Tissue Type Differences in ABCB1 Expression and Paclitaxel Tissue Pharmacokinetics in Patients With Esophageal Cancer

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Background: Data from previous work suggests that there is no correlation between systemic (plasma) paclitaxel exposure and efficacy in patients treated for esophageal cancer. In this trial, we investigated ATP-binding cassette efflux transporter expression and intratumoral pharmacokinetics of paclitaxel to identify changes which could be a first sign of chemoresistance.

Methods: Patients with esophageal cancer treated with paclitaxel and carboplatin (± concomitant radiotherapy) were included. During the first and last cycle of weekly paclitaxel, blood samples and biopsies of esophageal mucosa and tumor tissue were taken. Changes in paclitaxel exposure and expression of ABCB1 (P-glycoprotein) over time were studied in both tumor tissue and normal appearing esophageal mucosa.

Results: ABCB1 was significantly higher expressed in tumor tissue compared to esophageal tissue, during both the first and last cycle of paclitaxel (cycle 1: p < 0.01; cycle 5/6: p = 0.01). Interestingly, ABCB1 expression was significantly higher in adenocarcinoma than in squamous cell carcinoma (p < 0.01). During the first cycle, a trend towards a higher intratumoral paclitaxel concentration was observed compared to the esophageal mucosa concentration (RD:43%; 95%CI: −3% to 111%; p = 0.07). Intratumoral and plasma paclitaxel concentrations were significantly correlated during the first cycle (AUC0–48h: r = 0.72; p < 0.01).

Conclusion: Higher ABCB1 expression in tumor tissue, and differences between histological tumor types might partly explain why tumors respond differently to systemic treatment. Resistance by altered intratumoral paclitaxel concentrations could not be demonstrated because the majority of the biopsies taken at the last cycle of paclitaxel did contain a low amount of tumor cells or no tumor.

Keywords: tissue pharmacokinetics, intratumoral, paclitaxel, pharmacokinetics, esophageal cancer, ABCB1
INTRODUCTION

Esophageal cancer is the 7th most common cause of cancer-related mortality worldwide (Global Burden of Disease Cancer et al., 2018). Paclitaxel in combination with carboplatin and radiotherapy is highly effective in the curative setting of esophageal cancer, and in combination with carboplatin alone it has shown moderate efficacy both during induction chemotherapy and in the palliative setting of this tumor type (Polee et al., 2004; van Hagen et al., 2012; Shapiro et al., 2015; de Man et al., 2019). Nonetheless, a substantial part of the patients with esophageal cancer do not benefit from this treatment or show progression of disease short after their treatment has stopped (Chirieac et al., 2005; de Man et al., 2019; Toxopeus et al., 2019).

Paclitaxel acts by the inhibition of cell proliferation, by promoting the stabilization of cellular microtubules and the concentration-dependent induction of multipolar spindles which eventually leads to apoptosis (Jordan and Wilson, 2004; Weaver, 2014; Zasadil et al., 2014). Paclitaxel is also known for its induction of drug resistance (Barbuti and Chen, 2015), although the exact mechanisms are unknown. Major factors probably causing paclitaxel resistance are alterations in stability of the microtubule network, reduced function of apoptotic proteins (e.g., B-cell leukemia/lymphoma 2 (Bcl-2), cellular tumor antigen (p53)), and overexpression of transmembrane efflux pumps of the ATP-binding cassette (ABC) subfamily (Gottesman et al., 2002; Huisman et al., 2005; Barbuti and Chen, 2015).

ABC-efflux transporters are essential in the protection of the cell against xenobiotics (Schinkel and Jonker, 2003). ABCB1 (P-glycoprotein) is one of the subtypes in the ABC-efflux transporter family (Schinkel and Jonker, 2003). ABCB1 is expressed in the plasma membrane of human cells and is known for its diversity in substrates that can be transported via this efflux transporter (Schinkel and Jonker, 2003). Overexpression of ABCB1 contributes to chemotherapy resistance of cancer cells in vitro and was related to worse survival of cancer patients in several studies (Trock et al., 1997; Schinkel and Jonker, 2003; Schach et al., 2005; Haber et al., 2006; Stordal et al., 2012; Barbuti and Chen, 2015). In vivo studies demonstrated that inhibition or induction of ABCB1 in multidrug resistant tumor cells influences the intratumoral paclitaxel exposure (Huisman et al., 2005; Tiwari et al., 2013). Nevertheless, intratumoral pharmacokinetics of chemotherapeutical agents, and the relation between intratumoral chemotherapy exposure and ABC efflux transporter activity remains largely unknown, especially in the clinical setting.

In contrast to tissue pharmacokinetics, the systemic pharmacokinetics of paclitaxel are well known and characterized by a large inter-individual variability (Henningsson et al., 2003; de Graan et al., 2013). Moreover, commonly seen hematological toxicity and peripheral neuropathy have been linked with the time above a specific paclitaxel plasma concentration (i.e., >0.05 µM) (Gianni et al., 1995; Mielke et al., 2005). To determine the best dose for an individual patient it is often suggested to tailor the dose of paclitaxel based on the systemic pharmacokinetic exposure. This strategy improved the risk-benefit profile of non-small cell lung cancer patients treated with paclitaxel (Joerger et al., 2016). However, this is probably only a surrogate for the intratumoral exposure (Mathijssen et al., 2011). Additionally, in a previous study no correlation between systemic paclitaxel clearance and esophageal cancer response was shown (Toxopeus et al., 2019).

Currently, knowledge about the intratumoral concentrations of paclitaxel, the influence of intratumoral paclitaxel concentration on the effectiveness of the treatment and the correlation between ABC efflux transporters and intratumoral paclitaxel is lacking. Therefore, there is an urgent need to investigate and elucidate the intratumoral paclitaxel pharmacokinetics.

In this exploratory study we assessed both ABC efflux transporter expression, and intratumoral and esophageal mucosa paclitaxel concentrations over time, to identify changes in paclitaxel concentrations and/or differences between tissue types which could potentially be a sign of the development of drug resistance in esophageal carcinoma.

METHODS

We performed a single center pharmacokinetic study in patients diagnosed with esophageal cancer for whom treatment with weekly paclitaxel and carboplatin was indicated. The study was performed between October 2017 and September 2019 at the Erasmus MC Cancer Institute, Rotterdam, Netherlands. The Medical Ethics Committee and the board of directors of the Erasmus MC approved the study protocol. The study was performed in accordance with the International Conference on Harmonization Good Clinical Practice guidelines, the Declaration of Helsinki, and all applicable regulations. The trial is registered at the Dutch Trial Registry (www.trialregister.nl number NL5990). All patients provided written informed consent before any study related procedure was pursued.

Patients

Patients, 18 years or older, were eligible if they were diagnosed with a histologically proven malignancy of the esophagus that was safely accessible by upper endoscopy. They were treated with weekly paclitaxel and carboplatin with or without concomitant radiotherapy in a standard regimen (Supplementary Methods 1) (Polee et al., 2004; van Hagen et al., 2012; de Man et al., 2019). Patients had to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. Patients were excluded if the tumor caused esophageal stenosis prohibiting upper endoscopy, if they previously received radiotherapy on the esophagus, if they had a history of bleeding diathesis, or if they used medication or supplements which could interact with paclitaxel during the study period.
Study Design
The primary objective of the study was to demonstrate a 25% reduction of the intratumoral concentration of paclitaxel in the last cycle of weekly paclitaxel compared to the first cycle of paclitaxel in esophageal cancer patients. Secondary objectives of our study were to: 1. compare intratumoral paclitaxel concentrations with paclitaxel concentrations in normal appearing esophageal mucosa, 2. compare paclitaxel concentrations in non-tumoral mucosa per study cycle, 3. correlate intratumoral concentrations of paclitaxel with systemic paclitaxel pharmacokinetics per study cycle, 4. to investigate ABCB1 expression over time, 5. compare ABCB1 expression between tumor tissue and non-tumoral esophageal mucosa tissue, and 6. compare ABCB1 expression between different histological types of esophageal cancer.

All included patients were seen at the outpatient clinic prior to each chemotherapy cycle. During cycle 1 and the last cycle (i.e., cycle 5 or 6), patients were admitted to the hospital to perform blood withdrawals for pharmacokinetic purposes and to undergo an upper endoscopy to obtain biopsies of the tumor and normal appearing esophageal mucosa for pharmacokinetic purposes and pathological assessments. Patients were evaluable for the primary endpoint if the biopsies were successfully obtained during the first and the last cycle of their weekly paclitaxel treatment.

Biopsy Procedure
Upper endoscopy was planned at 4 h after the start of paclitaxel administration. Sedation with midazolam and fentanyl was allowed during the endoscopy procedure. During the procedure, a total of 2–4 biopsies of the tumor -- with a mean diameter of 6 mm -- were taken by an experienced and dedicated gastroenterologist. Biopsies of normal appearing esophageal mucosa (visual inspection by the gastroenterologist) were taken at least 5 cm proximal or distally from the visible tumor area. These biopsies were of the same size and same numbers as the tumor biopsies. Half of the biopsies were directly frozen in liquid nitrogen and stored at −70°C for pharmacokinetic analysis. The other half of the biopsies were formalin-fixed for pathological assessment. If the gastroenterologist could not identify a macroscopic tumor during the last treatment cycle, samples were taken at the same location as during the first cycle.

Pharmacokinetic Analysis
Plasma samples were taken before start of the paclitaxel infusion, 30 min after start of administration, 5 min prior to the end of infusion, and 1.5 and 3 h after the end of the administration of paclitaxel. The timing of blood sampling as well as tissue sampling were comparable when the anti-allergic infusion regimen used. Blood samples were collected in 4 ml lithium heparin tubes and plasma was collected after centrifugation at 2,500* g at 4°C for 10 min and stored at < −70°C until analysis. Paclitaxel concentrations were measured using a validated liquid chromatography-mass spectrometry method (Sparreboom et al., 1998). Systemic exposure was expressed as area under the curve from pre-infusion to 48 h (AUC₀–48h) and estimated using a previously developed population PK model developed in NONMEM (Henningsson et al., 2003). The analysis took the anti-allergic infusion regimen into account.

Tissue biopsies were homogenized in 400 µL of blank human plasma with a tissue-lyser (Qiagen, Germany) and a stainless-steel bead (5 mm) for 90 s at 60 Hz. Homogenized tissue samples were further processed as plasma samples as described above.

Pathological Analysis
To determine the expression of ABCB1 an automated immunostainer (the Ventana Benchmark ULTRA, Ventana Medical Systems Inc., Arizona, United States) was used. Sequential 4 µm thick (FFPE) sections were stained for ABCB1 using Optimivew universal DAB detection Kit (#760–700, Ventana).

In brief, following deparaffinization and heat-induced antigen retrieval with CC1 (#950–500, Ventana) for 32 min, the tissue samples were incubated with the ABCB1 antibody (Company: NovusBio; Type: anti mouse; Clone: OTIIA7; Lot number: W001; Dilution: 1/9,600) for another 32 min at 37°C. Incubation was followed by hematoxylin II counter stain for 8 min and then a blue coloring reagent for 8 min according to the manufactures instructions (Ventana). Positive controls were used on every slide.

After immunohistochemical staining the percentage of positive stained cells of interest and the intensity of the staining per biopsy were evaluated (by R.A.G.V.E. and M.D.). The biopsies were scored according to the immunoreactive score (IRS) described by Remmele and Stegner (Remmele and Stegner, 1987).

Statistical Analysis
This study was powered to detect a 25% decrease of the intratumoral concentrations of paclitaxel in the last treatment cycle compared to the first treatment cycle. Since we had no information on beforehand on the variability of the intratumoral paclitaxel concentrations, we assumed an intrapatient standard deviation of 30% in intratumoral paclitaxel concentrations. Given a power of 80% and two-sided significance level of 5%, at least 14 evaluable patients were required for the primary objective.

Log-transformation was used for data regarding tissue (tumor and normal appearing esophagus mucosa) tissue) paclitaxel concentrations and AUC₀–48h, since we assumed that these data followed a lognormal distribution. A paired t-test was used to compare tissue paclitaxel concentrations, and systemic exposure (i.e., AUC₀–48h) for the total study population. Mean differences with corresponding 95% confidence intervals (CI) were exponentiated to calculate the geometric mean ratio with 95% CI for these ratios. Geometric mean (GEM) ratios represent relative differences (RD) as a percentage. Comparisons between the first cycle and last cycle were made for intratumoral concentrations, healthy esophageal mucosa tissue concentrations, and plasma AUC₀–48h using paired t-test. The intratumoral paclitaxel concentration was also compared with normal appearing esophageal tissue concentration during cycle 1 and the last cycle using the same test. The intratumoral concentrations observed in adenocarcinoma and squamous cell carcinoma were compared to each other using an independent t-test. To compare the ABC efflux transporter expression between the types of tissues and the cycles of chemotherapy the Wilcoxon signed-rank test was used. The
In total 15 patients were included, of whom 14 patients were evaluable. One patient withdrew informed consent after the first cycle of chemotherapy and gastroscopy within the study. Table 1 displays all baseline characteristics. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**RESULTS**

**Patient Characteristics**

In total 15 patients were included, of whom 14 patients were evaluable. One patient withdrew informed consent after the first cycle of chemotherapy and gastroscopy within the study. Table 1 displays all baseline characteristics. The correlation between systemic pharmacokinetics and tissue paclitaxel concentrations was estimated using Pearson’s correlation coefficients. The correlation between immunohistochemical expression and intratumoral paclitaxel concentrations was estimated using Spearman’s correlation coefficient given the ordinal immunohistochemical data used for this analysis.

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

TABLE 1 | Baseline characteristics.

| Characteristics | No. (%) |
|-----------------|---------|
| **Gender**      |         |
| Male            | 13 (93%)|
| Female          | 1 (7%)  |
| **Age (years)** |         |
| Median [IQR]    | 27.7 [25.3 – 33.8] |
| **BMI (kg/m²)** |         |
| Median [IQR]    | 27.7 [25.3 – 33.8] |
| **BSA (m²)**    |         |
| Median [IQR]    | 2.1 [1.85 – 2.20] |
| **Tumor location** |     |
| Mid esophageal tumora | 3 (21%) |
| Distal esophageal tumorb | 11 (79%) |
| **Histological subtype** |   |
| Adenocarcinoma   | 9 (64%) |
| Squamous cell carcinoma | 5 (36%) |
| **Treatment regimen** | |
| CTx             | 3 (21%) |
| dCRT            | 2 (14%) |
| nCRT            | 9 (64%) |
| **Characteristic** | No. (%) |
| **Immunohistochemical expression and intratumoral paclitaxel** request. are available from the corresponding author on reasonable this analysis.

aMid esophageal tumor is defined as tumor located at 24–32 cm from the teeth row.
bDistal esophageal tumor is located between 32 and 40 cm from the teeth row.

Abbreviations: BMI, body mass index; BSA, body surface area; CTx, chemotherapy; dCRT, definitive chemoradiotherapy; IQR, interquartile range; nCRT, neoadjuvant chemoradiotherapy; No., number of cases.

The tumors were predominantly located in the distal esophagus (79%). Nine out of the 14 patients (67%) were diagnosed with an adenocarcinoma, while the remaining patients were diagnosed with a squamous cell carcinoma of the esophagus. The majority of the included patients were male (93%) and were treated with paclitaxel (50 mg/m²), carboplatin (AUC2) and concomitant radiotherapy (78%).

**Tissue Biopsies**

The time between start of infusion and biopsies was comparable between cycle 1 (median 4.8 h; IQR 4.3–5.1 h) and the last cycle (median 4.3 h; IQR 3.7–4.8 h). A summary of the location of the analyzed biopsies and pathological assessments is presented in Supplementary Table S1. The amount of tissue obtained during the biopsy procedure differed between normal esophageal mucosa and tumor tissue, and between cycles (cycle 1 tumor tissue: median 6.4 mg (IQR: 4.3–7.9 mg); cycle 1 esophageal mucosa: median 2.9 mg (IQR: 2.5–4.3 mg); last cycle tumor tissue: median 5.6 mg (IQR: 2.0–7.1 mg); last cycle esophageal mucosa: median 2.0 mg (IQR: 0.99–2.3 mg)). All biopsies of the tumor at the first cycle contained cancer cells (median cancer cell percentage 60%; IQR 30–85%). Biopsies of normally appearing esophageal mucosa during cycle 1 nonetheless contained tumor cells in two patients: subject 4 (20% tumor cells) and subject 15 (30% tumor cells). Of the tumor biopsies taken at the last treatment cycle, only the biopsies of six patients (43%) contained tumor cells, which is probably a positive result of the treatment. Of these six biopsies containing tumor cells, 5 samples contained maximum 10% tumor cells and one sample contained 80% tumor cells. The esophageal mucosa samples taken at the last cycle were all tumor cell negative, except one which contained 1% tumor cells. Necrosis was present in a minority of the biopsies, i.e., in 5 tumor samples and 1 normal mucosal sample during cycle 1 and in 4 tumor samples and 3 normal mucosal samples during the last cycle. In patients treated with concomitant radiotherapy, tumor samples showed limited necrosis percentages but instead showed active inflammation or ulceration.

**Tissue Pharmacokinetics**

Paclitaxel could be measured in all biopsy samples. One sample (esophageal mucosa cycle 5; subject 12) was excluded from all analyses due to a low amount of tissue (i.e., 0.04 mg) resulting in an unreliable quantification of the paclitaxel concentration. No statistical analyses were performed involving the tumor samples taken during the last cycle given the low amount of tumor cells observed in these biopsies. During the first cycle, a trend towards a higher intratumoral paclitaxel concentration was seen compared to the esophageal mucosa paclitaxel concentration (RD: 43.44%; 95% CI: −2.60–111.22%; p = 0.07; Table 2) (excluding Barrett’s esophagus biopsies; RD: 58%; 95% CI: 3–145%; p = 0.04). The GEM paclitaxel concentration in normal esophageal mucosa during the first cycle was 2.03 ng/mg (95% CI: 1.38–2.98 ng/mg) while the intratumoral GEM paclitaxel concentration was 2.91 ng/mg (95% CI: 2.22–3.83 ng/mg). The intratumoral paclitaxel concentration in adenocarcinoma samples was not significantly different from the concentrations measured in squamous cell carcinoma samples during the first cycle (RD: −11%; 95% CI: −53–70%; p = 0.70; Table 2). The paclitaxel concentration in esophageal mucosa during the last cycle of chemotherapy (GEM) was 1.89 ng/mg (95% CI: 1.38–2.85 ng/mg)) was not significantly different from the concentration measured during the first cycle in esophageal mucosa (RD: −10%; 95% CI: −47–53%; p = 0.68; Table 2).

**Immunohistochemical Staining**

A summary of all immunohistochemical scores per biopsy is presented in Table 3. Figure 1 depicts the H&E staining and the ABCB1 staining of a general representable biopsy of an adenocarcinoma (Figures 1A,B), squamous cell carcinoma (Figures 1C,D), healthy esophageal mucosa tissue (Figures
TABLE 2 | Comparisons of tissue pharmacokinetics.

| Tissue pharmacokinetics                                      | Relative difference | 95% confidence interval | p-value |
|--------------------------------------------------------------|---------------------|-------------------------|---------|
| Esophageal PTX last cycle vs. esophageal PTX first cycle     | −10%                | −47% to 53%             | 0.68    |
| Tumoral PTX first cycle vs. esophageal PTX first cycle       | 43%                 | −3% to 111%             | 0.07    |
| Adenocarcinoma PTX first cycle vs squamous cell carcinoma PTX first cycle | −11%                | −53% to 70%             | 0.70    |

DISCUSSION

In this study, we demonstrated that ABCB1 efflux transporter expression is significantly higher in adenocarcinoma of the esophagus compared to squamous cell carcinoma of the esophagus. Moreover, the expression of ABCB1 by esophageal carcinomas is higher compared to normal-appearing esophageal mucosa. We could not demonstrate an alteration of intratumoral paclitaxel as first sign of resistance due to the low tumor cell percentage in the second biopsies. Nevertheless, we may have (partly) explained the effectivity of this taxane in esophageal cancer since the paclitaxel concentration in non-tumoral esophageal mucosa is lower than in tumor tissue, and a strong correlation between plasma pharmacokinetics and intratumoral paclitaxel concentration was seen.

We have tried to identify pharmacokinetic mechanisms of resistance to paclitaxel in esophageal cancer. A major factor contributing to the occurrence of paclitaxel resistance in solid tumors is overexpression of ABC efflux transporters, which could potentially lower the intratumoral drug concentration (Gottesman et al., 2002; Huisman et al., 2005; Barbuti and Chen, 2015). Previous studies have reported expression of ABCB1 in adenocarcinoma of the esophagus, as well as in squamous cell carcinoma, while no expression of ABCB1 was described in esophageal mucosa (Atlas;Vrana et al., 2018). In line with these results, we have demonstrated that ABCB1 expression was higher in esophageal carcinoma than in normal esophageal mucosa. However, we have also demonstrated a significantly higher expression of ABCB1 in adenocarcinoma than in squamous cell carcinoma of the esophagus. Interestingly, in the CROSS trial a significantly higher complete response rate in patients with squamous cell carcinoma of the esophagus than in those with esophageal adenocarcinoma was found (van Hagen et al., 2012). Further, in the long-term data of the CROSS trial also a clinically relevant difference (adenocarcinoma: median overall survival of 43 months versus squamous cell carcinoma: 82 months median overall survival) between the two histological types seems to exist (Shapiro et al., 2015). Therefore, it could be speculated that ABCB1 expression might have contributed to the differences in complete response rate and median survival between the two histological types. Several other studies investigating different regimens of repeated preoperative chemotherapy and radiotherapy in esophageal carcinoma could not identify a survival difference between those two histological subtypes (Cooper et al., 1999; Reynolds et al., 2007; Xi et al., 2017). This difference could possibly be explained by the fact that those studies administered cisplatin and fluoropyrimidines as
### TABLE 3 | Immunohistochemical score of ABCB1 per biopsy.

| Subject | Cycle  | Tumor | Percentage positive cells | Score positive cells | Intensity score | IRS score | Percentage positive cells | Score positive cells | Intensity score | IRS score |
|---------|--------|-------|---------------------------|---------------------|----------------|-----------|---------------------------|---------------------|----------------|-----------|
| 1       | Last cycle | no tumor | 0 | no tumor | NA | 0% | 0 | 0 | 0 |
| 2       | First cycle | 100% | 4 | 3 | 12 | 0% | 0 | 0 | 0 |
| 3       | First cycle | 40% | 2 | 3 | 6 | 0% | 0 | 0 | 0 |
| 4       | First cycle | 40% | 2 | 3 | 6 | 0% | 0 | 0 | 0 |
| 5       | First cycle | no tumor | 0 | no tumor | NA | 0% | 0 | 0 | 0 |
| 6       | First cycle | 5% | 1 | 3 | 3 | 1% | 1 | 3 | 3 |
| 7       | First cycle | no tumor | 0 | no tumor | NA | 0% | 0 | 0 | 0 |
| 8       | First cycle | 100% | 4 | 3 | 12 | 1% | 1 | 3 | 3 |
| 9       | First cycle | 100% | 4 | 3 | 12 | 0% | 0 | 0 | 0 |
| 10      | Last cycle | 100% | 4 | 3 | 12 | 0% | 0 | 0 | 0 |
| 11      | First cycle | 5% | 1 | 1 | 1 | 0% | 0 | 0 | 0 |
| 12      | Last cycle | no tumor | 0 | no tumor | NA | 0% | 0 | 0 | 0 |
| 13      | First cycle | 100% | 4 | 3 | 12 | no tissue | no tissue | no tissue | NA |
| 14      | Last cycle | 100% | 4 | 3 | 12 | 0% | 0 | 0 | 0 |
| 15      | First cycle | 100% | 4 | 3 | 12 | 0% | 0 | 0 | 0 |
| 16      | Last cycle | 100% | 4 | 3 | 12 | 0% | 0 | 0 | 0 |

The IRS (immunoreactive score) indicates different categories of ABCB1 expression (i.e., IRS 0–1 = negative for ABCB1; IRS 2–3 = mild ABCB1 expression; IRS 4–8 = moderate ABCB1 expression; IRS 9–12 = strong ABCB1 expression).
FIGURE 1 | Haematoxylin and eosin (H&E) staining and immunohistochemical staining of ABCB1 in different types of investigated tissue. (A) H&E staining of adenocarcinoma (B) ABCB1 immunohistochemical staining of adenocarcinoma (C) H&E staining of squamous cell carcinoma (D) ABCB1 immunohistochemical staining of squamous cell carcinoma (E) H&E staining of healthy esophageal mucosa and Barrett esophagus (F) ABCB1 immunohistochemical staining of healthy esophageal mucosa and Barrett esophagus.

FIGURE 2 | The correlation between intratumoral pharmacokinetics and plasma pharmacokinetics of paclitaxel. (A) Intratumoral paclitaxel concentration and AUC_{0-48 h}. (B) Intratumoral paclitaxel concentration and concentration at 4 h after start infusion.
that inhibition of this ABC ef
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esophageal mucosa does not express ABCB1, it is not expected
treatment with paclitaxel and carboplatin. Since normal
esophageal mucosa (EMBL-EBI Expression Atlas, 2021).
increased chemotherapeutical exposure in the healthy
in patients (Choi and Yu, 2014). Furthermore, it is always
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exposure by inhibition of ABCB1 expression might enhance
Tiwari et al., 2013). Increasing the intratumoral paclitaxel
substantial part of patients who do not benefit from the
treatment with paclitaxel and carboplatin. Since normal
esophageal mucosa does not express ABCB1, it is not expected
that inhibition of this ABC ef
 transporter results in an
increased chemotherapeutical exposure in the healthy
esophageal mucosa (EMBL-EBI Expression Atlas, 2021).
 Nevertheless, previous research demonstrated that the use
of MDRT (multidrug resistance transporters) inhibitors are
complicated by several factors (Choi and Yu, 2014). The first
generation of these inhibitors are characterized by the high doses
needed with only limited efficacy, the severe toxicity profile of
those compounds and the pharmacokinetic effects on other drugs
(Choi and Yu, 2014). Since these drugs affect drug transporters they
have an (potentially negative) effect on the absorption,
distribution, metabolism and elimination of others drugs used
in patients (Choi and Yu, 2014). Furthermore, it is always
important to realize that these transporters are also expressed
at other sites than tumors. ABCB1 transporters are also expressed
by liver tissue and kidney tissue which could increase paclitaxel
related toxicity in those organs which is undesirable given their
essential function (EMBL-EBI Expression Atlas, 2021). Newer
generations of MDRT inhibitors are characterized by milder
toxicity profiles and reduced effects on the overall
pharmacokinetics properties and therefore also the pharmacokinetics of other drugs (Choi and Yu, 2014). Nonetheless, the efficacy of these newer generation of MDRT inhibitors remained also limited which might be caused by heterogeneity of the tumor cells regarding ABCB1 expression, drug penetration, and other simultaneous existing resistance mechanisms (Choi and Yu, 2014).

Contrary to the aforementioned expected influence of ABCB1 expression on tissue paclitaxel exposure, the intratumoral paclitaxel concentration is higher than the paclitaxel concentration in esophageal mucosa despite the higher ABCB1 expression in tumor tissue. One of the factors that might explain the discrepancy between the expectations and the observed results could be tumor vessel permeability. The permeability of vessels in the tumor is higher compared to healthy esophageal tissue that could make it more easily for paclitaxel to distribute into the tumor tissue (Pasqualini et al., 2002). The fact that we identified a strong correlation between systemic paclitaxel pharmacokinetics and intratumoral pharmacokinetics could also point to a high vessel permeability in the tumor. Moreover, in line with our findings, it was previously
demonstrated that the intratumoral cisplatin concentration in
tumor tissue of patients diagnosed with esophagus carcinoma and
treated with cisplatin and 5-fluorouracil (5-FU) was higher
compared to the concentration in healthy esophagus tissue
(Troger et al., 1991). Increased permeability of tumor tissue
may also be induced by fractionated radiotherapy (Debbage
et al., 2000; Ng et al., 2007). In line with the described effects
of radiotherapy, the intratumoral doxorubicin distribution was
improved by radiotherapy (Potiron et al., 2019).

Alterations over time in ABCB1 expression or
intratumoral paclitaxel concentrations might also be a first
sign of resistance of the tumor. Nonetheless, we could not
identify an alteration in ABCB1 expression over time. This
may be the result of the relatively short treatment period in
our study. In addition, we used a low chemotherapeutic dose. In
contrast, Di Nicolantonio et al. did observe a significant
increase in mRNA levels of ABCB1 in paired samples of
adenocarcinoma of the esophagus after chemotherapy in an
in vitro experiment and therefore may not be concordant
with our clinical study results (Di Nicolantonio et al., 2005).
Moreover, Langer et al. also reported no alterations in ABCB1
expression after neoadjuvant chemotherapy in their clinical
study (Langer et al., 2007).

A comparison between the intratumoral paclitaxel
concentration during the first cycle and last cycle was
hampered by a low amount of tumor cells observed in tumor
biopsies taken during the last cycle. Previous studies
demonstrated that up to 28% of the patients who undergo
chemoradiotherapy have a complete pathological response
after completion of their treatment (van Hagen et al., 2012;
Shapiro et al., 2014). Therefore, it is most likely that the low
amount of tumor cells observed in the biopsies taken during the
last cycle is an effect of the chemoradiotherapy treatment. Due to
this low tumor cell percentage in the tumor biopsies it could be
doubted if the paclitaxel concentrations measured represents the
intratumoral paclitaxel concentration. Given that biopsies are
homogenized before paclitaxel quantification, it is likely that the
paclitaxel concentration measured represents the concentration
inside the most dominant type of tissue, which is probably non
tumorous tissue in the intended tumor biopsies of the last cycle.
Therefore, we could not investigate the alteration in intratumoral
paclitaxel concentrations over time which could be a sign of
chemotherapy resistance.

Previously, it has also been attempted to investigate the
intratumoral paclitaxel pharmacokinetics via several
mathematical models which predict the distribution of the
drug inside the tumor (Popilski et al., 2015). However, these models have limited accuracy probably due to
simplification of the multiple factors involved in intratumoral
drug distribution and can therefore not replace tumor biopsies for
intratumoral pharmacokinetic analysis (Popilski and Stepensky,
2015). However, bioanalytical methods should be further
improved so that even if a low amount of tumor tissue has
been obtained, the intratumoral paclitaxel could be accurately
measured without the influence of paclitaxel in the surrounding
tissue on the measured intratumoral paclitaxel concentration.
Matrix-assisted laser desorption/ionization (MALDI) mass
spectrometry might be a tool to achieve such an analytical improvement.

In conclusion, we found a significantly higher ABCB1 expression in esophageal adenocarcinomas than in squamous cell carcinomas, which might be causally related to a better treatment effectiveness of paclitaxel in the latter. Resistance by reduced intratumoral paclitaxel concentrations could not be demonstrated because of the low tumor percentage at the last cycle of paclitaxel. Further research investigating the ABCB1 expression in esophageal carcinoma and esophageal mucosa tissue is warranted to elucidate the relationship between response and ABCB1 status.

**DATA AVAILABILITY STATEMENT**

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

**ETHICS STATEMENT**

The studies involving human participants were reviewed and approved by Medical Ethics Committee of the Erasmus MC. The patients/participants provided their written informed consent to participate in this study.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.759146/full#supplementary-material
