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The Molecular and Clinical Impact of Atorvastatin Exposure on Paclitaxel Neurotoxicity in Sensory Neurons and Cancer Patients

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ABSTRACT

Recent evidence suggests that atorvastatin exacerbates paclitaxel neurotoxicity via P-glycoprotein inhibition. We used a translational approach to investigate if atorvastatin or simvastatin exacerbates (i) paclitaxel neurotoxicity in human sensory neurons and (ii) paclitaxel-induced peripheral neuropathy (PIPN) in cancer patients. Paclitaxel neurotoxicity was assessed by quantifying neuronal networks of human induced pluripotent stem cell-derived sensory neurons (iPSC-SNs) with and without atorvastatin or simvastatin exposure. We estimated the odds ratio (OR) of early paclitaxel discontinuation due to PIPN in a nationwide cohort of paclitaxel-treated women (2014–2018), comparing atorvastatin users to simvastatin users and nonusers of statins. Only the highest concentration of atorvastatin (100 nM) significantly exacerbated paclitaxel neurotoxicity in iPSC-SNs ($p < 0.05$). Among 576 paclitaxel-treated women, atorvastatin use was not significantly associated with early paclitaxel discontinuation due to PIPN, with adjusted ORs of 0.80 [95% confidence interval (CI) 0.34–1.88] compared with simvastatin, and 1.24 [95% CI 0.44–3.53] compared with nonuse. Supplementary analyses showed varying but statistically nonsignificant results. Our in vitro findings suggest that atorvastatin, not simvastatin, significantly worsens paclitaxel neurotoxicity. However, no link was found between atorvastatin use and early paclitaxel discontinuation due to PIPN. Larger, well-designed studies are required to clarify the discrepancy between in vitro and clinical data and the inconsistencies with previous clinical evidence.

1 | Introduction

A common adverse effect of many cancer drugs, especially taxanes [1], is chemotherapy-induced peripheral neuropathy (CIPN) [2]. CIPN is primarily caused by damage to sensory neurons of the peripheral nervous system, and its clinical manifestations include neuropathic pain and sensory disturbances, such as numbness and paraesthesia in hands and feet. Although paclitaxel is essential in the treatment of solid tumours like breast and ovarian cancers, it leads to CIPN in a large proportion of patients [3–5]. Because CIPN cannot be treated effectively with currently available medications [5], it can develop into a chronic condition [6] with significant impact on quality of life [7]. Consequently, severe CIPN is managed by treatment adjustments, such as dose reduction, treatment delay or treatment discontinuation, which can reduce therapeutic efficacy [8] and influence the prognosis of cancer patients.

Summary

- Paclitaxel frequently causes peripheral sensory neuropathy, resulting in neuropathic pain and sensory disturbances in patients receiving treatment for cancer.
- Our limited understanding of pathogenesis and risk factors of paclitaxel-induced peripheral neuropathy (PIPN) impedes the development of preventative and therapeutic strategies.
- Recent evidence suggests that atorvastatin exacerbates paclitaxel neurotoxicity via P-glycoprotein inhibition.
- In our study, only the highest concentration of atorvastatin exacerbated paclitaxel neurotoxicity in vitro, but we found no association between atorvastatin use and early paclitaxel discontinuation due to PIPN.
- Future studies using objective measures of different PIPN phenotypes are required to clarify this discordance between in vitro and clinical data, as well as the inconsistencies with previous evidence.

An improved understanding of the pathogenesis and risk factors of paclitaxel-induced peripheral neuropathy (PIPN) is essential for the development of preventative and therapeutic strategies. Commonly prescribed drugs such as statins and beta blockers have been associated with an increased risk of PIPN [9, 10]. Importantly, paclitaxel is a substrate for the efflux transporter P-glycoprotein (P-gp), which is expressed in human dorsal root ganglia [10]. P-gp is responsible for limiting the accumulation of toxic substances within cells and as such, concomitantly administered P-gp inhibitors may exacerbate paclitaxel neurotoxicity [10]. Notably, the mRNA expression of P-gp has been observed in the context of neuronal exposure to substances like paclitaxel, suggesting its role in drug transport [11]. A potential inhibitor of P-gp is atorvastatin [10]. In a clinical setting, users of atorvastatin have been found to be at greater risk of dose-adjustments of paclitaxel treatment due to sensory neuropathy compared with users of simvastatin [10]. However, before clinicians can be advised to switch paclitaxel-treated patients from atorvastatin to another statin, further clinical confirmation is required. Here, a translational approach was used to determine if atorvastatin exacerbates paclitaxel neurotoxicity in a human cell model and in patients with gynaecologic cancer or breast cancer.

2 | Methods and Materials

We (i) determined if paclitaxel neurotoxicity is exacerbated by atorvastatin or simvastatin in iPSC-derived sensory neurons (iPSC-SNs) and (ii) evaluated the risk of paclitaxel treatment adjustment due to PIPN among atorvastatin users, simvastatin users and nonusers of statins by accessing registry data on paclitaxel use and manually reviewing medical records. The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies [12].

Cell Model

2.1 | Sensory Neuron Differentiation

Sensory neurons were differentiated from a healthy iPSC donor (A18945, ThermoFisher, Roskilde, Denmark, hpscreg.eu/cell-line/TMOi001-A), as described in detail elsewhere [13]. Briefly, iPSCs were maintained in mTeSR1 medium (85850, StemCell Technologies, Vancouver, BC, Canada) on Matrigel (354277, Corning, NY, USA) with daily medium change. Upon 70%–80% confluency, iPSCs were clump-passaged using Accutase (00455556, ThermoFisher). The differentiation was performed using 5 small molecule inhibitors for 12 days followed by maturation with four neurotrophic growth factors and ascorbic acid (A4403, Sigma-Aldrich, Saint Louis, MO, USA) for 23–33 days. The small molecule inhibitors LDN193189 (S7507), SB431542 (S1067), CHIR99021 (S1263), SU5402 (S7667) and DAPT (S2215) were purchased from Selleck Chemicals (Houston, TX, USA). The neurotrophic growth factors NGF-β (450-01), BDNF (450-02), GDNF (450-10) and NT-3 (450-03) were obtained from Peprotech (Cranbury, NJ, USA). On day 12, immature sensory neurons were seeded as single cells at a density of 150000 cells/cm^2 on culture plates coated with poly-L-ornithine hydrobromide (20 μg/mL, P3655, Sigma-Aldrich), laminin (10 μg/mL, 23017015, ThermoFisher) and fibronectin (2 μg/mL, F1141, Sigma-Aldrich). On day 14, nonneuronal cells were removed using Mitomycin-C (1 μg/mL, M4287, Sigma-Aldrich) for 2 h. On day 16, all medium was replaced and afterwards, 50% of the medium was changed every 3–4 days. The mature sensory neurons were used for experiments between days 35 and 45.

2.2 | Compound Preparations and Considerations

Paclitaxel (T7402, Sigma-Aldrich, Søborg, Denmark), atorvastatin (PZ0001, Sigma-Aldrich) and simvastatin (S6196, Sigma-Aldrich) were dissolved and serially diluted in dimethyl sulfoxide (DMSO, D8418, Sigma-Aldrich). The final concentration of DMSO was maintained at 0.2% for all conditions, and the same concentration of DMSO was included as a vehicle control. The concentrations of paclitaxel and statins were ensured to be clinically relevant. We selected the concentrations based on their maximum observed plasma concentration after commonly used dosing regimens, while accounting for their high plasma protein binding and interindividual variability in clinical pharmacokinetic profiles (Table S1) [14, 15]. iPSC-SNs seeded in 24-well plates were treated with 0.1-, 1.0- and 10-μM paclitaxel. We used atorvastatin concentrations at 10 and 100 nM and simvastatin concentrations at 5 and 50 nM. iPSC-SNs were pretreated with statins for 1 h to allow binding to drug transporters. After pretreatment, iPSC-SNs were exposed to vehicle, or paclitaxel with and without concomitant exposure to statins for 48 h.

2.3 | Immunolabelling and Neurotoxicity Assessment

Following exposure to paclitaxel and statins, iPSC-SNs were fixed with 4% paraformaldehyde for 10 min. Specifically, 200 μL of medium was removed from each well and 16% paraformaldehyde (28906, ThermoFisher) was diluted 1:4 into the medium to minimize cell detachment. After two washing

steps with phosphate buffered saline containing Ca^{2+} and Mg^{2+} (PBS+, D8662, Sigma-Aldrich), iPSC-SNs were permeabilized with 0.25% Triton X-100 for 15 min. Unspecific binding was subsequently blocked using 1% bovine serum albumin (A9418, Sigma-Aldrich) for 1 h. iPSC-SNs were labelled with peripherin (1:200, SC-377093, Santa Cruz) overnight at 4°C. The following day, iPSC-SNs were labelled with Alexa Fluor 488-conjugated anti-mouse (1:400, A11001, ThermoFisher) for 1 h at room temperature. Importantly, all medium was only removed at the final washing step after fixation and before addition of primary and secondary antibodies. For all other steps, 10% of the washing or blocking buffer remained in each well. Labelled cells were stored in PBS+ until image acquisition. A template was created in CellReporterXpress (Molecular Devices, San Jose, CA, USA) to systematically acquire three images per well for all plates. Images were acquired using ImageXpress Pico Automated Imaging System with the 10× objective (Molecular Devices, San Jose, CA, USA). Several in vitro studies have demonstrated that axons, rather than cell bodies, are sensitive to paclitaxel toxicity [16]. As such, we assessed neurotoxicity by measuring the number of axons emanating from each ganglion using Sholl analysis (ImageJ software 2.0.0). The analysis was blinded to the person performing Sholl analysis to ensure no bias. All images were converted to 8-bit, and a threshold of 38 was applied. The centre of the ganglion was defined using the straight-line tool, and Sholl analysis was performed with an appropriate end-radius for all ganglia in each image. The end-radius was adjusted manually according to the size of the ganglia. Figure S1 shows threshold adjustment and Sholl analysis. The experiment was repeated in iPSC-SNs from three individual differentiations.

Health Registers and Medical Records

2.4 | Study Cohort

To ensure a high retrieval rate of medical records and comparability between atorvastatin and simvastatin users, we restricted the study period to 1 January 2014 to 31 December 2018, a period during which atorvastatin and simvastatin were used equally in Denmark [17]. During this period, we identified all women with at least one paclitaxel procedure code recorded in the Danish National Patient Registry [18]. The population was further limited to people with a valid civil registration number in the Civil Registration System [19]. Finally, we restricted to women diagnosed with gynaecologic cancer (International Classification of Diseases, 10^{th} revision [ICD10], C53-57) or breast cancer (C50) within 1 year prior to starting paclitaxel treatment (Table S2). For this cohort, we identified all filled statin prescriptions, defined by Anatomical Therapeutic Chemical (ATC) code C10AA*, from the Danish National Prescription Registry [20] and identified women who, prior to starting paclitaxel treatment, had redeemed atorvastatin, simvastatin or neither of these drugs. Statin users were classified as such by having redeemed a prescription on either atorvastatin or simvastatin within 120 days before starting paclitaxel treatment. In Denmark, when medical treatment is stable, patients normally receive a 3-month supply of medication, which typically consists of 100 tablets for medication taken once daily [21]. And as such, we chose an exposure period of 120 days. For users of both statins, the latest exposure was used. For each atorvastatin user, we aimed to identify one simvastatin user and one nonuser, matching on age (in 5-year bands) and hospital department. Due to data limitations, the final count of simvastatin users was 315 instead of 350.

The women were treated in oncology departments at 11 hospitals across all five regions of Denmark: Rigshospitalet, Herlev and Gentofte Hospital, North Zealand Hospital, Region Zealand Hospital Service, Odense University Hospital, Hospital Sønderjylland, Hospital South West Jutland, Vejle Hospital, Aarhus University Hospital, Hospital Unit West and Aalborg University Hospital.

The civil registration numbers linked data from health registers and data from medical records. Both patient characteristics and information on paclitaxel treatment, including treatment adjustments, were collected from medical records. The review was blinded for medication status, including the women's statin exposure status. Only notes entered by medical doctors or nurses within the study period were disclosed to the reviewer. All study data were collected and stored in REDCap [22] hosted by OPEN (Open Patient data Explorative Network).

2.5 | Patient Characteristics

The patient characteristics collected included height, weight, cancer diagnosis, cancer stage and potential confounders such as Eastern Cooperative Oncology Group (ECOG) performance status, alcohol, smoking and neurological comorbidity. Here, 'neurological comorbidity' included neurological disease (e.g., multiple sclerosis and neurofibromatosis) and preexisting sensory disturbances. At baseline, sensory disturbances could be due to conditions like diabetes, infections, nerve compression syndromes or traumatic injuries. Lastly, paclitaxel dose (mg) and treatment schedule were collected.

Women who, at any time prior to starting paclitaxel treatment, had redeemed a prescription for a glucose-lowering drug (defined by ATC code A10*, i.e., drugs used in diabetes) were categorized as having diabetes.

2.6 | Treatment Adjustment

For each paclitaxel treatment adjustment (i.e., reduction and discontinuation), it was noted whether it was due to toxicity or another reason. The toxicities recorded included: PIPN, haematological toxicity and febrile neutropenia. The other reasons recorded included: poor health, allergic reaction, wishes of the patient or limited treatment response. The reason 'poor health' included factors such as old age, fatigue/weariness and common gastrointestinal symptoms (e.g., diarrhoea and nausea). Some adjustments were due to both toxicity and another reason.

Because standard paclitaxel treatment is six cycles for gynaecologic cancer patients and ≤ 12 cycles for most breast cancer patients, we did not follow patients beyond the 12^{th} cycle. This only restricted

the data capture for those breast cancer patients who had distant metastases and therefore received > 12 paclitaxel treatments.

The composite endpoint 'paclitaxel treatment modification' encompassed all-cause dose reduction and treatment discontinuation, unless a specific cause was provided. A dose reduction was defined as receiving less than standard dose (i.e., 175 mg/m^2 once every 3 weeks for gynaecologic cancer and 80 mg/m^2 once every week for breast cancer). All breast cancer patients received paclitaxel as a monotherapy administered once weekly. All gynaecologic cancer patients received paclitaxel in combination with carboplatin every 3 weeks. A treatment discontinuation was defined as termination of paclitaxel prior to completing scheduled treatment, including cessation during infusion of paclitaxel.

Statistical Analysis

2.7 | Cell Data

Analysis of cell data was performed in R (version 4.0.2; R Statistical Foundation for Statistical Computing). Graph was created using the ggplot2 package.

Number of axons was calculated relative to the mean of the vehicle control for each individual differentiation. The mean of relative ratios for each condition was subsequently calculated for the three independent differentiations. Differences between relative ratios were tested for statistical significance using nonparametric Kruskal–Wallis test because the assumption of normality of observations could not be met with so few data points.

2.8 | Clinical Data

The collected clinical data were uploaded to a server hosted by the Danish Health Data Authority, and calculations were performed using STATA 17 (Stata-Corp, College Station, TX). To ensure data confidentiality, the number of patients below five was presented as '< 5'.

We used logistic regression to estimate odds ratios (ORs) and 95% confidence intervals (Cs) for the association between atorvastatin use and risk of early paclitaxel discontinuation due to PIPN, while adjusting for age, alcohol, smoking, neurological comorbidity and diabetes. We defined early discontinuation as treatment cessation before the fourth treatment cycle for

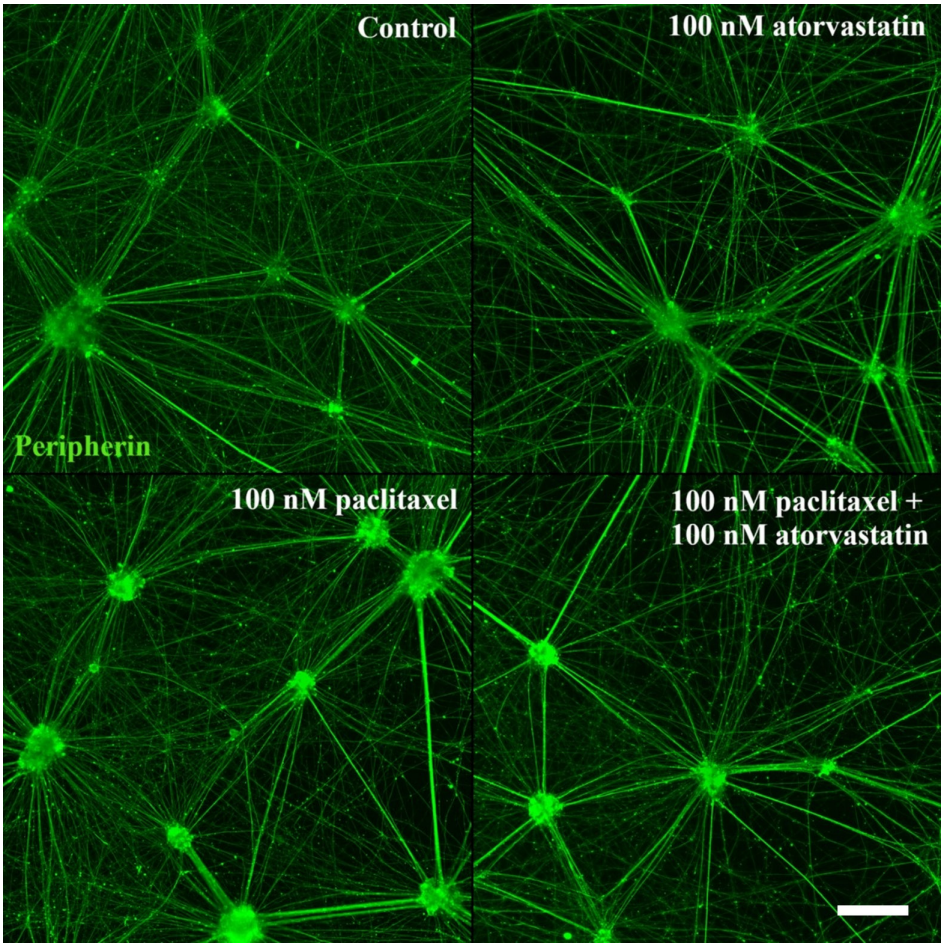

FIGURE 1 | Exposure to paclitaxel alone reduces the neuronal network of iPSC-derived sensory neurons, and this effect is exacerbated by concomitant exposure to atorvastatin. Atorvastatin alone also causes minimal neurotoxicity in iPSC-derived sensory neurons. Cells were treated with 100-nM paclitaxel for 48 h with and without concomitant treatment with 100-nM atorvastatin. The control was treated with vehicle (0.2% DMSO). Cells were labelled with peripherin, and images were acquired using ImageXpress Pico with the 10× objective. The end-radius was adjusted manually according to the size of the ganglia. Representative images are shown. Scale bar represents 200 μm.

gynaecologic cancer patients and before the seventh treatment cycle for breast cancer patients. As a supplementary analysis, the association was also evaluated for all recorded treatment cycles. In our primary analysis, atorvastatin users were compared with simvastatin users, and to put our primary analysis in perspective, users of atorvastatin and users of simvastatin were also compared with respect to all-cause paclitaxel discontinuation.

As secondary outcomes, users of atorvastatin and users of simvastatin were compared with respect to all-cause paclitaxel treatment modification, as well as paclitaxel treatment modification due to PIPN, haematological toxicity, poor health or allergic reaction, individually.

3 | Results

3.1 | Paclitaxel Neurotoxicity in iPSC-SNs

Atorvastatin exacerbated 0.1-, 1.0- and 10-μM paclitaxel neurotoxicity at the applied concentration of 100 nM (Figure 1, Figure 2; $p < 0.05$). Simvastatin, however, did not affect paclitaxel neurotoxicity in a statistically significant manner (Figure 2). Exposure to both statins alone did not significantly increase neurotoxicity in iPSC-SNs compared with vehicle (Figure 2).

3.2 | Study Cohort

We identified 8235 women who started paclitaxel treatment within 1 year of being diagnosed with either gynaecologic cancer or breast cancer during 2014–2018. Of these, 350 women (4.3%) were classified as using atorvastatin, and 315 matched simvastatin users and 350 matched nonusers were also eligible for inclusion in the final stage with review of medical records (Figure 3).

A total of 846 out of 1015 women were included in the review process due to exclusion at hospital-level; Hospital Unit West declined to participate in the study, and North Zealand Hospital was unable to retrieve medical records locally. During the review process, 270 women were excluded from the study due to the following reasons: received paclitaxel before 2014, received other chemotherapy less than 5 years before starting paclitaxel treatment, missing data for scheduled treatment, not meeting the inclusion criteria, including

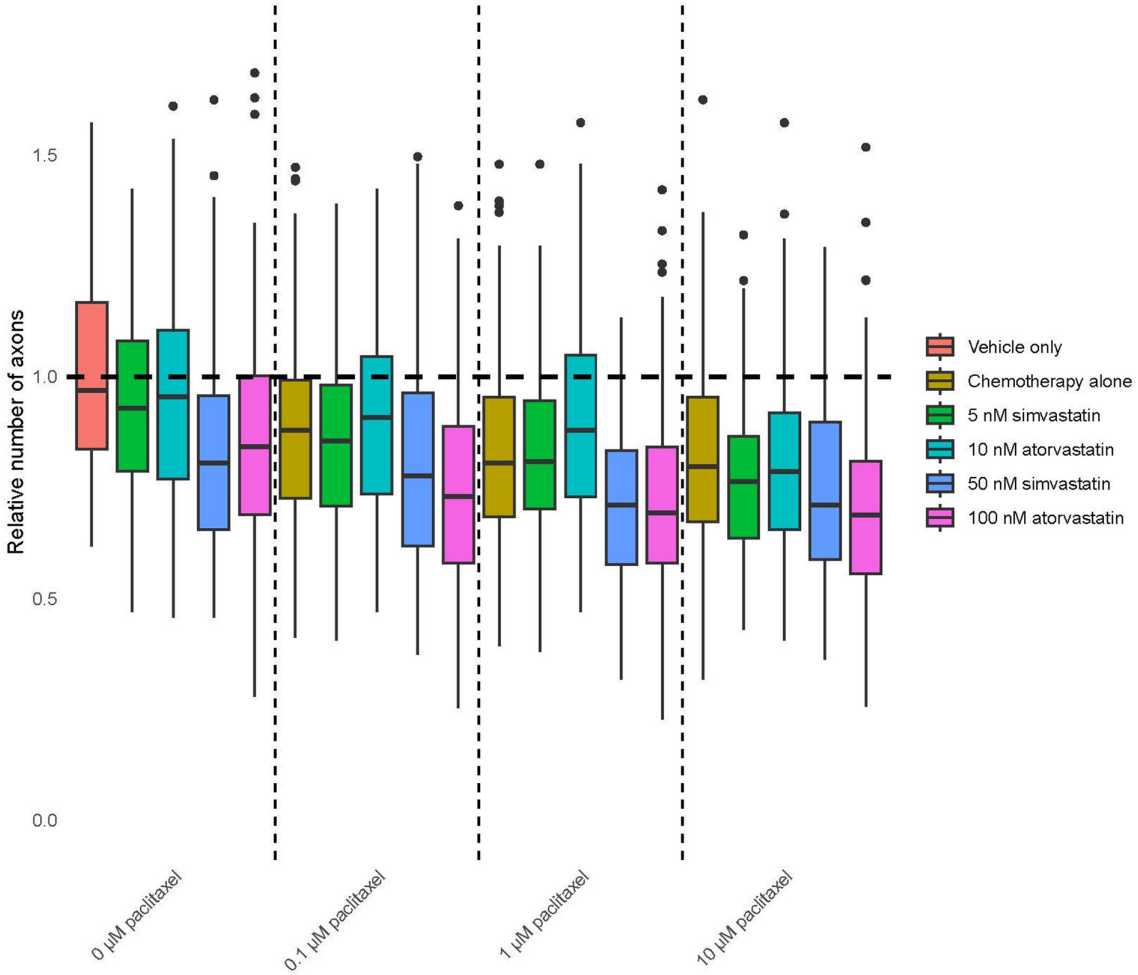

FIGURE 2 | Atorvastatin exacerbated paclitaxel neurotoxicity at the applied concentration of 100 nM ($p < 0.05$, nonparametric Kruskal–Wallis test). Simvastatin did not significantly exacerbate paclitaxel neurotoxicity at the applied concentrations of 5 and 50 nM. Both statins alone caused no neurotoxicity in iPSC-derived sensory neurons. The number of axons emanating from each ganglion was quantified using Sholl analysis, and three images were acquired for each condition from three independent differentiations.

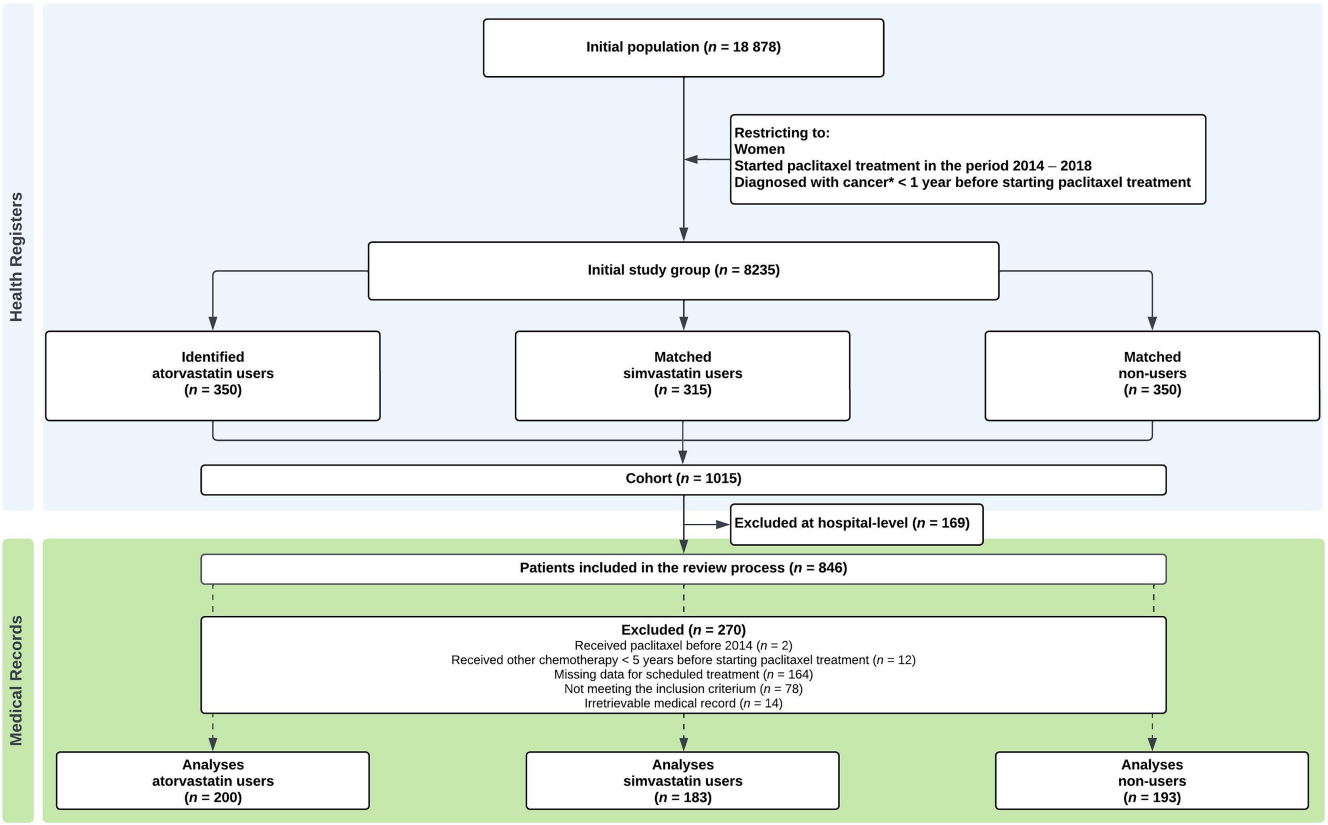

FIGURE 3 | CONSORT diagram. *Patients diagnosed with either gynaecologic cancer or breast cancer.

first-line treatment with paclitaxel and irretrievable medical record (Figure 3). All women diagnosed with cervical cancer were excluded because they received paclitaxel as minimum second-line treatment. The final cohort available for analysis included 200 atorvastatin users, 183 simvastatin users and 193 nonusers (Figure 3, Table 1).

3.3 | Paclitaxel Treatment Discontinuation

Among 576 paclitaxel-treated women, 131 discontinued paclitaxel treatment due to PIPN; 8 of the women had other concurrent causes. Specifically, early paclitaxel discontinuation due to PIPN occurred in 17 of 200 (8.5%) atorvastatin users; 14 of 183 (7.7%) simvastatin users; and 8 of 193 (4.1%) nonusers.

The risk of early paclitaxel discontinuation due to PIPN was not increased among atorvastatin users compared with simvastatin users (adjusted OR 0.80, 95% CI 0.34–1.88; Table 2). The corresponding adjusted OR for all recorded treatment cycles was 0.87 [95% CI 0.51–1.48]. The exclusion of patients with diabetes and patients with preexisting sensory disturbances primarily reduced the ORs related to PIPN, but the findings remained statistically nonsignificant (Tables S3, S5 and S6).

The risk of early paclitaxel discontinuation due to PIPN was increased among atorvastatin users compared with nonusers (adjusted OR 1.24, 95% CI 0.44–3.53; Table 2), although not statistically significantly. The corresponding adjusted OR for all recorded treatment cycles was 1.24 [95% CI 0.68–2.25].

When patients with breast cancer and patients with gynaecologic cancer were analysed separately with respect to statin use and risk of early paclitaxel discontinuation due to PIPN, the results remained statistically nonsignificant (Table S7).

3.4 | Paclitaxel Treatment Modification

In our secondary analysis, atorvastatin use was not associated with paclitaxel treatment modification due to PIPN with an adjusted OR of 0.71 [95% CI 0.44–1.15] (Table 3) and 1.22 [95% CI 0.72–2.06] (Table 3) when compared with simvastatin use and nonuse, respectively.

After excluding patients with diabetes from the analyses, users of atorvastatin were less than half as likely to experience paclitaxel treatment modification due to PIPN compared with simvastatin users (adjusted OR 0.47, 95% CI 0.26–0.82; Table S4).

4 | Discussion

In our study, the highest concentration of atorvastatin exacerbated paclitaxel neurotoxicity in vitro, but we found no association between atorvastatin use and early paclitaxel discontinuation due to PIPN. Some variation was observed when our clinical observations included all recorded treatment cycles, and the secondary outcomes were analysed; however, all associations remained statistically nonsignificant.

TABLE 1 | The cohort's baseline characteristics, mostly based on medical records; use of anti-diabetic drugs is register-based.

	Atorvastatin ($n=200$)	Simvastatin ($n=183$)	Nonuse ($n=193$)
Age (years, median, IQR)	65 (58–71)	67 (61–72)	67 (58–72)
Body surface area (m^2, median, IQR)	1.83 (1.70–1.97)	1.83 (1.72–1.99)	1.75 (1.64–1.92)
ECOG performance status, (%)			
0	155 (78%)	146 (80%)	145 (75%)
1	36 (18%)	23 (13%)	36 (19%)
2+	5 (2.5%)	8 (4.4%)	6 (3.1%)
Cancer diagnosis, (%)			
Breast cancer	131 (66%)	118 (64%)	131 (68%)
Uterine cancer	30 (15%)	21 (11%)	16 (8.3%)
Ovarian cancer	36 (18%)	43 (23%)	44 (23%)
Cancer of other and unspecified female genitals	$n<5$	$n<5$	$n<5$
Stage breast cancer, (%)			
Local	68 (34%)	57 (31%)	62 (32%)
Locally advanced	53 (27%)	49 (27%)	59 (31%)
Metastatic	($n<5$)	5 (2.7%)	5 (2.6%)
Stage gynaecologic cancer (FIGO), (%)			
I	6 (3.0%)	5 (2.7%)	($n<5$)
II	($n<5$)	($n<5$)	5 (2.6%)
III	32 (16%)	32 (17%)	23 (12%)
IV	24 (12%)	19 (10%)	25 (13%)
Alcohol, (%)	8 (4.0%)	10 (5.5%)	5 (2.6%)
Smoking, (%)	37 (19%)	36 (20%)	30 (16%)
Neurological comorbidity, (%)	30 (15%)	16 (8.7%)	15 (7.8%)
Preexisting sensory disturbances, (%)	24 (12%)	16 (8.7%)	14 (7.3%)
Prescription within ATC group AT10 (i.e., drugs used in diabetes), (%)	55 (28%)	45 (25%)	10 (5.2%)

Abbreviation: FIGO, Fédération Internationale de Gynécologie et d'Obstétrique (International Federation of Obstetrics and Gynecologic).

To our knowledge, the evidence for atorvastatin use as a risk factor for PIPN is limited to one study. Stage et al. [10] found that the risk of paclitaxel dose modification due to peripheral neuropathy significantly increased to 7.0-fold in patients treated with atorvastatin after adjusting for age, body surface area, tumour type, cancer stage, treatment schedule and previous chemotherapy. We found that the risk of peripheral neuropathy among users of atorvastatin increased to 1.2-fold compared with nonusers of statins. This difference might be caused by several factors. Firstly, the stronger association previously found might be due to residual confounding by diabetes or other potential risk factors for peripheral neuropathy, including smoking. Secondly, phenotyping of PIPN is complicated and challenging, even for prospective clinical studies, with the existing subjective methods. Currently, there is no objective tool to grade PIPN [23]. Thirdly, use of statins has in itself been linked to peripheral neuropathy [24, 25], although the evidence is highly inconsistent [26]. Lastly, using register data to identify statin exposure status might have misclassified some individuals, which in turn will have diluted the associations observed.

The major strength of this study is the translational approach that combines in vitro findings with clinical data. Through an interdisciplinary collaboration, we have highlighted that our in vitro findings involving statins and paclitaxel neurotoxicity cannot be detected clinically in our setting. We differentiated iPSCs into human sensory neurons to recapitulate the characteristic morphologic, transcriptional and functional properties of sensory neurons. Compared with SH-SY5Y-derived neurons that were previously used to study this drug–drug interaction (DDI), iPSC-SNs constitute multiple sensory neuron subtypes

TABLE 2 | Risk of paclitaxel discontinuation with use of atorvastatin compared with simvastatin and nonuse, respectively, and specified by cause.

Risk of paclitaxel discontinuation with use of atorvastatin compared with simvastatin					
Cause	**Atorvastatin ($n=200$)**	**Simvastatin ($n=183$)**	**Crude OR [95% CI]**	**Adjusted[a] OR [95% CI]**	***p*-value**
Any discontinuation (all)	83 (41.5%)	61 (33.3%)	1.42 (0.94–2.15)	1.03 (0.63–1.67)	0.917
Any discontinuation (early)	35 (17.5%)	23 (12.6%)	1.48 (0.84–2.61)	1.15 (0.59–2.25)	0.688
Neurotoxicity (all)	54 (27.0%)	42 (23.0%)	1.24 (0.78–1.98)	0.87 (0.51–1.48)	0.608
Neurotoxicity (early)	17 (8.5%)	14 (7.7%)	1.12 (0.54–2.34)	0.80 (0.34–1.88)	0.609
Haematological toxicity (all)	0 (0.0%)	$n<5$	—	—	—
Poor health (all)	22 (11.0%)	16 (8.7%)	1.29 (0.66–2.54)	0.76 (0.33–1.74)	0.519
Allergic reaction (early)	11 (5.5%)	$n<5$	3.49 (0.96–12.72)	4.02 (0.82–19.67)	0.086
Risk of paclitaxel discontinuation with use of atorvastatin compared with nonuse					
Cause	**Atorvastatin ($n=200$)**	**Nonuse ($n=193$)**	**Crude OR [95% CI]**	**Adjusted[a] OR [95% CI]**	***p*-value**
Any discontinuation (all)	83 (41.5%)	65 (33.7%)	1.40 (0.93–2.11)	1.01 (0.61–1.66)	0.977
Any discontinuation (early)	35 (17.5%)	26 (13.5%)	1.36 (0.79–2.36)	0.89 (0.46–1.73)	0.736
Neurotoxicity (all)	54 (27.0%)	35 (18.1%)	1.67 (1.03–2.70)	1.24 (0.68–2.25)	0.487
Neurotoxicity (early)	17 (8.5%)	8 (4.1%)	2.15 (0.90–5.10)	1.24 (0.44–3.53)	0.682
Haematological toxicity (all)	0 (0.0%)	5 (2.6%)	—	—	—
Poor health (all)	22 (11.0%)	16 (8.3%)	1.37 (0.69–2.69)	0.97 (0.41–2.29)	0.940
Allergic reaction (early)	11 (5.5%)	8 (4.1%)	1.35 (0.53–3.42)	1.07 (0.38–2.97)	0.902

[a]Adjusted for age, alcohol, smoking, neurological comorbidity and diabetes.

and express receptors involved in the perception of pain [13]. Furthermore, we included a large patient cohort involving major hospitals spread across all five regions in Denmark. We included an active comparator group (i.e., simvastatin users) to help establish the effects of atorvastatin, and by blinding the review process to statin exposure status, the risk of bias was reduced. Finally, by incorporating early paclitaxel discontinuation due to PIPN as a clinically relevant main endpoint, clearly documented in medical records, we avoided relying on often inadequate recording of PIPN occurrence and severity.

There are many practical challenges in obtaining reliable data on the incidence and extent of PIPN, both retrospectively and prospectively. As previously mentioned, objective measures of different PIPN phenotypes do not exist, and therefore, it is difficult for clinicians to reliably assess the extent of PIPN. Even though grading systems for PIPN exist, they have not been systematically incorporated in clinical practice and rely solely on patient or physician descriptions of symptoms. Consequently, it was not possible to correlate atorvastatin use directly to the degree of PIPN by reviewing medical records. We have recently shown that neurofilament light chain measured in serum (sNFL) is a useful and objective biomarker of PIPN in ovarian cancer patients [13] and as such, sNFL may serve as an tool for phenotyping PIPN in future clinical studies.

A limitation of our in vitro work is that we only focused on pharmacodynamic DDIs, so our results do not account for pharmacokinetic DDIs occurring in the liver. Although genome-wide association studies have found that genetic variants in *ABCB1* are associated with an increased risk of PIPN [27], in vitro studies show that clinically relevant concentrations of statins are not able to inhibit CYP2C8, the main drug-metabolizing enzyme of paclitaxel [28]. Nonetheless, a hepatic-specific interaction at clinically relevant concentrations cannot be entirely disregarded if P-gp is involved in hepatic and renal elimination of paclitaxel. Another limitation of our in vitro work is that we only assessed possible interactions between paclitaxel and the parent compounds of the statins. Therefore, the contribution of atorvastatin and simvastatin metabolites in exacerbating neurotoxicity during paclitaxel treatment was not assessed. Additionally, possible DDIs were assessed using a single iPSC donor with an unknown clinical background, and this might limit the generalizability of our in vitro results.

In conclusion, we found no evidence of an increased risk of PIPN requiring paclitaxel treatment adjustment with use of atorvastatin as compared with simvastatin use in a nationwide cohort of paclitaxel-treated cancer patients. However, our in vitro results showed that the highest concentration of atorvastatin increased paclitaxel neurotoxicity in iPSC-SNs. The limitations of the clinical study, including register-based exposure status and reliance on

TABLE 3 | Risk of paclitaxel treatment modification with use of atorvastatin compared with simvastatin and nonuse, respectively, and specified by cause.

Risk of paclitaxel treatment modification with use of atorvastatin compared with simvastatin					
Cause	**Atorvastatin ($n=200$)**	**Simvastatin ($n=183$)**	**Crude OR [95% CI]**	**Adjusted[a] OR [95% CI]**	***p*-value**
Any modification (all)	123 (61.5%)	110 (60.1%)	1.06 (0.70–1.60)	0.86 (0.53–1.40)	0.549
Any modification (early)	84 (42.0%)	81 (44.3%)	0.91 (0.61–1.37)	0.83 (0.52–1.34)	0.449
Neurotoxicity (all)	70 (35.0%)	74 (40.4%)	0.79 (0.52–1.20)	0.71 (0.44–1.15)	0.163
Neurotoxicity (early)	45 (22.5%)	44 (24.0%)	0.92 (0.57–1.47)	0.89 (0.51–1.57)	0.694
Haematological toxicity (all)	0 (0.0%)	$n<5$	—	—	—
Poor health (all)	22 (11.0%)	16 (8.7%)	1.29 (0.66–2.54)	0.76 (0.33–1.74)	0.519
Allergic reaction (early)	11 (5.5%)	$n<5$	3.49 (0.96–12.72)	4.02 (0.82–19.67)	0.086
Risk of paclitaxel treatment modification with use of atorvastatin compared with nonuse					
Cause	**Atorvastatin ($n=200$)**	**Nonuse ($n=193$)**	**Crude OR [95% CI]**	**Adjusted[a] OR [95% CI]**	***p*-value**
Any modification (all)	123 (61.5%)	107 (55.4%)	1.28 (0.86–1.92)	1.00 (0.62–1.62)	0.998
Any modification (early)	84 (42.0%)	69 (35.8%)	1.30 (0.87–1.95)	1.19 (0.73–1.94)	0.481
Neurotoxicity (all)	70 (35.0%)	52 (26.9%)	1.46 (0.95–2.25)	1.22 (0.72–2.06)	0.456
Neurotoxicity (early)	45 (22.5%)	30 (15.5%)	1.58 (0.95–2.63)	1.50 (0.82–2.77)	0.192
Haematological toxicity (all)	0 (0.0%)	5 (2.6%)	—	—	—
Poor health (all)	22 (11.0%)	16 (8.3%)	1.37 (0.69–2.69)	0.97 (0.41–2.29)	0.940
Allergic reaction (early)	11 (5.5%)	8 (4.1%)	1.35 (0.53–3.42)	1.07 (0.38–2.97)	0.902

[a]Adjusted for age, alcohol, smoking, neurological comorbidity and diabetes.

medical records for all outcomes (i.e., phenotyping of PIPN), might have hindered detection of a clinical effect. Therefore, although our findings indicate no increased risk, it would be premature to firmly conclude that atorvastatin does not exacerbate PIPN.

Author Contributions

The study was designed by authors ES, CM, TBS and AP. All clinical data were collected by ES. All cell data were collected by CM. The analyses were carried out by CM, MO and MTE. The first draft was written by ES, CM and AP. and revised for important intellectual content by all authors. The final version of the paper was approved by all authors.

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Ethics Statement

The study was approved by the Danish Health Data Authority (record no. 00004814), Region of Southern Denmark (record no 21/50988), the Danish Patient Safety Authority (record no. 31-1521-179), OPEN (ID: OP_1137) and the repository of the University of Southern Denmark (record no 10.900). All hospital departments involved consented to the study's review of medical records. According to Danish law, neither patient consent nor approval from an ethical committee was needed for this study, as it did not involve biological material or patient contact. We performed this study in accordance with the Declaration of Helsinki.

Conflicts of Interest

Tore Bjerregaard Stage has given paid lectures for Pfizer and Eisai and done consulting for Pfizer, all unrelated to the present work. Anton Pottegård reports participation in research projects funded by Alcon, Almirall, Astellas, Astra-Zeneca, Boehringer-Ingelheim, Novo Nordisk, Servier and LEO Pharma, all regulator-mandated phase IV-studies, all with funds paid to the institution where he was employed (no personal fees) and with no relation to the work reported in this paper. The remaining authors report no potential conflicts of interest.

Data Availability Statement

The clinical dataset supporting the conclusions of this article is kept at the Danish Health Data Authority (record no. 00004814). The dataset is pseudonymized at the Danish Health Data Authority. The clinical dataset cannot be made public due to GDPR and ethical restrictions. The cell data are available in a separate file.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.