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Influence of Short-term use of Dexamethasone on the Pharmacokinetics of Ifosfamide in Patients

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Running title: **Ifosfamide Pharmacokinetics and Dexamethasone in Patients**

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abbreviations: IFO = ifosfamide, 4-OH-IFO = 4-hydroxyifosfamide,

                   CAA = chloroacetaldehyde, DEX = dexamethasone

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Abstract

Dexamethasone induces the hepatic cytochrome P450 3A and therefore, is predicted to change the pharmacokinetics, activities and side effects of drugs metabolized by cytochrome P450 3A. The aim of this study was to determine if the pharmacokinetics of the cytochrome-P450-3A-dependent oxazaphosphorine cytostatic drug ifosfamide is influenced by short-term antiemetic use of dexamethasone in patients. The peak-concentration and AUC were determined for the parent compound and the metabolites 4-hydroxyifosfamide and chloroacetaldehyde in 8 patients who received two cycles of ICE-chemotherapy (ifosfamide 5 g/m^2 day 1, carboplatin 300 mg/m^2 day 1, etoposide 100 mg/m^2 day 1-3). One cycle included concomitant administration of dexamethasone (40 mg over 30 min 16 hours and 1 hour prior to chemotherapy), whereas the other did not. The half-lives of ifosfamide, 4-hydroxyifosfamide and chloroacetaldehyde were shorter with concomitant administration of dexamethasone, but the differences were not statistically significant. In addition, there were no significant differences in the ifosfamide and active 4-hydroxyifosfamide peak-concentrations and AUCs when dexamethasone was included. After dexamethasone administration, the chloroacetaldehyde peak-concentration was slightly increased by 1.5-fold and the AUC by 1.3-fold, however, these increases were not statistically significant. In conclusion, dexamethasone comedication in ICE-chemotherapy did not change the ifosfamide pharmacokinetics. Thus, dexamethasone can be used safely as an antiemetic drug in ifosfamide chemotherapy.

Dexamethasone (DEX) is frequently used as an antiemetic with highly emetogenic chemotherapy regimens, enhancing the antiemetic potential of 5-HT3-antagonists (Perez EA, 1998). DEX is known to induce hepatic cytochrome P450 3A via the SXR nuclear receptor (Xie W et al., 2000) and thus could alter the pharmacokinetics, activities and side effects of other cytochrome-P450-dependent drugs, including oxazaphosphorine cytostatic ifosfamide (IFO) and cyclophosphamide. Both these prodrugs require hepatic activation to form 4-hydroxymetabolites (Furlanut M and Franceschi L, 2003). For IFO, a second metabolic pathway catalyzed by cytochrome P450 3A4 and 2B6 (Granvil CP et al., 1999; Huang Z et al., 2000) leads to the release of chloroacetaldehyde (CAA). CAA is responsible for some side effects of IFO (Aleksa K et al., 2004; Nicolao P and Giometto B, 2003) and has been reported to exert cytotoxic effects on tumour cells in vitro and in vivo, by inhibition of DNA-synthesis and induction of DNA-strand-breaks (Börner K et al., 2000; Brueggemann SK et al., 2002; Brüggemann SK et al., 2006; Brüggemann SK et al., 1997). Changes in the metabolism of IFO could result in unpredictable antitumour activities and side effects. Using cyclophosphamide in male rats, Yu et al. (Yu LJ et al., 1999) found DEX to increase the CAA values almost ten-fold when compared with the controls without DEX administration. The peak-levels and AUC of 4-hydroxycyclophosphamide were reduced by DEX administration. In humans, CAA generation is low after cyclophosphamide administration (Yu LJ et al., 1999), but considerably increased with IFO. However, so far there are no published reports documenting the influence of DEX on IFO-metabolism in cancer patients.

The aim of this study was to test if short-term use of DEX as an antiemetic influences the pharmacokinetics of the oxazaphosphorine cytostatic IFO in patients, and thereby to determine if these drugs can be safely combined without changing the metabolism, antitumoural potentials and side effects of ifosfamide.

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Methods

Patients. Nine patients (6 males, 3 females), 18 to 70 years of age, 0-2 on the WHO performance scale, took part in this prospective randomized trial. All patients suffered from solid tumours (5 bronchial carcinomas, 1 breast cancer, 1 neuroendocrine tumour, 1 hepatic metastasized cancer of unknown primary and 1 neuroendocrine cancer of unknown primary with axillar, cervical, infraclavicular, mediastinal and abdominal metastases) and had a life expectancy of three months or more, and the indication for IFO-containing polychemotherapy. Patients with florid gastric or duodenal ulcer were excluded from the study, and one patient dropped out because of antiepileptic medication, which is known to influence hepatic cytochrome P450. The study was approved by the ethics committee of the University of Lübeck and was carried out in accordance with the Declaration of Helsinki. All patients gave their written informed consent prior to the start of the therapy.

Patient Therapy. In order to minimize inter- and intraindividual differences, the patients received two cycles of ICE-polychemotherapy (IFO 5 g/m^2 day 1, carboplatin 300 mg/m^2 day 1 and etoposide 100 mg/m^2 day 1-3 with 8 mg ondansetron as standard antiemetic prior to chemotherapy) and were randomized to receive DEX concomitantly in cycle one or two. When DEX was administered, 40 mg were infused over 30 min 16 hours and 1 hour prior to chemotherapy. Three ml blood were taken from a venous catheter 0, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 24 and 30 hours after the start of IFO infusion and IFO, 4-hydroxyifosfamide (4-OH-IFO) and CAA were measured as described below.

IFO Assay. For IFO determination, the blood samples were centrifuged and the plasma spiked with an internal standard solution containing the oxazaphosphorine derivative trofosfamide (for details see (Kurowski V and Wagner T, 1993)). After dichloromethane extraction, IFO was measured by gas chromatography with a HP5-column (5% phenyl-methylsilicone) and helium serving as carrier gas. Temperatures of the oven, injector and

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detector were set at 130°C (-> 210°C), 190°C and 300°C. LOQ and LOD were 1.5 µmol/l and 0.5 µmol/l, respectively. Inter-assay CV at 50 µmol/l was 5.2% (for more details see Kurowski and Wagner (Kurowski V and Wagner T, 1993)).

CAA Assay. To determine the CAA concentrations, the blood samples were processed as described by Kurowski and Wagner (Kurowski V and Wagner T, 1993). Briefly, CAA was measured by gas chromatography with an electron capture detector, a capillary column (Hewlett-Packard 5, cross-linked 5% phenyl-methylsilicone) and helium serving as carrier gas. LOQ and LOD were 1.0 µmol/l and 0.33 µmol/l, respectively. Inter-assay CV at 5 µmol/l were 13.7% (for more details see Kurowski and Wagner (Kurowski V and Wagner T, 1993)).

4-OH-IFO Assay. To measure 4-OH-IFO, the blood samples were processed as previously described (Alarcon RA, 1968) with minor modifications. The derivatisation mixture with fluorescent 7-hydroxyquinoline was separated by high performance liquid chromatography without the extraction procedure and quantified by fluorescence detection (see (Bohnenstengel F et al., 1997)). LOQ and LOD were 0.16 µmol/l and 0.05 µmol/l, respectively. Inter-assay CV at 2 µmol/l was 5.7% (for more details see Kurowski and Wagner (Kurowski V and Wagner T, 1993)).

Calculations and Statistical Evaluation. The terminal half-life ($t_{1/2}$), the highest measured concentration (c_{max}), the time point of highest concentration (t_{max}) and the area under the concentration-time-curve (AUC_{0-24} and $AUC_{0-\infty}$) were calculated with the TOPFIT 2.0 program. The Wilcoxon-Test (Wilcoxon matched pair signed rank statistic) was used for statistical evaluation.

Results and Discussion

DEX is known to induce hepatic cytochrome P450 3A via the SXR nuclear receptor (Xie W et al., 2000). Used as an antiemetic, DEX could alter the pharmacokinetics, activities and side effects of cytochrome-P450-dependent oxazaphosphorine cytostatic IFO. Therefore, the pharmacokinetics of the prodrug IFO and the active metabolites 4-OH-IFO and CAA were determined after ICE-chemotherapy, with or without concomitant DEX administration (Figure 1). The plasma concentrations were similar for IFO and 4-OH-IFO, respectively, and no significant differences were identified in the peak-concentrations and AUC (see Table 1). The standard deviations can likely be explained by the known high interindividual differences in cytochrome P450 activities (Lamba JK et al., 2002). Small differences cannot be ruled out, however, statistically significant and clinically relevant differences, were not observed in IFO and 4-OH-IFO peak-concentrations and AUC between the two groups. The half-lives of all three substances were shorter when DEX was administered, but the differences were not statistically significant (Table 1). Different to IFO and 4-OH-IFO, the CAA peak-concentration and AUC were increased 1.5 and 1.3-fold, respectively, with concomitant DEX administration. However, due to the number of patients and the high standard deviation, these increases could not be statistically confirmed (Figure 1 and Table 1).

The IFO, 4-OH-IFO and CAA AUCs of each patient are shown in Figure 2. The individual AUC-ratios after ICE-chemotherapy, without vs. with DEX, ranged from 0.64 to 1.95, 0.63 to 2.01, and 0.27 to 1.64 for IFO, 4-OH-IFO and CAA, respectively. No differences were observed between administering DEX in cycle one or two.

Currently, there is no information about the pharmacokinetics of ifosfamide after short-term use of dexamethasone in humans. Our results contrast those from animal studies in rats (Brain EG et al., 1998) where a proportional decrease in the AUCs for both IFO metabolites was observed after DEX administration with no net impact on the fraction of IFO undergoing

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4-hydroxylation. Furthermore, our results differ from those of Yu et al. (Yu LJ et al., 1999), who found that DEX-pretreatment enhances the CAA levels. These authors reported that Cmax of CAA was increased ~8.5-fold, while the AUC of CAA was increased 4-fold. The concomitant peak-level and AUC of 4-hydroxycyclophosphamide were reduced when DEX was given prior to the cyclophosphamide application. However, unlike in rats, in humans the generation of CAA is low after cyclophosphamide treatment (Yu LJ et al., 1999).

Wang et al. investigated the pharmacokinetics of carboplatin and gemcitabine after DEX pretreatment in mice (Wang H et al., 2004b); (Wang H et al., 2004a). They found small but significant differences in the plasma pharmacokinetics of gemcitabine. The plasma AUC of gemcitabine was decreased by 40% with the DEX-pretreatment, due to a reduced half-life (Wang H et al., 2004a). These data are inconsistent with earlier results from the same group, which did not show significant changes in the gemcitabine pharmacokinetics (Wang H et al., 2004b). In both studies, DEX-pretreatment resulted in no significant changes in the carboplatin pharmacokinetics and AUC.

In this study, we compared the pharmacokinetics of IFO and its metabolites in patients, with or without the short-term use of DEX as an antiemetic. Taken together, our results show that DEX administration prior to chemotherapy has no important effect on the IFO pharmacokinetics, which suggests that DEX may be safely used with ifosfamide chemotherapy. The induction of cytochrome P450 3A by DEX may require 2-3 days and therefore, the long-term use of DEX could induce significant changes. However, there are no data to document the effects of continuous DEX application for example in the therapy of cerebral metastases in bronchial carcinoma.

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Legend for Figures

Fig. 1: Pharmacokinetics of ifosfamide (IFO) (1a) and the metabolites 4-hydroxyifosfamide (4-OH-IFO) (1b) and chloroacetaldehyde (CAA) (1c) after ifosfamide-containing chemotherapy ± dexamethasone (DEX) (n=8; mean ± SD).

Fig. 2: Area under the concentration-time-curve (0 – ∞) of ifosfamide (IFO) (2a), 4-hydroxyifosfamide (4-OH-IFO) (2b) and chloroacetaldehyde (CAA) (2c) of patients 1 to 8 ± concomitant application of dexamethasone (DEX).
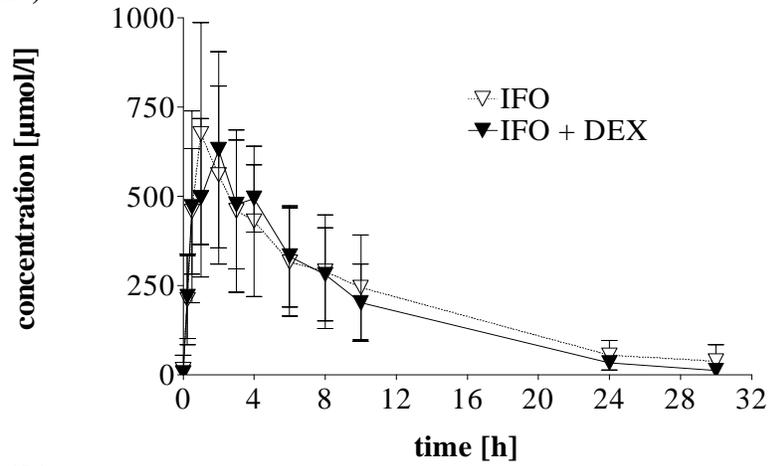
Table 1 Pharmacokinetics of ifosfamide (IFO) and the metabolites 4-hydroxyifosfamide (4-OH-IFO) and chloroacetaldehyde (CAA) after ifosfamide-containing chemotherapy ± dexamethasone (DEX).
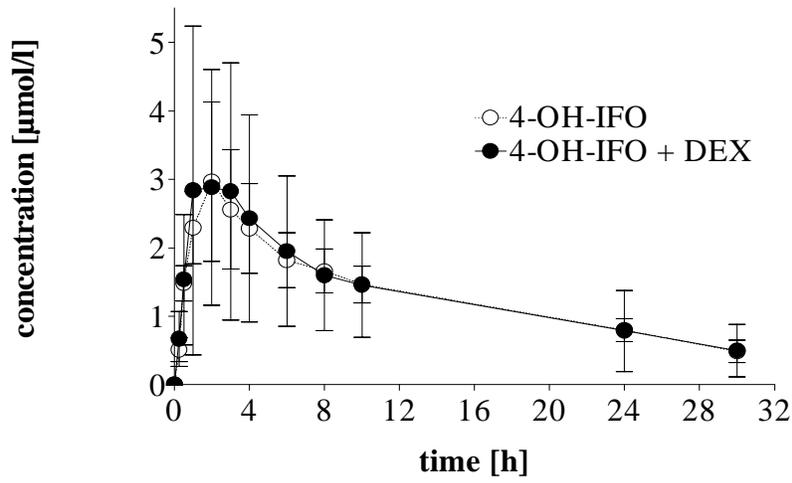
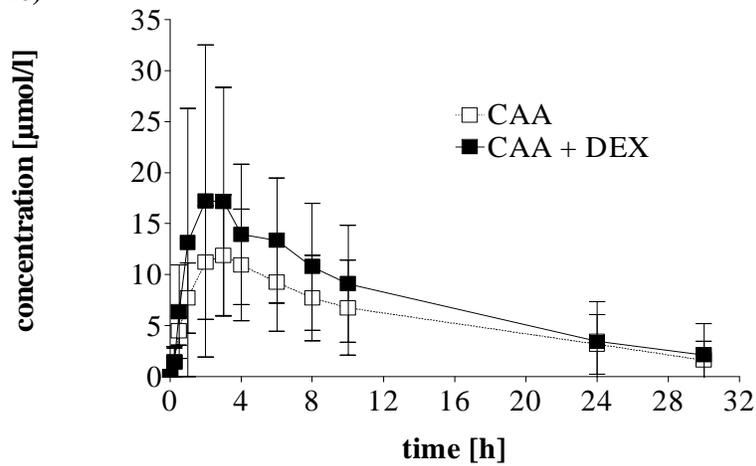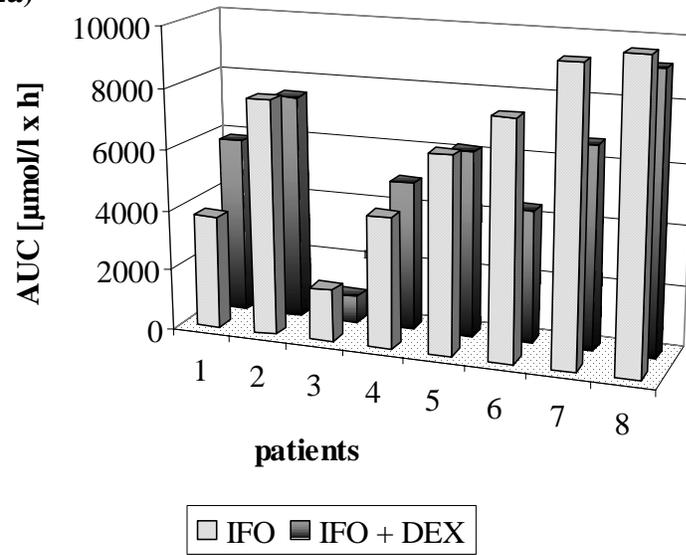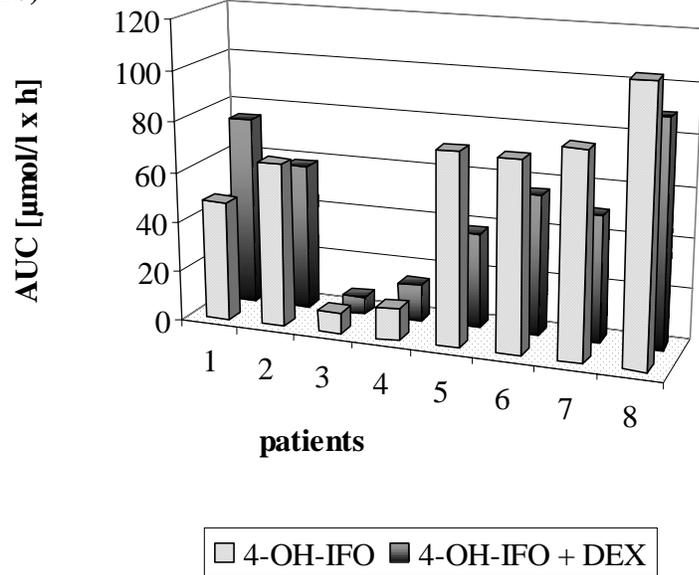|	t1/2 [h]	Cmax [µmol/l]	AUC 0-24 [µmol x l^{-1} x h]	AUC 0-∞ [µmol x l^{-1} x h]
IFO	5.86 (2.71)	766.5 (274.5)	5890 (2555)	6450 (2945)
IFO + DEX	4.86 (0.49)	715.3 (263.0)	5444 (2288)	5699 (2449)
4-OH-IFO	16.02 (8.81)	2.94 (3.34)	34.44 (19.88)	59.39 (34.60)
4-OH-IFO + DEX	11.22 (4.72)	3.39 (2.10)	35.61 (19.25)	48.62 (28.18)
CAA	10.74 (7.22)	12.68 (5.52)	158.14 (77.07)	272.65 (230.01)
CAA + DEX	9.68 (5.58)	19.50 (15.45)	213.42 (98.37)	285.62 (179.51)

n = 8, mean (± SD)

Fig. 1a)

1b)

1c)

Fig. 2a)

2b)

2c)