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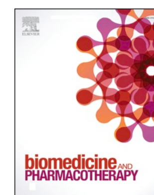

Identification of prophylactic drugs for oxaliplatin-induced peripheral neuropathy using big data

Yoshito Zamami[a,b,1], Takahiro Niimura[c,1], Takehiro Kawashiri[d,1], Mitsuhiro Goda[e], Yutaro Naito[e], Keijo Fukushima[f], Soichiro Ushio[b], Fuka Aizawa[c], Hirofumi Hamano[g], Naoto Okada[c], Kenta Yagi[g], Koji Miyata[e], Kenshi Takechi[h], Masayuki Chuma[i], Toshihiro Koyama[j], Daisuke Kobayashi[d], Takao Shimazoe[d], Hiromichi Fujino[f], Yuki Izawa-Ishizawa[k], Keisuke Ishizawa[c,e,*]

[a] Department of Clinical Pharmacy, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan
[b] Department of Pharmacy, Okayama University Hospital, Okayama, Japan
[c] Department of Pharmacy, Tokushima University Hospital, Tokushima, Japan
[d] Department of Clinical Pharmacy and Pharmaceutical Care, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan
[e] Department of Clinical Pharmacology and Therapeutics, Tokushima University Graduate School of Biomedical Sciences, Tokushima, Japan
[f] Department of Pharmacology for Life Sciences, Tokushima University Graduate School of Biomedical Sciences, Tokushima, Japan
[g] Clinical Research Center for Developmental Therapeutics, Tokushima University Hospital, Tokushima, Japan
[h] Department of Drug Information Analysis, College of Pharmaceutical Sciences, Matsuyama University, Matsuyama, Japan
[i] Department of Hospital Pharmacy and Pharmacology, Asahikawa Medical University, Asahikawa, Japan
[j] Department of Pharmaceutical Biomedicine, Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama University, Okayama, Japan
[k] Department of Pharmacology, University of Tokushima Graduate School of Biomedical Sciences, Tokushima, Japan

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ABSTRACT

Background: Drug repositioning is a cost-effective method to identify novel disease indications for approved drugs; it requires a shorter developmental period than conventional drug discovery methods. We aimed to identify prophylactic drugs for oxaliplatin-induced peripheral neuropathy by drug repositioning using data from large-scale medical information and life science information databases.
Methods: Herein, we analyzed the reported data between 2007 and 2017 retrieved from the FDA's database of spontaneous adverse event reports (FAERS) and the LINCS database provided by the National Institute of Health. The efficacy of the drug candidates for oxaliplatin-induced peripheral neuropathy obtained from the database analysis was examined using a rat model of peripheral neuropathy. Additionally, we compared the incidence of peripheral neuropathy in patients who received oxaliplatin at the Tokushima University Hospital, Japan. The effects of statins on the animal model were examined in six-week-old male Sprague–Dawley rats and seven or eight-week-old male BALB/C mice. Retrospective medical chart review included clinical data from Tokushima University Hospital from April 2009 to March 2018.
Results: Simvastatin, indicated for dyslipidemia, significantly reduced the severity of peripheral neuropathy and oxaliplatin-induced hyperalgesia. In the nerve tissue of model rats, the mRNA expression of *Gstm1* increased with statin administration. A retrospective medical chart review using clinical data revealed that the incidence of peripheral neuropathy decreased with statin use.
Conclusion and relevance: Thus, drug repositioning using data from large-scale basic and clinical databases enables the discovery of new indications for approved drugs with a high probability of success.

Abbreviations: AE, Adverse event; DRG, dorsal root ganglion; FAERS, FDA's database of spontaneous adverse event reports; GST, glutathione S-transferase; LINCS, Library of Integrated Network-based Cellular Signatures; MedDRA, Medical Dictionary for Regulatory Activities; OIPN, oxaliplatin-induced peripheral neuropathy; PWT, paw withdrawal threshold; ROR, reporting odds ratio; ROS, reactive oxygen species.

* Correspondence to: Department of Clinical Pharmacology and Therapeutics, Tokushima University Graduate School of Biomedical Sciences, 3-18-15 Kuramoto, Tokushima 770-8503, Japan.
E-mail address: ishizawa@tokushima-u.ac.jp (K. Ishizawa).

[1] These authors contributed equally to this study.

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1. Introduction

Drug development is an expensive and time-intensive process. Frequently, compounds that show efficacy in the early developmental stages, fail in clinical trials because of low efficacy, unexpected adverse effects, and inappropriate pharmacokinetic results. This gap between basic research and clinical development is known as "the valley of death"—a major bottleneck in drug discovery and approval. The investment in drug development and cost of drug discovery continue to rise annually [1].

Drug repositioning could overcome these obstacles by discovering new uses of approved drugs [2,3]. As information on adverse effects and pharmacokinetics of approved drugs already exists, drug repositioning is less likely to stall drug development compared with conventional drug discovery processes; moreover, it can reduce the associated time and cost [4].

Large-scale drug discovery research has been conducted using large-scale medical information including medical electronic records [5]. A meta-analysis of clinical trial data revealed that the antiepileptic drug eslicarbazepine could be effective against partial epileptic seizures, thereby expanding its applications [6]. Large-scale medical information contains abundant clinical data on approved drugs and is suitable for drug repositioning. Several drug repositioning studies have utilized large-scale medical information [7–9]. Previously, we demonstrated in vivo that teprenone, an approved drug for peptic ulcers, improves depressive symptoms by upregulating heat shock protein expression. Data analyzed from an adverse event (AE) reporting database showed that the reporting rate of depression may decrease in patients treated with teprenone [10].

Big data on life sciences, such as gene expression data, have been accumulating and are used to systematically evaluate complex pathological conditions in humans. The Library of Integrated Network-based Cellular Signatures (LINCS) is a gene expression database developed by the National Institutes of Health. It contains data obtained using human-derived cell lines and facilitates the search for compounds that may cause similar or opposite variations in the expression of genes of interest [11]. Using the connectivity map, the predecessor of LINCS, it was demonstrated that the antiepileptic drug topiramate counteracts gene expression changes in inflammatory bowel disease [12]. In a rat inflammatory bowel disease model, tissue damage and severity were reduced by topiramate.

We hypothesized that combining big data on clinical information and gene expression would help predict drug efficacy in humans and infer action mechanism. Herein, we aimed to establish a new drug development platform to overcome the "valley of death", the gap between early-stage discoveries and translation to novel therapeutics. We focused on the effect of non-treatment to verify the usefulness of this approach. As an example, we investigated strategies to prevent oxaliplatin-induced peripheral neuropathy (OIPN). Oxaliplatin, a key anticancer drug, causes peripheral neuropathy with numbness in approximately 90% of the patients [13]. Although various drugs are promising prophylactic agents for OIPN, no preventive method has been established owing to barriers in clinical practice [14,15]. We evaluated the usefulness of an efficient drug repositioning method that integrates data from a large-scale medical information database and life science information database by identifying approved drugs that serve as OIPN prophylactic agents.

2. Methods

2.1. Prophylactic drug candidate selection

From the FDA website, we downloaded 7,738,415 spontaneous AE reports that were submitted to FDA's database of spontaneous adverse event reports (FAERS) between the first quarter of 2007 through to the first quarter of 2017. Duplicate data were excluded following FDA recommendations; the remaining 6,994,117 reports were analyzed. Access 2016 (Microsoft, Redmond, WA, USA) was used to process the data and R ver. 3.2.1 (R Foundation for Statistical Computing, Vienna, Austria) for statistical analysis. Peripheral neuropathy was defined as "peripheral sensory neuropathy" and "neuropathy peripheral" according to the Medical Dictionary for Regulatory Activities (MedDRA) ver. 21.0.

The risk of AEs was assessed using the reporting odds ratio (ROR) and 95% confidence interval (CI) [16]. Patients using oxaliplatin were classified into four groups: (A) patients who used drug A and reported peripheral neuropathy; (B) patients who used drug A and did not report peripheral neuropathy; (C) patients who did not use drug A and reported peripheral neuropathy; and (D) patients who did not use drug A and did not report peripheral neuropathy. ROR and 95% CI were calculated using the following equations:

$$\mathrm{ROR} = (\mathrm{A}/\mathrm{B})/(\mathrm{C}/\mathrm{D})$$

$$95\%CI = exp\left(lnROR \pm 1.96\sqrt{\frac{1}{A}+\frac{1}{B}+\frac{1}{C}+\frac{1}{D}}\right)$$

Drugs with an ROR < 0.5 and significantly lower frequency of OIPN were selected as candidates.

As the AE database is an anonymized open-access database, institutional review board approval and informed consent were not required according to the Ministry of Health, Labor, and Welfare's Ethical Guidelines for Epidemiological Research.

We screened review articles on OIPN and selected nine genes associated with the development or exacerbation of peripheral neuropathy [17–20]. Five genes associated with promoting peripheral neuropathy were classified as "up genes" and four genes associated with suppression were "down genes" (eTable 1 in the Supplement). These genes were entered in a web-based search application LINCS L1000CDS2, a search engine that identifies compounds that mimic or reverse the gene expression entered by the user [21]. The search is based on the gene expression data of 3924 compounds collected from the National Institute of Health's LINCS project [22]. When entering a list of up and down genes into the LINCS L1000CDS2, cosine distance score is calculated for the gene expression data of each compound against the entered gene list. The top 50 compounds with the highest cosine distance score are presented along with their associated genes as candidate compounds that inhibit the expression of the input genes. Among the compounds in the search results, compounds already approved as drugs were selected as prophylactic drug candidates.

Therefore, the drugs extracted using both FAERS and LINCS analyses were selected as candidate drugs and validated using in vitro and in vivo experiments.

2.2. Animals and ethical considerations

Six-week-old male Sprague–Dawley rats were purchased from Kyudo, Saga, Japan, while seven- or eight-week-old male BALB/C mice were purchased from Clea Japan, Tokyo, Japan. All animals had free access to food and water and were maintained under a 12-h light/dark cycle.

All animal experiments were approved by the Ethical Committee for Animal Research of Tokushima University (No. T28–69). We followed the "Animal Research: Reporting of In Vivo Experiments" reporting guidelines.

2.3. Validating peripheral neuropathy mitigation

Sprague–Dawley rats were divided into four groups—vehicle, 4 mg/kg oxaliplatin (Tokyo Chemical Industry, Tokyo, Japan), oxaliplatin + 1 mg/kg statins (simvastatin, atorvastatin, and rosuvastatin; Tokyo Chemical Industry), and oxaliplatin + 10 mg/kg statins (N = 6 per

group). The effects of statins on the animal model were examined. Oxaliplatin 4 mg/kg (Tokyo Chemical Industry, Tokyo, Japan) prepared in 5% glucose solution was intraperitoneally administered twice a week for 4 weeks (days 1, 2, 8, 9, 15, 16, 22, and 23). Statins (simvastatin, atorvastatin, and rosuvastatin; Tokyo Chemical Industry) dispersed in 0.5% methylcellulose were administered orally five times a week for four weeks (days 1–5, 8–12, 15–19, and 22–26) at a dose of 1 or 10 mg/kg/day. The vehicle group was administered 5% glucose and 0.5% methylcellulose solution.

Mechanical pain threshold was assessed via the von Frey test before the first dose (day 0) and on days 5, 12, 19, and 26. The von Frey filament (Touch Test Sensory Evaluator Set, Linton Instrumentation, Norfolk, UK) was in contact with the center of the hind paw for 6 s each. The paw withdrawal threshold (PWT) was calculated using the up–down method [23].

2.4. Sciatic nerve axonal degeneration

Sciatic nerves were collected from rats on day 30. They were processed with 2% (w/v) glutaraldehyde and then with 8% (w/v) sucrose. The samples were then embedded in Epon, sliced, and stained with toluidine blue. The sliced samples were evaluated by light microscopy (BX51; Olympus, Tokyo, Japan) and analyzed using ImageJ 1.51. Circularity was calculated using the following formula:

$$Circularity = \frac{4\pi \times (axonalarea)}{(axonalperimeter)^2}$$

2.5. Real-time polymerase chain reaction (PCR) of OIPN rat model tissues

The bilateral dorsal root ganglion (DRG) of the lumbar vertebrae (L4–L6) was collected from the OIPN rats. RNA was extracted according to the manufacturer's instructions using an RNA extraction solution (NIPPON GENE, Tokyo, Japan). The RNA was reverse transcribed using the PrimeScript RT Reagent Kit (Takara Bio, Shiga, Japan) and PCR Thermal Cycler Dice (Takara). In each sample, the cDNA and forward and reverse primers (eTable 2 in the Supplement) were mixed with Thunderbird SYBR qPCR Mix (Toyobo, Osaka, Japan), and PCR was performed using Applied Biosystems StepOnePlus (Applied Biosystems, Waltham, MA, USA). Using rat *GAPDH* as the internal standard, gene expression level was determined by the $^{\Delta\Delta}$Ct method.

2.6. Cell culture and cell viability assay

We used PC12 cells (rat adrenal gland cell; American Type Culture Collection, Manassas, VA, USA) to investigate the effect of statins on oxaliplatin-induced cell death. The cells were maintained and subcultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin in 95% air/5% CO_2 at 37 °C, according to the culture protocol [24].

Cell viability was assessed using the Cell Counting Kit-8 (Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's instructions (N = 8–16). Briefly, the cells were seeded in 96-well plates at a concentration of 5×10^4 cells/well and incubated for 24 h at 37 °C. Subsequently, the cells were cultured in DMEM containing 3 μM oxaliplatin and statin for 24 h. Thereafter, the cells (with and without oxaliplatin) were subjected to statin treatment. Cell viability was assessed by measuring the absorbance of WST-8 formazan at 450 nm using a plate reader (Model680 Microplate Reader; Bio-Rad Laboratories, Hercules, CA, USA). For experiments using small-interfering RNA (siRNA), the cells were seeded at 3×10^4 cells/well, treated with or without 3 μM oxaliplatin and statins (0.1, 1, and 10 μM for 24 h after siRNA treatment, and assessed by measuring WST-8 absorbance.

2.7. Transfection and gene silencing with siRNAs

To perform siRNA transfection, the cultured PC12 cells were inoculated with DMEM containing 10% FBS and incubated for 24 h. siRNA targeting rat *Gstm1* (Predesigned siRNA, rat *Gstm1*, 24423; Bioneer, Oakland, CA, USA) or scrambled control (sc-37007; Santa Cruz) was transfected using Lipofectamine RNAiMAX (Life Technologies, Carlsbad, CA, USA) for 24 h according to the manufacturer's instructions (100 nM final density). The silencing efficiency was measured by immunoblotting.

2.8. Western blotting

Western blot analysis was performed as described previously [25]. Briefly, PC12 cells cultured in 24-well plates were lysed with cell lysis buffer (CelLytic MT Cell Lysis Reagent; Sigma-Aldrich, St. Louis, MO, USA) and then homogenized using HANDY SONIC UR-20 P (Tommy, Tokyo, Japan) on ice. Following centrifugation at 15,294 × *g* (4 °C), the supernatant was used as the protein sample. The samples were added to the sodium dodecyl sulfate sample buffer, boiled, and loaded onto 4–20% polyacrylamide gels (Mini-PROTEAN TGX Precast Gels; Bio-Rad Laboratories) to be separated by electrophoresis at a constant current of 0.02 A/gel. The samples were electro-transferred onto polyvinylidene fluoride (PVDF) membranes at a constant voltage of 24 V for 10 min, and the membranes were blocked with Bullet Blocking One for Western Blotting (Nacalai Tesque, Kyoto, Japan). Anti-*Gstm1* (Anti *Gstm1* Rabbit-Poly, GTX113448; GeneTex, Irvine, CA, USA) antibody diluted at 1:1000 with Can Get Signal Immunoreaction Enhancer Solution 1 (Toyobo, Tokyo, Japan) was used as the primary antibody. Anti-rabbit IgG antibody (ECL Anti-rabbit IgG horseradish peroxidase-linked whole antibody (from donkey), NA934V; GE Healthcare, Chicago, IL, USA) diluted at 1:1000 with Can Get Signal Immunoreaction Enhancer Solution 1 (Toyobo) was used as the secondary antibody. The PVDF membranes were treated using the Amersham ECL western blotting analysis system (GE Healthcare) for 5 min prior to visualization of the bands. Band luminescence was photographed using ChemiDoc (Bio-Rad Laboratories) and quantified using ImageJ. Protein expression levels were standardized with anti-beta-actin antibody (1:1000, β-actin (E4D9Z) Mouse mAb; Cell Signaling Technology, Danvers, MA, USA), and anti-mouse IgG antibody was used as the secondary antibody (1:1000, ECL Anti-mouse IgG horseradish peroxidase-linked whole antibody (from sheep), GE Healthcare).

2.9. Tumor-bearing model mouse experiment

Mouse colorectal cancer cells, Colon26 (1×10^6 cells), were suspended in DMEM (Nacalai Tesque) and subcutaneously injected into the right abdomen of BALB/C mice, which showed no tumor expansion 5 days post implantation. The mice whose tumor volume could not be measured were excluded from the experiment. The included mice were divided into three groups—vehicle, 6 mg/kg oxaliplatin, and oxaliplatin + 15 mg/kg statins.

Oxaliplatin and statins were administered 5 days after tumor cell implantation. Oxaliplatin (6 mg/kg/day) was administered intraperitoneally (10 mL/kg twice a week for 2 weeks; days 1, 2, 8, and 9). Simvastatin, atorvastatin, and rosuvastatin (15 mg/kg/day) were administered orally five times a week for 2 weeks (days 1–5 and 8–12). Oxaliplatin was diluted in 5% glucose solution and statins in 0.5% methylcellulose. The vehicle group was treated with 10 mL/kg 0.5% methylcellulose solution. Tumor volume was measured every 2 days (days 1, 3, 5, 7, 9, 11, 13, and 15) for tumor length (a: mm), short diameter (b: mm), and height (h: mm). Tumor volume (V: mm^3) was calculated using the following formula [26]:

$$V = (\pi abh)/6$$

2.10. Retrospective medical chart review

We retrospectively collected patient data from Tokushima University Hospital from April 2009 to March 2018. Patients aged ≥ 18 years who received oxaliplatin were included in the medical chart review. The following patients were excluded: patients who had previously received oxaliplatin; patients with a history of peripheral neuropathy due to other anticancer agents or diseases; clinical trial participants; patients who had undergone nerve block therapy; regimens containing oxaliplatin of one cycle or less. OIPN was defined as grade 1 or higher, based on the Common Terminology Criteria for Adverse Events ver. 5 [27]. We also analyzed patient background (age and sex), laboratory data, cancer types, and concomitant drugs.

Medical charts were reviewed in compliance with the Ministry of Health, Labor, and Welfare's Ethical Guidelines for Epidemiological Research. The study was approved by the Ethics Committee of Tokushima University Hospital (No. 3275) and conformed to the guidelines of the Declaration of Helsinki.

2.11. Statistics

Differences between groups in medical chart review were compared using Mann-Whitney U test for continuous variables and Fisher's exact test for categorical variables. The effect of statin use on peripheral neuropathy was calculated with a Cox proportional hazards model adjusted for age. A one-way analysis of variance was performed for comparisons involving three or more groups, and Tukey's test was performed as a post hoc analysis. R ver. 3.2.1 was used for statistical analysis, and results with two-tailed $P < 0.05$ were considered significant.

3. Results

3.1. Identification of candidate drugs

Thirteen drugs with an ROR < 0.5 were identified using data from the FAERS (Table 1), including allopurinol, an antipodagric drug, and metformin, an antidiabetic drug. For the LINCS analysis, 22 drugs including the local anesthetic lidocaine and the antihypertensive drug losartan were extracted (Table 2). Simvastatin was included as a common drug in both FAERS and LINCS analyses. Simvastatin has relatively fewer reports of peripheral neuropathy according to the FAERS analysis and of increased expression of glutathione S-transferase (GST)-related genes according to the LINCS analysis.

The reporting frequency of OIPN was significantly lower for not only simvastatin but also all HMG-CoA reductase inhibitors (eTable 3 in the Supplement). Therefore, to clarify whether this effect is common among HMG-CoA reductase inhibitors, we conducted in vitro/in vivo experiments of atorvastatin and rosuvastatin in addition to simvastatin.

Table 2
Drug candidate selection using the LINCS analysis.

Cosine distance score	Perturbation	Cell line	Dose (μM)	Time (h)
0.2857	Anagrelide hydrochloride	A375	10.0	6.0
0.2857	Bromhexine hydrochloride	MCF7	10.0	6.0
0.2857	Bromocryptine mesilate	A375	10.0	24.0
0.2857	Cimetidine	HA1E	10.0	6.0
0.2857	Dihydroergocristine	HT29	10.0	24.0
0.2857	Docosahexaenoic acid	HA1E	10.0	24.0
0.2857	Etodolac	PC3	10.0	6.0
0.2857	Guanabenz acetate	PC3	10.0	6.0
0.2857	Ipratropium bromide	HT29	10.0	6.0
0.2857	Isoxsuprine hydrochloride	HCC515	10.0	6.0
0.2857	Labetalol hydrochloride	PC3	10.0	24.0
0.2857	Lidocaine hydrochloride	HA1E	10.0	24.0
0.2857	Losartan	HCC515	10.0	24.0
0.2857	Nabumetone	HCC515	10.0	6.0
0.2857	Olaparib	PC3	10.0	24
0.2857	Ondansetron hydrochloride	A549	10.0	24.0
0.2857	Perindopril erbumine	HA1E	10.0	24.0
0.2857	Rimexolone	VCAP	10.0	24.0
0.2857	**Simvastatin**	**A375**	**10.0**	**6.0**
0.2857	Talipexole	PC3	10.0	24.0
0.2857	Ticlopidine hydrochloride	HCC515	10.0	6.0
0.2857	Trimipramine maleate	PC3	10.0	24.0

Compounds that promote the expression of suppressor genes and suppress the expression of OIPN-related promoter genes were identified using the LINCS analysis. Three kinds of approved drugs were extracted. Cell line, Dose, and Time indicate the experimental conditions under which the gene expression data stored in LINCS L1000CDS2 were acquired. OIPN, oxaliplatin-induced peripheral neuropathy; LINCS, Library of Integrated Network-based Cellular Signatures.

3.2. Effect of statins on rat OIPN model

The von Frey test in OIPN model rats revealed no difference in the PWT between the groups on day 0. The PWT of the oxaliplatin group decreased with an increase in the cumulative dose; on day 26, the PWT was significantly lower than that of the vehicle group. However, the PWT of the vehicle and oxaliplatin + statin groups was not significantly different over 26 days. On day 26, the PWT of the oxaliplatin + statin group was significantly higher than that of the oxaliplatin group and similar to that of the vehicle group (Fig. 1A–C). We focused on statins as candidate drugs for OIPN prevention and used toluidine blue staining to determine their protective effect, if any, against axonal degeneration of the sciatic nerve. In oxaliplatin-treated rats, axon circularity was significantly reduced. Conversely, statin treatment suppressed the oxaliplatin-induced degeneration of nerve axons (eFig. 1).

Table 1
Drug candidate selection using the FAERS analysis.

Drug name	Peripheral neuropathy reporting rate (number of reports)		ROR	P value
	Without the drug of interest	With the drug of interest	(95% CI)	
Allopurinol	5.48% (1271/23192)	2.03% (4/197)	0.36 (0.13–0.96)	.03
Bisoprolol	5.48% (1273/23234)	1.29% (2/155)	0.23 (0.06–0.91)	.02
Cytarabine	5.49% (1274/23226)	0.61% (1/163)	0.11 (0.01–0.76)	< .01
Enoxaparin sodium	5.49% (1270/23139)	2% (5/250)	0.35 (0.14–0.85)	.01
Levothyroxine sodium	5.48% (1270/23165)	2.23% (5/224)	0.39 (0.16–0.96)	.03
Metformin hydrochloride	5.53% (1269/22953)	1.38% (6/436)	0.24 (0.11–0.53)	< .01
Metoprolol tartrate	5.48% (1274/23256)	0.75% (1/133)	0.13 (0.02–0.94)	.01
Morphine	5.49% (1273/23198)	1.05% (2/191)	0.18 (0.05–0.73)	< .01
Omeprazole	5.53% (1258/22762)	2.71% (17/627)	0.48 (0.29–0.77)	< .01
Pantoprazole sodium	5.53% (1265/22868)	1.92% (10/521)	0.33 (0.18–0.63)	< .01
Prochlorperazine	5.48% (1273/23233)	1.28% (2/156)	0.22 (0.06–0.9)	.02
Ramipril	5.49% (1272/23170)	1.37% (3/219)	0.24 (0.08–0.75)	< .01
Simvastatin	**5.52% (1267/22967)**	**1.9% (8/422)**	**0.33 (0.16–0.67)**	**< .01**

Statistical analysis was conducted using Fisher's exact test. FAERS, FDA Adverse Event Reporting System; ROR, reporting odds ratio; CI, confidence interval.

Biomedicine & Pharmacotherapy 148 (2022) 112744

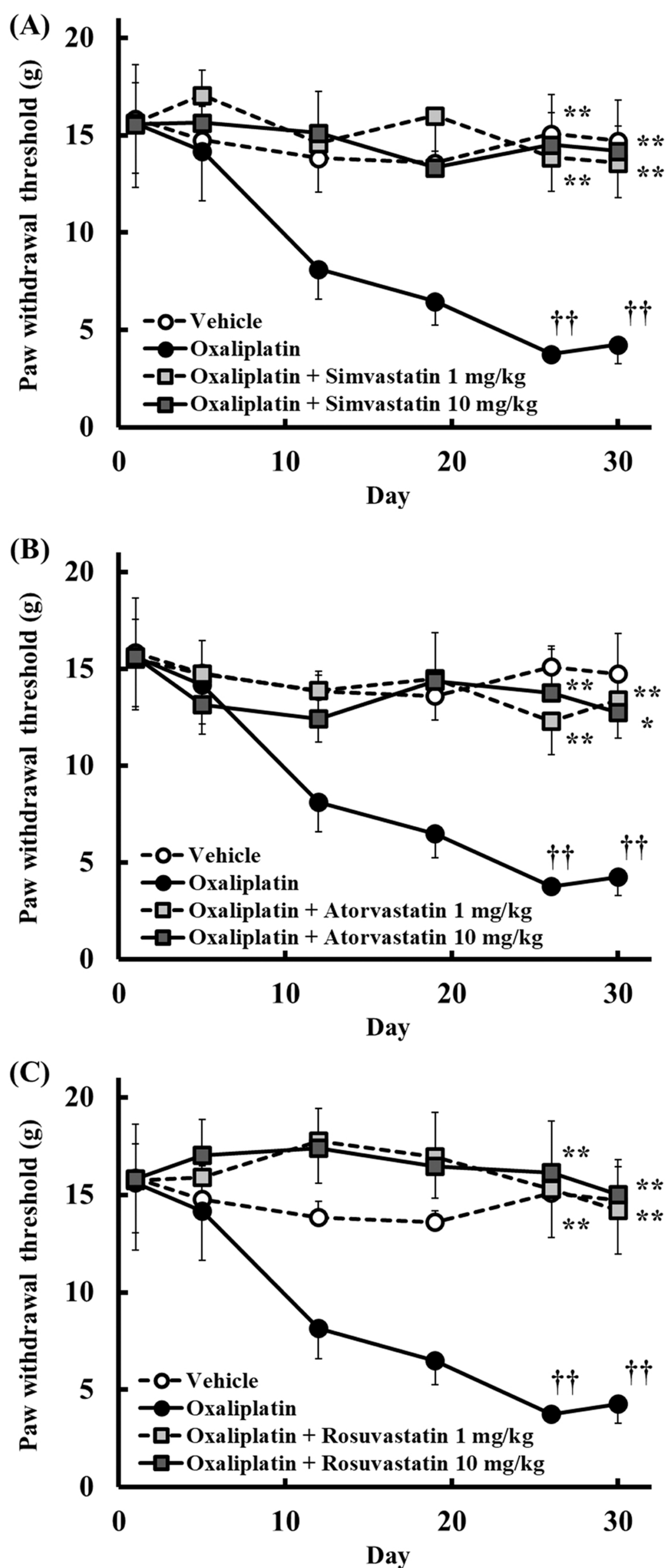

Fig. 1. Effect of statins on mechanical allodynia in the OIPN rat model. Time course of paw withdrawal threshold (PWT) on oxaliplatin-induced mechanical allodynia in the von Frey test of (A) simvastatin, (B) atorvastatin, or (C) rosuvastatin administration. On day 26, the PWT of the oxaliplatin group was significantly lower than that of the vehicle group, and the PWT of the oxaliplatin + statin group was significantly higher than that of the oxaliplatin group. Data are expressed as mean ± standard error. (N = 6, *$P < .05$, **$P < .01$ vs. vehicle, ††$P < .01$ vs. oxaliplatin alone; Tukey's test). OIPN, oxaliplatin-induced peripheral neuropathy; Sim, simvastatin; Ato, atorvastatin; Ros, rosuvastatin.

3.3. Effect of statins on Gstm1 expression

We evaluated *Gstm1* to determine its neuroprotective effect against OIPN using LINCS L1000CDS2 and the DRG rat model. Real-time PCR was conducted with RNA samples extracted from the DRG of the rats. The oxaliplatin group showed a significant decrease in *Gstm1* mRNA expression compared with the vehicle group. All oxaliplatin + statin groups showed a significant increase in *Gstm1* expression compared with the oxaliplatin group (Fig. 2A). *In vitro* PC12 cell viability significantly reduced within 24 h of oxaliplatin exposure, with cell viability being subsequently elevated by simvastatin (≥ 1 μM), atorvastatin (10 μM), or rosuvastatin (10 μM) (Fig. 2B). In *Gstm1* knockdown (eFig. 2A), PC12 cell viability was reduced in an oxaliplatin concentration-dependent manner (eFig. 2B); statins did not improve PC12 cell viability (Fig. 2C).

3.4. Effects of statins on oxaliplatin properties

In the tumor-bearing mouse model experiment, the tumor volume of the oxaliplatin group was significantly lower than that of the vehicle group on day 15. Furthermore, the tumor volume of the simvastatin and atorvastatin groups was similar to that of the oxaliplatin group. The tumor volume of the rosuvastatin group was significantly lower than that of the vehicle and oxaliplatin groups (eFig. 3).

3.5. Retrospective medical chart review of oxaliplatin-treated patients receiving statins

The medical charts of 392 patients treated with oxaliplatin from April 2009 to March 2018 were reviewed. Among these, 115 patients were excluded, including 10 patients who had received oxaliplatin, 15

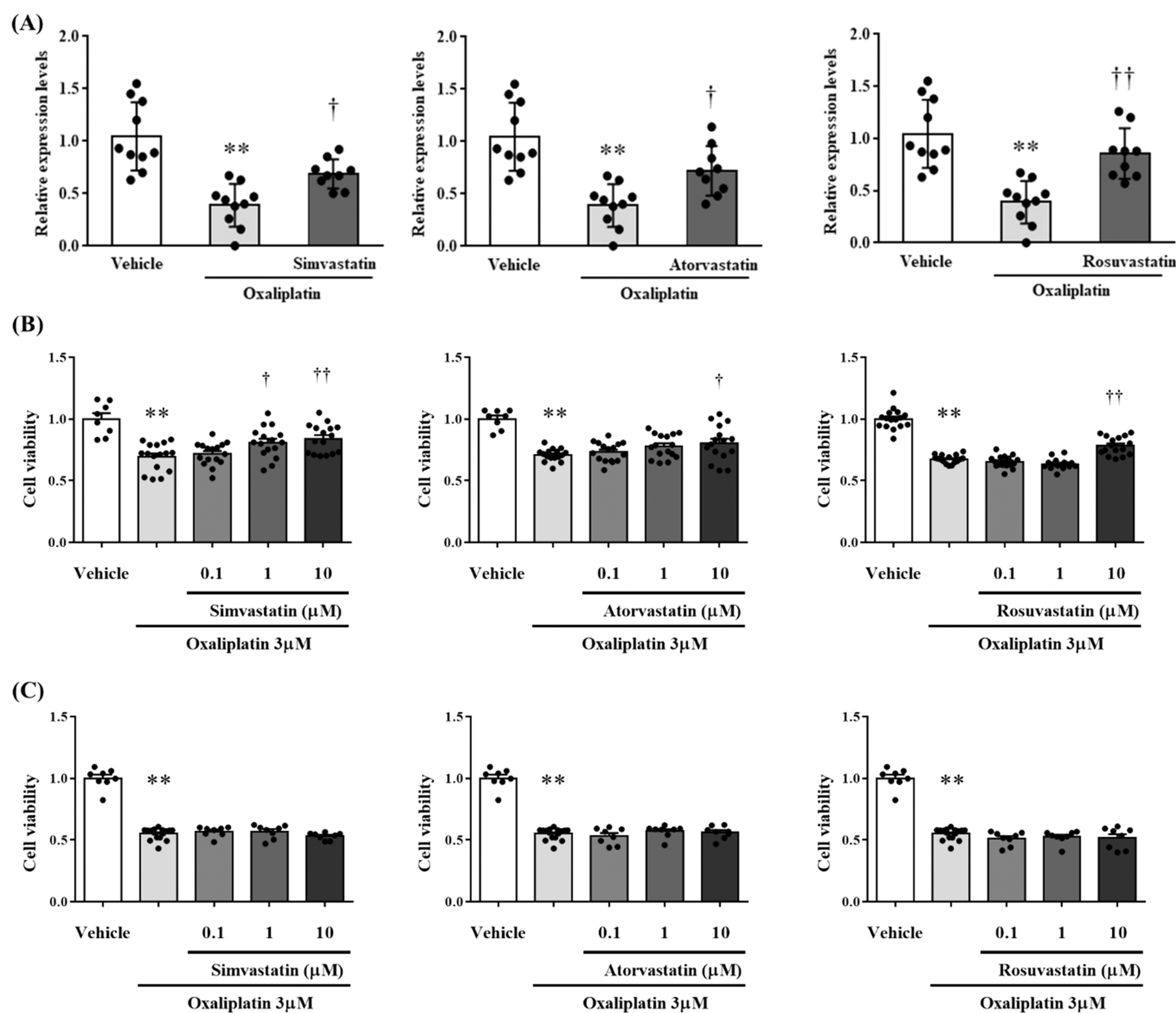

Fig. 2. Effects of oxaliplatin and statins on Gstm1 mRNA expression. The expression of Gstm1 mRNA in the rats of the (A) simvastatin, atorvastatin, and rosuvastatin groups. The oxaliplatin group showed a significant decrease in Gstm1 mRNA expression in DRG neuron compared with the vehicle group. The oxaliplatin + statin group showed a significant increase in Gstm1 mRNA expression compared with the oxaliplatin group. Data are expressed as mean ± standard error. (N = 9–10, **P < .01 vs. vehicle. †P < .05, ††P < .01 vs. oxaliplatin alone; Tukey's test). PC12 cell viability following GST knockdown in the (B) simvastatin, atorvastatin, and rosuvastatin groups. PC12 cell viability was reduced by exposure to oxaliplatin, but the reduction in cell viability was attenuated by the addition of statins. Data are expressed as mean ± standard error. (N = 8–16, **P < .01 vs. vehicle. †P < .05, ††P < .01 vs. oxaliplatin alone; Tukey's test). (C) Effect of statins on oxaliplatin-induced Gstm1 knockdown cell death (N = 8–16, **P < .01 vs. vehicle; Tukey's test). Statins did not attenuate oxaliplatin-induced decrease in the viability of PC12 cells with Gstm1 knockdown. Data are expressed as mean ± standard error. DRG, dorsal root ganglion.

Table 3
Peripheral neuropathy incidence between the non-statin and statin groups.

	Non-statin user	Statin user	Odds ratio	*P* value
Peripheral neuropathy	220 (87.65%)	17 (65.38%)	0.31	.012
Without peripheral neuropathy	31 (12.35%)	9 (34.62%)		

Statistical analysis was performed using the logistic regression analysis with age and statin used as covariates.

patients with a history of peripheral neuropathy, 42 clinical trial participants, and 48 patients who discontinued treatment within one cycle or less. The data of the remaining 277 subjects were analyzed; only 26 of these patients were treated with statins. The patients' age in the statin group was significantly higher than that in the non-statin group. There were no significant differences in sex, renal function, hepatic function, cancer type, or cancer stage between the groups (eTable 4 in the Supplement). The incidence of peripheral neuropathy was 87.6% in the non-statin group versus 66.7% in the statin group (Table 3). The Kaplan-Meier analysis showed that peripheral neuropathy incidence in the statin group was lower than that in the non-statin group (eFig. 4).

4. Discussion

We aimed to identify prophylactic drugs for OIPN by drug repositioning using information hosted on medical and life science databases. We verified the usefulness of the proposed research method for investigating OIPN, which decreases the quality of life of cancer survivors. Simvastatin was examined as a therapeutic drug candidate based on the AE report database and drug discovery tool analyses. In OIPN model rats, statins including simvastatin significantly suppressed the hyperalgesic reaction. PCR using neural tissue samples from OIPN model rats and knockdown experiments suggested that the inhibitory effect of statins on OIPN was mediated by increased *Gstm1* expression. We further confirmed in a tumor-bearing mouse model that statins do not reduce antitumor effects, even in combination with oxaliplatin. A retrospective chart review of clinical patient data suggested that statins inhibit OIPN. Thus, statins may suppress peripheral neuropathy that occurs as an AE without interfering with the therapeutics of oxaliplatin.

The United States has more than 13 million cancer survivors, with 2 million surviving for $\geq$ 15 years. However, the AEs of anticancer drugs are associated with decreased quality of life and increased duration of hospital stay and medical costs, resulting in significant economic loss [28]. The frequency of drug-induced AEs is difficult to predict. Predicting AEs in drug discovery is more difficult than achieving appropriate treatment efficacy; consequently, progress in the development of approaches to avoid and treat drug-induced AEs is lacking.

Drug repositioning enables the development of new therapeutic agents for rare and intractable diseases. The medical information database searched in this study contains extensive clinical data on approved drugs; therefore, it can be used to identify new indications for approved drugs, anticipate AEs and their occurrence rate, and predict drug safety and efficacy.

The AE report database analysis revealed that statins including simvastatin tended to suppress OIPN. A retrospective observational study using medical chart data suggested that the occurrence of peripheral neuropathy may be low in patients treated with statins, suggesting that this may be a class effect of statins. However, more detailed studies on the inhibitory effect of statins on OIPN are needed, including randomized controlled trials. OIPN is a dose-limiting factor that interferes with treatment, but there are no effective preventive drugs [29]. In 2020, the American Society of Clinical Oncology published guidelines on the management of drug-induced neurotoxicity, but made no recommendations for preventive drugs [30]. Statins are widely used, and their safety is well-established. Therefore, statins may serve as new agents for OIPN management.

Herein, we elucidated the mechanism underlying OIPN management with statins. GST promotes the detoxification of reactive oxygen species (ROS) via glutathione conjugation [31]. Oxidative stress induction through mitochondrial dysfunction is a mechanism underlying OIPN pathogenesis, and antioxidants reportedly suppressed OIPN in animal studies and clinical trials [29]. Statins may suppress peripheral neuropathy by reducing ROS levels in nerve cells via increased *Gstm1* expression. Therefore, Gstm1 may be involved in the neuroprotective mechanism of statins. We examined this using gene expression data and performing in vitro and in vivo experiments. Statins increased *Gstm1* expression in DRG tissue from model rats. *Gstm1* knockdown in PC12 cells reduced the inhibitory effect of statins on peripheral neuropathy. Thus, our method can predict drug efficacy in humans and infer action mechanism.

This study had some limitations. First, the gene expression data included in LINCS were based on cell line experiments and did not fully reflect the condition in humans. Second, the FAERS database curates reports that may contain biases including underreporting and overreporting. Therefore, we conducted a retrospective survey using electronic medical record data providing more detailed patient information. The results also suggest that statins suppressed OIPN development. Currently, we are conducting a multicenter retrospective study to investigate statin efficacy in more detail.

The candidate drugs identified using a single method are yet to be validated; however, utilizing information obtained through multiple methods and from multiple databases can mitigate these limitations. Using data from a gene expression database, relevant genes may be identified, and their detailed action mechanisms can be examined using basic experiments. Additionally, clinical data can be used to verify drug efficacy and predict corresponding therapeutic and AEs.

This study shows that repositioning conventional drugs may be useful as a rapid and inexpensive drug development platform. All the databases used in this study are publicly available. Additionally, numerous other drug-related databases are available nowadays. Moreover, these databases are continuously updated as new drugs or data become available, thereby providing a continuously growing source of information that may be used for drug repositioning. The application of our approach to investigate drug-induced AEs other than peripheral neuropathy may contribute to improving the quality of life of cancer survivors.

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CRediT authorship contribution statement

Yoshito Zamami: Conceptualization, Investigation, Writing – original draft, Funding acquisition. **Takahiro Niimura:** Investigation, Formal analysis, Writing – review & editing. **Takehiro Kawashiri:** Investigation, Writing – review & editing. **Mitsuhiro Goda:** Investigation, Formal analysis, Writing – review & editing. **Yutaro Naito:** Investigation, Formal analysis, Writing – review & editing. **Keijo Fukushima:** Investigation, Writing – review & editing. **Soichiro Ushio:**

Writing – review & editing. **Fuka Aizawa:** Investigation, Writing – review & editing. **Hirofumi Hamano:** Formal analysis, Writing – review & editing. **Naoto Okada:** Writing – review & editing. **Kenta Yagi:** Writing – review & editing. **Kenshi Takechi:** Writing – review & editing. **Masayuki Chuma:** Writing – review & editing. **Toshihiro Koyama:** Investigation. **Daisuke Kobayashi:** Writing – review & editing. **Takao Shimazoe:** Writing – review & editing. **Hiromichi Fujino:** Writing – review & editing. **Yuki Izawa-Ishizawa:** Writing – review & editing. **Keisuke Ishizawa:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

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

Data and material availability

The data and code that support the findings of this study are available from the corresponding authors upon request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2022.112744.

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