aglycones are identical molecules. This small difference in C4 atom of the aminosugar implicates quite different metabolism, pharmacological efficacy [5] and toxicity [6].

Epimerization at the C7 atom position of the baccatin moiety (axial or equatorial position of the hydroxy group) is a common in-vitro pathway for PTX [7] and DTX [8], including the natural precursor 10-

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deacetyl baccatin-III. The formation of the epimer of PTX is considered to be due to a retroaldol/aldol intramolecular rearrangement mechanism, involving the neighbouring keto moiety at position 9 of the molecule, and occurs spontaneously in aqueous solution [7]. Furthermore epiDTX is generated at various concentrations in different DTX preparations: for instance, in an HPLC analysis of a crude DTX preparation of unspecified origin, epiDTX was present at 0.46 area % [9]. Interestingly this epimerization is decreased at about 80 percent in the presence of aluminium sulphate [10].

The addition of DTX to the standard mixture of human liver microsomes resulted in the occurrence of a high concentration of epiDTX [11,12]. Furthermore, in a study on the metabolism of DTX by recombinant human CYP 1B1, epiDTX was found to be present shortly after the start of the incubations, its concentrations being influenced by the concentration of DTX and the type of buffer used [3, 13]. That epiDTX undergoes metabolic reactions (oxidation by CYP 3A4 and 4A5 isoenzymes) identical to those of the parent compound DTX.

Contrary to PTX [14], to date the epimerization of DTX in patients during chemotherapy not yet has been described, only in-vitro and animal data are available [15].

DTX epimerization has been implicated in loss of potency of the drug and as well as in the development of resistance in tumour cells [16]. From this point of view it is important to know the amount of DTX epimerization in man. Furthermore, concerning new formulations and investigations in new generic taxanes drugs as well as the development of new DTX derivatives, it is of high interest to verify the extent of epimerization.

## CLINICAL STUDY

### Study Subjects

The trial was a national, hypothesis- confirming investigation of patient material (blood, urine) at two oncology centres. The investigation was non-interventional, there was no significant additional burden to patients, because the sampling of urine and blood occurred within the clinical standard setting and blood sampling had been performed mostly through central or peripheral venous lines without additional punctures. Patient inclusion criteria were: male or female, at least 18 years old. No restriction in terms of gender, tumour entity, stage-histology or co-medication. Patient exclusion criterion was concomitant or previous platinum-based chemotherapy.

### Study Design and Treatment

Twelve patients suffering from mamma carcinoma, lung cancer or prostate cancer were included in this trial. Written informed consent has been obtained prior study specific procedure; vote E 943, top4 of the ethics

**Table 1: Patient Characteristics and Drug Administration**

| pat ID | tumor | sex | chemotherapy | dose [mg/m$^2$] | infusion time [min] | Infusion rate [mg/min] | drugs of pre- and co-medication |
|---|---|---|---|---|---|---|---|
| A | Mamma Ca | f | Taxotere | 180 | 90 | 3,00 | Emend, Fortecortin, Kytril |
| B | Mamma Ca | f | Epirubicin /Taxotere | 130/130 | 90 | 1,44 | Kytril, Dibondrin |
| C | Mamma Ca | f | Taxotere | 180 | 90 | 3,00 | Dexamethason, Kytril, Dibondrin |
| D | Prostate Ca | m | Bicalutamid / Taxotere | 40/70 | 60 | 1,17 | Furosemid, Pamidronat |
| E | Mamma Ca | f | Taxotere | 130 | 60 | 2,17 | Dexamethason, Hydromorphon, Metamizol, Pamidronat |
| F | Prostate Ca | m | Bicalutamid / Taxotere | 40/70 | 60 | 1,17 | Mefenaminsäure, Furosemid, Pantoprazol, Pamidronat |
| G | Bronchus Ca | f | Taxotere | 75 | 60 | 1,25 | Diclofenac, Dexamethason, Pantoprazol, Metamizol, Pregabalin |
| H | Mamma Ca | f | Epirubicin /Taxotere | 120/120 | 90 | 1,33 | Kytril, Dexamethason |
| I | Mamma Ca | f | Herceptin/ Taxotere | 80/40 | 60 | 0,67 | Kytril, Dibondrin |
| J | Mamma Ca | f | Taxotere | 180 | 90 | 3,00 | Kytril, Dibondrin |
| K | Mamma Ca | f | Taxotere | 130 | 60 | 2,17 | Kytril, Dibondrin |
| L | Mamma Ca | f | Taxotere | 120 | 60 | 2,00 | Kytril, Dexamethason, Dibondrin |

committee of the University of Salzburg. Patients received various conventional taxotere® - based schedules as a mono or combination chemotherapy, depending on tumour entity and staging. Table **1** lists the individual information about the included patients, the chemotherapeutic schedule and drugs of the co-medication.

### Drugs

For chemical standard samples and for calibration purposes DTX (P 20 mg/2 ml, batch No 714058, Probe 2.093) and epiDTX (batch No V2008083/N) was supplied as pure chemical substance from Ebewe Pharmaeuticals Inc. (Unterach, Austria). Taxotere® concentrated solution was used for preparation of infusion, each vial containing 20 mg DTX (as trihydrat) per 1 ml anhydrous ethanol. The preparation has been diluted with isotonic sodium hydrochloride solution in a 250 ml infusion bag to final concentrations ranging from 0.6 to 3.0 mg DTX per ml according to the chemotherapeutic schedule.

### Sample Collection

Blood samples of 5 ml have been collected through central or peripheral venous lines without additional punctures. Times of collection were before infusion (= 0 min), at the end of infusion (EOI) and 20 min thereafter. After centrifuging the blood samples for 5 min at 5000 rpm, 2.0 ml of the supernatant plasma was frozen immediately at – 80 $^{o}$C. Urine was collected over a time period of 6 hours, pooled and after mixing 6.0 ml aliquots were frozen at -80 $^{o}$C until analyzed.

### Analytical Assay

DTX and epiDTX were separated from matrix compounds and co-administered drugs by solid phase extraction using Oasis® HLB extraction cartridges especially designed for taxanes (1ccm volume, 100 mg packing, 30 µm, Waters Inc.). DTX and epiDTX were quantified by use of a validated reversed phase HPLC method using a taxane specific HPLC column (Symmetry® C8, Waters Inc., USA) as described in the literature [17].

### Biometrics

Statistical analysis (descriptive statistics, scattergrams and correlation analysis) was performed by the scientific software GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla California USA). From urine and plasma data an apparent epimerization coefficient ($R_{epi}$) for epiDTX has been calculated by dividing the concentration [µg/ml] of epiDTX by the amount of the parent compound DTX [µg/ml].

## RESULTS

Chromatographic separation of the both epimers was surprisingly quite simple using our specific reversed phase HPLC method for taxanes [17]. Figure **1** depicts chromatograms obtained from a patient's blood and urine sample, respectively. Both compounds are well separated from plasma and urine matrix compounds in front of the chromatogram. DTX elutes at 8.5 minutes, epiDTX peak elutes about 4 minutes later at 12.3 min and was base line separated from DTX peak. The whole analytical procedure for one plasma or urine sample could be performed within 30 minutes including sample preparation. The peak area of epiDTX of 3 of 12 patients (blood) and 5 of 12 patients (urine) was too small for quantification and below LOQ, however, significant peaks of the epimer were detectable in the other blood and urine samples.

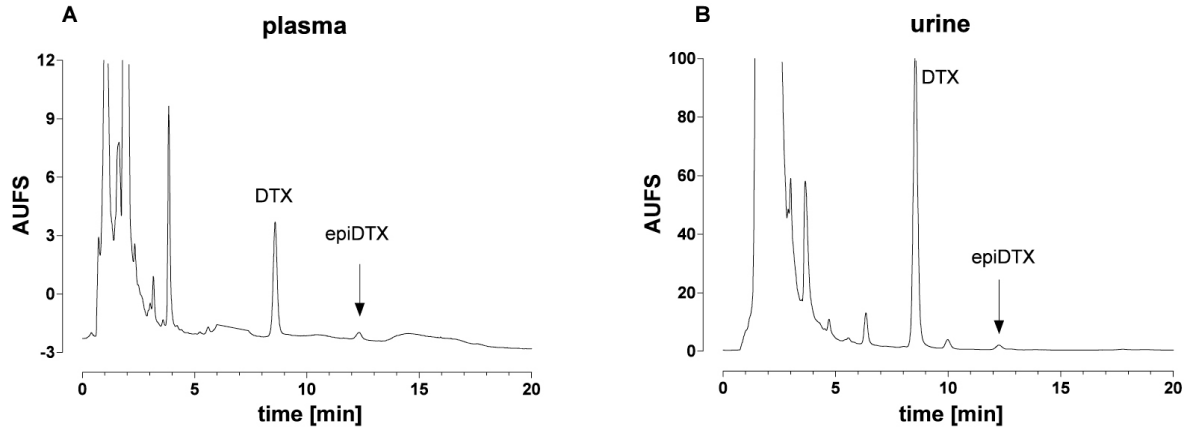


**Figure 1:** plasma (insert **A**) and urine (insert **B**) sample chromatograms from a patient.

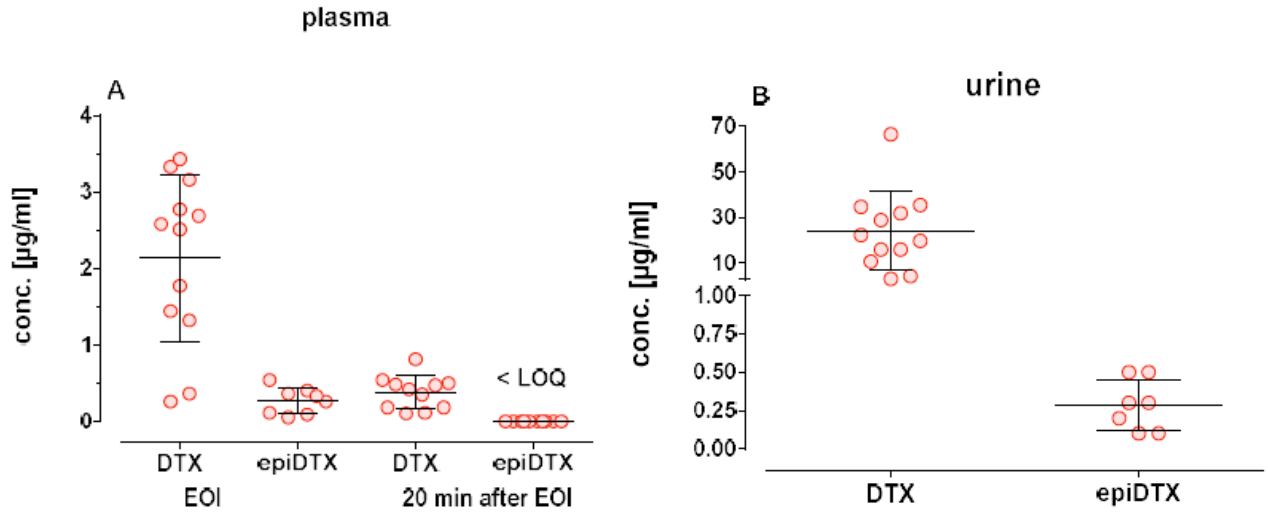


**Figure 2:** Scattergrams of DTX and epiDTX concentrations in plasma (insert **A**) and urine (insert **B**).

As anticipated in pre dose plasma samples DTX was not detectable. Plasma concentrations of DTX at EOI could be measured in each single patient (mean concentration 2.14 ± 1.09 µg/ml, range from 0.26 to 3.43 µg/ml). 20 min after EOI DTX plasma concentrations could be measured in 11 patients (mean 0.376 ± 0.172 µg/ml, range from LOQ to 0.81 µg/ml). Sample concentrations 20 min after EOI were significantly lower than that at EOI giving evidence for a rapid distribution of the drug from blood into the adjacent tissue as well as for extensive elimination processes.

At EOI, in 8 of 12 patients mean epiDTX plasma concentrations were 0.267 ± 0.172 µg/ml ranging from 0.05 µg/ml to 0.54 µg/ml. 20 minutes after EOI quantification of epiDTX failed due plasma concentrations below the limit of the analytical assay. *In vitro* incubation of DTX with liver microsomes preparations showed a rapid formation of epiDTX [11, 12]. During DTX liver passage in our investigated patients a similar procedure may occur: formation of the epimer resulting in measurable plasma concentrations of epiDTX.

**Table 2: Statistics of Percent epiDTX in Plasma and Urine Samples and Apparent Epimerization Coefficient ($R_{epi}$)**

| subject | plasma %epi EOI | urine % epi | $R_{epi}$ plasma | $R_{epi}$ urine |
|---|---|---|---|---|
| A | nc | 1,0 | nc | 0.010 |
| B | nc | 0,6 | nc | 0.006 |
| C | nc | 0,8 | nc | 0.007 |
| D | 1,9 | nc | 0.19 | nc |
| E | 10,1 | 1,6 | 0.10 | 0.016 |
| F | 1,3 | 0,6 | 0.11 | 0.006 |
| G | 6,2 | 0,9 | 0.06 | 0.009 |
| H | 21,5 | nc | 0.22 | nc |
| I | 27,7 | 8,2 | 0.28 | 0.234 |
| J | 25,0 | nc | 0.25 | nc |
| K | 25,0 | nc | 0.25 | nc |
| L | nc | nc | nc | nc |
| sample size | 8 | 7 | 8 | 7 |
| mean | 14,8 | 2,0 | 0,18 | 0,041 |
| SD | 11,1 | 2,8 | 0,08 | 0,085 |
| CV [%] | 75 | 142 | 45 | 207 |

mean… mean value, SD … standard deviation, CV … coefficient of variation, nc … not calculable.

In 6 hours pooled urine, mean DTX concentrations were 24.12 ± 17.25 µg/ml (range 3.21 – 66.37 µg/ml), epiDTX could be found in much lower concentrations (mean 0.285 ± 0.167 µg/ml) ranging from 0.1 – 0.5 µg/ml in 7 of 12 patients. Figure **2** depicts the scattergrams for plasma and urine data for each single patient.

By dividing the concentration of epiDTX by the concentration of DTX it is possible to calculate an apparent epimerization coefficient $R_{epi}$. Table **2** lists the percent extent of DTX epimerization and the corresponding coefficient in blood and urine. In plasma $R_{epi}$ ranged from 0.06 to 0.28 in urine $R_{epi}$ ranged distinctly decreased from 0.006 to 0.234. A high $_{Repi}$ value points out a high epimerization rate of DTX.

We also attempted a correlation analysis between DTX or epiDTX concentrations at the EOI and the taxotere rate of infusion in mg/min, yet this analysis was not successful. As can be seen from Figure **3**, DTX and epiDTX plasma concentrations at EOI did not correlate with the infusion rate. These results give evidence that epiDTX formation is independent from taxotere dose.

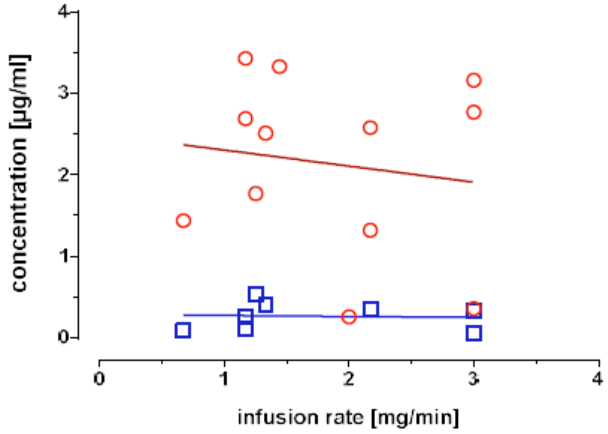


**Figure 3:** correlation plot between infusion rate and plasma concentration of DTX (circles) and epiDTX (squares) at EOI.

## DISCUSSION

The aim of this study was to determine the extent of epimerization of DTX into epiDTX in plasma and urine of 12 patients (two male and 10 female) receiving conventional taxotere chemotherapy against various tumours. All included patients were evaluable for this pharmacokinetic investigation. Depending on the staging of the patients, they were treated by a 60 or 90 min intravenous infusion (freshly prepared) with infusion rates ranging from 0.67 to 3.00 mg DTX per minute. It must be pointed out that the taxotere formulation administered to the patients did not contain epiDTX, this has been controlled by HPLC measurements of the solutions for infusion. Drugs of the chemotherapeutic co-medication were bicalutamid (prostate cancer), epirubicin or herceptin (mamma carcinoma). For additional drugs of the pre-medication or co-medication compare Table **1**.

Rationale for blood sampling was as follows: DTX pharmacokinetics preferably can be described by a two or three compartment model depending on the time period of sample collection [18]. The rapid distribution of DTX from blood into tissue ensures only very small amounts of the drug in the blood within few minutes after the EOI. Therefore we decided to collect three blood samples: pre dose for control purposes, at the EOI representing DTX peak plasma concentration and 20 minutes thereafter, when distribution of the drug has finished and terminal elimination of the drug predominates. By this procedure a direct comparison between high plasma concentration at EOI and low plasma concentration 20 min after EOI for both, DTX and epiDTX is possible. For urine a sampling period of six hours seemed to be appropriate in order to prevent degradation of DTX during storage.

The pharmacokinetic profile of DTX is rather complex as a result of excessive plasma protein binding as well as high metabolic conversion and tissue distribution of DTX. These factors contribute to a high variability of plasma and urine data of DTX in our investigated subjects. Nevertheless we could quantitate epiDTX in 8 out of 12 samples collected at EOI whereas 20 min thereafter plasma concentrations were below LOQ. Compared to DTX, mean percent amount of epiDTX in plasma was 14.4 ± 11.1 % and in urine 1.96 ± 2.77 %. The presence of the epimer in urine gives evidence for epiDTX excretion *via* the renal route according do to the parent compound, although renal excretion only plays a minor role in DTX elimination.

The extent of this epimerization seemed to be independent from tumour entity. Further, by comparing medication schedules, no clues for a probable influence of co-administered drugs on the epimerization could be found. Future investigations including a larger sample of subjects will be necessary to answer this question.

## CONCLUSION

So our knowledge, this is the first time that quantification of epiDTX in blood and urine of patients

receiving taxotere chemotherapy has been reported. Our results are comparable with existing in-vitro and animal investigations: epiDTX is a significant DTX metabolite in man.

It has been shown that DTX metabolism leads to a remarkable reduction in both cytotoxic and myelotoxic properties compared with the parent compound [19]. For epiDTX it has been also discussed, whether this compound shows identical pharmacologic activity as the parent compound or not. It remains open whether this epimerization leads to a loss of potency of the drug and as well as in the development of resistance in tumour cells [16].

From the pharmacokinetic point of view it is important to know the extent of DTX epimerization especially in the blood (and urine) of patients. Loss of pharmacologic activity could necessitate a dose adjustment of DTX. Beyond that, the extent of epimerization may play an important role concerning the development of new taxane derivatives and new galenic formulations of DTX (generic drugs) to ensure a safe and effective therapy of this class of compounds.

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