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2 **Renin-angiotensin-aldosterone system inhibitors prevent the onset of oxaliplatin-induced**

3 **peripheral neuropathy: A retrospective multicenter study and *in vitro* evaluation**

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SUMMARY

Oxaliplatin (OXA) is used in chemotherapy for various cancer types and is associated with acute and chronic neurotoxicity. However, a preventive strategy for OXA-induced peripheral neuropathy (OIPN) and its underlying mechanism remain unclear. We examined the effects of renin-angiotensin-aldosterone system inhibitors (RAASIs) on OIPN by performing a retrospective multicenter study and an *in vitro* assay. We retrospectively evaluated electronic medical records of 976 patients who underwent one or more courses of OXA-containing regimens at Ehime, Okayama, and Tokushima University Hospitals. The primary endpoint was the incidence of OIPN during or after OXA administration. The effects of RAASIs and OXA on the neurite length in PC12 cells were determined. The combined administration of an OXA-containing regimen and RAASI significantly inhibited the cumulative incidence grade-2 or higher OIPN (log-rank test; $P=0.0001$). RAASIs markedly suppressed the development of

both acute and chronic OIPN (multivariate analysis; $P=0.017$ and $P=0.011$). In an *in vitro* assay, 10 μM OXA suppressed the neurite length; treatment with 1 μM aliskiren, spironolactone, 10 μM candesartan, and enalapril significantly restored neurite length to the control level. Moreover, 1 μM SCH772984 (a selective inhibitor of extracellular signal-regulated kinase, ERK1/2) and 500 μM SQ22536 (a cell-permeable adenylate cyclase [AC] inhibitor) markedly abolished neuroprotective effects of candesartan and enalapril. These results indicate that RAASIs possess preventive or therapeutic effects in acute and chronic OIPN, candesartan and enalapril may directly increase in the activity of ERK1/2 and AC in PC12 cells.

Keywords: Adenylate cyclase, Extracellular signal-regulated kinase 1/2, Oxaliplatin-induced peripheral neuropathy, PC12 cells, renin-angiotensin-aldosterone system inhibitors.

INTRODUCTION

Oxaliplatin (OXA) is used in the standard treatment of colorectal, gastric, and pancreatic cancers, either in adjuvant settings or metastatic disease.¹⁻³⁾ OXA-induced peripheral neuropathy (OIPN) presents as acute cold-aggravated neurotoxicity and dose-dependent cumulative neuronal degeneration, characterized by transient and rapid onset neurological symptoms, as well as sensory ataxia, in most patients treated with 750-850 mg/m².⁴⁾ Although OIPN gradually recovers after treatment completion, it takes several months to years for complete recovery to be achieved, causing a marked deterioration in the patient's quality of life.⁵⁾ Several clinical trials have reported that pregabalin and duloxetine are effective for treating OIPN.^{6, 7)} In contrast, the inhibitory effects of goshajinkigan, a Japanese traditional medicine, vitamin E, calcium gluconate/magnesium sulfate, pregabalin, and antihyperalgesic drugs on chemotherapy-induced peripheral neuropathy (CIPN) are insufficient.⁸⁻¹³⁾

69 Accordingly, an effective and preventive strategy for CIPN or OIPN needs to be established.^{7,}

70 ¹¹⁾

71 Previous reports have demonstrated that OXA and oxalate (OXA metabolite) damage

72 sensory nerve cell bodies of the dorsal root ganglion, inducing peripheral neuropathy.¹⁴⁾

73 Hobara et al. have revealed that selective stimulation of angiotensin II (Ang II) type 2

74 receptor (AT2R) with Ang II facilitates reinnervation of mesenteric perivascular calcitonin

75 gene-related peptide (CGRP)-containing nerves, the primary sensory nerve, injured by topical

76 application of phenol in rats.¹⁵⁾ Furthermore, our previous study demonstrated that long-term

77 administration of temocapril (an angiotensin-converting enzyme inhibitor, ACEI) or losartan

78 (an Ang II receptor blocker, ARB) markedly ameliorated the age-related decrease in CGRP

79 nerve density observed in spontaneous hypertensive rats. These reports strongly suggest that

80 ACEIs and ARBs are highly likely to inhibit OIPN via long-term suppression of

renin-angiotensin system (RAS), AT1R and/or a selective stimulation of AT2R. Indeed, our previous single-center study has shown that RAS inhibitors (RASIs) confer protection against the onset of OIPN.¹⁶⁾ However, our sample size was limited, and our study was restricted to RASIs without aldosterone and renin antagonists. Moreover, the preventive effects of renin-angiotensin-aldosterone system inhibitors (RAASIs) on acute and chronic OIPN separately and its underlying mechanism remain unknown. Therefore, in the present study, we examined whether RAASIs have the preventive effects on acute and chronic OIPN in a multicenter investigation and evaluated the neuroprotective mechanism of RAASIs *in vitro* using rat adrenal pheochromocytoma PC12 cells, a commonly used neuronal cell model.

MATERIALS AND METHODS

Study participants

93 We retrospectively evaluated electronic medical records of patients (aged ≥ 20 years) who
94 underwent one or more courses of an OXA-containing regimen at Ehime, Okayama, and
95 Tokushima University Hospitals between May 2009 and December 2016. All patient records
96 were identified and analyzed. We extracted the necessary clinical information regarding
97 physical and hematological examinations, grade evaluation of OIPN, medical and
98 pharmacotherapy history with treatment cycles or dosage of OXA, the presence or absence of
99 dose reduction, interruption or molecular targeted drugs, regimen type, regular use of
100 combined drugs with RAASIs, calcium channel blockers, use of supportive drugs such as
101 goshajinkigan, vitamin B₁₂, pregabalin, gabapentin, an extract from inflammatory rabbit skin
102 inoculated with vaccinia virus, antiepileptic drugs, antidepressant drugs, duloxetine,
103 mexiletine, opioids, non-steroidal anti-inflammatory drugs, and acetaminophen. Patients
104 meeting any of the criteria shown in Fig. S1 were excluded from this study.

Definition of acute and chronic OIPN

In the present study, OIPN included paresthesias, dysesthesias, cold hypersensitivity, spontaneously numbness and pain around the limbs and lips, distal amyotrophy and muscle weakness, and decreased tendon reflex. We defined acute OIPN as a condition characterized by onset from immediately after OXA treatment to the second day and after administering a cumulative dose of OXA less than 540 mg/m². We also considered chronic OIPN to involve a cumulative OXA dosage exceeding 540 mg/m².¹⁷⁾

Endpoint

The primary endpoint was the incidence of severe peripheral neuropathy during or at any time after OXA administration.¹⁸⁾ OIPN was defined as grade 2 or higher according to the Common Terminology Criteria for Adverse Events, version 5.0.

117 ***Cell culture and drug treatment***

118 PC12 cell line was obtained from the RIKEN BioResource Center (Ibaraki, Japan).
119 Undifferentiated cells were cultured in RPMI 1640 medium (Nacalai Tesque Inc., Kyoto,
120 Japan) supplemented with 10% horse serum (Life Technologies NZ Ltd., Auckland, NZ), 5%
121 fetal bovine serum (Life Technologies Corporation, Grand Island, NY, USA), and 1%
122 penicillin-streptomycin (Nacalai Tesque), under 95% air and 5% CO₂ at 37°C. PC12 cells
123 (1×10^3 cells/well) were seeded in 96-well plates and cultured for 24 h; then, the serum-free
124 medium was replaced, supplemented with 100 ng/mL pro-NGF (nerve growth factor;
125 Alomone Labs, Jerusalem, Israel). After 48 h of pro-NGF treatment, differentiated cells
126 exhibiting an increased neurite outgrowth from cell bodies were cultured in serum-free
127 medium containing 0.1, 1, and 10 μ M OXA (Wako Pure Chemical Industries, Ltd., Osaka,
128 Japan), 1 and 10 μ M RAASIs with candesartan cilexetil, enalapril maleate, aliskiren (Wako

129 Pure Chemical), and spironolactone (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), 10
130 and 100 μ M duloxetine (Tokyo Chemical), 1 μ M SCH 772984 (Cayman Chemical Company,
131 Ann Arbor, MI, USA), or 500 μ M SQ 22536 (Tokyo Chemical) for 2 days. Changes in the
132 length of neurites were obtained with a phase-contrast microscope (CKX41, Olympus Co.,
133 Tokyo, Japan) equipped with a digital camera (Olympus E330-ADU12X) and expressed as
134 the ratio to that of the control group.

135 All drugs, except candesartan cilexetil, spironolactone, and SCH 772984, were dissolved in
136 RPMI 1640 medium. Candesartan cilexetil, spironolactone, and SCH 772984 were dissolved
137 in 10 mM dimethyl sulfoxide (DMSO) and diluted with RPMI 1640 medium to a final
138 concentration.

139

140 *Statistical analysis*

141 Cox proportional-hazards regression analysis was performed to evaluate risk factors
142 associated with the cumulative dosage of OXA and the onset of acute or chronic OIPN. The
143 cumulative dosage of OXA and the onset of acute or chronic OIPN were estimated using the
144 Kaplan-Meier method and analyzed by the log-rank test. Categorical variables were expressed
145 as numbers and compared using the χ^2 test or Fisher's exact test between RAASIs and
146 non-RAASIs groups.

147 All *in vitro* assay data are expressed as the mean $\pm$ standard error of the mean (SEM) of at
148 least three observations. All statistical analyses were performed using one-way ANOVA
149 followed by Student's t-test, Tukey's test, or Dunnett's test to determine significance.

150 Statistical significance was set at $P < 0.05$. Statistical analyses were performed using JMP 14.2
151 (SAS Institute Inc., NC, USA).

152

153 ***Ethical consideration***

154 The study protocol was approved by the ethics committee of Matsuyama University, Ehime,
155 Okayama, and Tokushima University Hospital (approval number: 21005, 1806003, 1910-007,
156 and 3275, respectively). The study was conducted in accordance with the principles of the
157 Declaration of Helsinki and the Ethical Guidelines for Medical and Health Research
158 Involving Human Subjects by the Ministry of Education, Culture, Sports, Science, and
159 Technology, and the Ministry of Health, Labour, and Welfare of Japan. Japanese law does not
160 require individual informed consent from participants in a non-invasive observational trial, as
161 in the present study. Therefore, we used our official website as an opt-out method rather than
162 acquiring written or oral informed consent.

163

164 **RESULTS**

Basic characteristics of the patients

This study included 641 patients treated with an OXA-containing regimen at three hospitals; the breakdown of 335 patients excluded from the study is shown in Fig. S1. As shown in Table S1, baseline patient characteristics in RAASIs and non-RAASIs groups have multiple biases, which include age ($P<0.001$), sex ($P<0.01$), body mass index (BMI; $P<0.001$), estimated glomerular filtration rate (eGFR; $P<0.001$), concomitant diabetes mellitus ($P<0.001$), and concomitant use of calcium channel blockers ($P<0.001$).

Oxaliplatin-induced peripheral neuropathy (OIPN)

Based on the univariate Cox proportional-hazards model analysis, we observed that the reduced risk of OIPN was undoubtedly related to the combined administration of RAASIs (crude hazards ratio [HR]: 0.50, 95% confidence interval [CI]: 0.34-0.71, $P<0.0001$), calcium

177 channel blockers (crude HR: 0.57, 95% CI: 0.40-0.79, $P=0.0005$), and concomitant diabetes
178 mellitus (crude HR: 0.53, 95% CI: 0.36-0.76, $P=0.0003$) (Table 1). In the multivariate Cox
179 proportional-hazards model analysis, the combination of RAASIs and diabetes mellitus
180 significantly suppressed the onset of OIPN (adjusted HR: 0.64, 95% CI: 0.42-0.95, $P=0.027$
181 and adjusted HR: 0.61, 95% CI: 0.41-0.89, $P=0.008$, respectively) (Table 1). As shown in Fig.
182 1, the combination of an OXA-containing regimen and RAASIs markedly inhibited the
183 cumulative incidence of grade 2 or higher OIPN (log-rank test; $P=0.0001$).

184

185 ***Acute OIPN***

186 Among 641 patients, 489 were assessed for acute OIPN treated with OXA ($< 540 \text{ mg/m}^2$).
187 The basic characteristics of patients with acute OIPN differed significantly in terms of age
188 ($P<0.001$), sex ($P<0.05$), BMI ($P<0.001$), eGFR ($P<0.001$), concomitant diabetes mellitus

189 ($P<0.001$), and co-administration of calcium channel blockers ($P<0.001$) between RAASIs
190 ($n=101$) and non-RAASIs group ($n=388$) (Table S2). RAASIs used in the patients with acute
191 OIPN included olmesartan (median dose [interquartile range]: 20 mg [20-20], number of
192 prescriptions: $n=18$), valsartan (80 mg [80-80], $n=16$), spironolactone (25 mg [25-25], $n=16$),
193 candesartan (8 mg [4-8], $n=15$), losartan (50 mg [43.8-50], $n=10$), azilsartan (20 mg [20-20],
194 $n=10$), telmisartan (40 mg [25-40], $n=9$), enalapril (5 mg [4.4-5], $n=5$), imidapril (5 mg [5-5],
195 $n=4$), and canrenoate (100 mg [100-100], $n=1$). Patients with combined use of double (losartan
196 and spironolactone, $n=1$) or triple (candesartan, enalapril and spironolactone, $n=1$) drug are
197 included.

198 The univariate analysis revealed a significant relationship between reduced OIPN and the
199 regular usage of RAASIs (crude HR: 0.45, 95% CI: 0.29-0.67, $P<0.0001$), calcium channel
200 blockers (crude HR: 0.54, 95% CI: 0.36-0.79, $P=0.001$), and concomitant diabetes mellitus

201 (crude HR: 0.57, 95% CI: 0.36-0.86, $P=0.007$). Additionally, the multivariate analysis
202 clarified that the combination of RAASIs and concomitant diabetes mellitus markedly
203 suppressed the onset of acute OIPN (crude HR: 0.59, 95% CI: 0.36-0.91, $P=0.017$ and crude
204 HR: 0.64, 95% CI: 0.40-0.99, $P=0.044$, respectively).

205

206 ***Chronic OIPN***

207 Among 641 patients, the development of chronic OIPN induced by OXA treatment (≥ 540
208 mg/m^2) was assessed in 152 patients. As shown in Table S3, basic patient characteristics,
209 including age ($P<0.05$), concomitant diabetes mellitus ($P<0.001$), and concomitant use of
210 calcium channel blockers ($P<0.001$), differed significantly between the RAASIs ($n=30$) and
211 non-RAASIs groups ($n=122$). RAASIs used in the patients with chronic OIPN included
212 olmesartan (median dose [interquartile range]: 20 mg [20-20], number of prescriptions: $n=8$),

213 valsartan (40 mg [40-80], $n=6$), telmisartan (40 mg [30-40], $n=4$), irbesartan (100 mg
214 [100-100], $n=3$), enalapril (5 mg [5-5], $n=3$), spironolactone (not detected, $n=3$), candesartan
215 (2 mg [2-2], $n=2$), losartan (not detected, $n=1$), azilsartan (20 mg [20-20], $n=1$). Patients with
216 combined use of double drug (valsartan and spironolactone, $n=1$) are included. The
217 co-administration of RAASIs and OXA significantly suppressed chronic OIPN in both
218 univariate and multivariate analyses (crude HR: 0.44, 95% CI: 0.17-0.94, $P=0.033$ and
219 adjusted HR: 0.37, 95% CI: 0.14-0.81, $P=0.011$), as shown in Table 3.

220

221 *Neuroprotective effects of RAASIs*

222 In differentiated PC12 cells, treatment with OXA (0.1-10 μ M) dose-dependently decreased
223 the elongation of neurite outgrowth, with significant differences ($P<0.01$) observed in the 10
224 μ M OXA-treated group when compared with that in the control group. Candesartan at 1 and

225 10 μ M and 10 μ M enalapril markedly ameliorated the neurodegeneration induced by 10 μ M
226 OXA (Figs. 2A and 2B). In addition, 1 μ M aliskiren and 1 μ M spironolactone significantly
227 improved the reduced neurite outgrowth induced by 10 μ M OXA (Figs. 2C and 2D); however,
228 no dose-dependent relationship was observed. However, 10 and 100 μ M duloxetine did not
229 impact the OXA-induced decrease in neurite outgrowth (Fig. 2E).

230

231 *Effect of ERK1/2 or AC inhibitor on RAASIs-induced neuroprotective effects*

232 Notably, 1 μ M SCH 772984, a selective ERK1/2 inhibitor, markedly inhibited
233 neuroprotective effects of candesartan and enalapril on neurite outgrowth in PC12 cells to the
234 levels observed in the OXA-treated group (Figs. 3A and 3B). In addition, the neuroprotective
235 effects of candesartan and enalapril were significantly inhibited following treatment with 500
236 μ M SQ 22536, an AC inhibitor (Figs. 4A and 4B). However, both SCH 772984 and SQ 22536

did not alter the neuroprotective effects of aliskiren and spironolactone (Figs. 3C, 3D, 4C, and 4D).

DISCUSSION

The present study revealed that combined treatment with an OXA-containing regimen and RAASIs significantly prevented the development of both acute and chronic OIPN, as assessed in a retrospective multicenter study. Moreover, we determined for the first time that the mechanism of neuroprotective effects afforded by RAASIs in PC12 cells. Acute neurotoxicity following OXA therapy reportedly occurs in 85-95% of patients and consists of cold intolerance and throat discomfort and largely abates after a few days.^{19, 20)} On the other hand, chronic OIPN is generally more problematic and dose-limiting,¹⁹⁾ its development usually occurs when the cumulative dosage exceeds 540 mg/m².^{17, 20)} It has been reported that an

249 OXA dosage of 1,170 mg / m² or higher can result in grade-3 OIPN in 50% of patients.²¹⁾

250 Pachman et al. have reported that the severity of acute OIPN is associated with that of chronic

251 symptoms.²²⁾ Recent clinical studies have reported that goshajinkigan, a traditional Japanese

252 herbal medicine, and the antidepressant duloxetine may improve OIPN.^{7, 23)} However,

253 breakthrough therapy and prevention of especially acute OIPN remain elusive.¹⁶⁾

254 In the present study, we defined a boundary concentration of 540 mg/m² between acute and

255 chronic OIPN.^{17, 20)} Moreover, we demonstrated that OXA induced cumulative

256 dose-dependent and marked grade-2 or higher OIPN, resulting in occurrence rates of 76% and

257 24% for acute and chronic OIPN, respectively, thus revealing novel evidence that RAASIs

258 markedly suppressed the occurrence of acute OIPN from a relatively early stage of OXA

259 therapy and chronic OIPN. In our previous study, however, we did not examine the preventive

260 effects of RAASIs on acute and chronic OIPN separately.¹⁶⁾

OIPN is frequently associated with discontinuation of chemotherapy, as well as a change or dosage reduction of anticancer drugs, posing a serious challenge to clinicians, interfering with cancer treatment, and resulting in a reduced patient quality of life.²⁴⁾ Previous reports have indicated that risk factors for developing acute and chronic OIPN include cumulative dose, young age, low body weight, body surface area > 2.0, persistent neuropathy in the past cycle, and surgery.^{25, 26)} The present multivariate analysis for acute OIPN reveals that regular administration of RAASIs and diabetic complications markedly suppressed OIPN, indicating a strong interaction between diabetes mellitus and the RAASIs group, as RASIs,¹⁶⁾ except for aldosterone and renin antagonists, are the first-line antihypertensive drugs for hypertensive patients with diabetes mellitus. However, diabetic neuropathy and diabetes may have less relevance to the present results because the neuropathy caused by diabetes mellitus is excluded in this study, and there is no correlation between incidence or duration of OIPN and diabetes.^{16,}

273 ²⁷⁾ Moreover, a prescription rate (43.2%) of RAASIs in diabetics is higher than that of
274 non-diabetics (16.6%), which may be the cause of decreasing acute OIPN. The present study
275 also demonstrated another novelty that the co-administration of RAASIs and OXA
276 significantly also suppressed chronic OIPN in both univariate and multivariate analyses. We
277 investigated various types and usage rates of RAASIs, including olmesartan (19.4%),
278 valsartan (16.4%), spironolactone (13.4%), candesartan (12.7%), telmisartan (9.7%),
279 azilsartan (8.2%), losartan (8.2%), enalapril (6.0%), imidapril (3.1%), irbesartan (2.2%), and
280 canrenoate (0.7%). Most RAASIs have ARBs and ACEIs, as in our previous investigation ¹⁶⁾;
281 however, the present study is the first report to reveal the involvement of aldosterone
282 antagonists with spironolactone and canrenoate.

283 To investigate the inhibitory effects and mechanism of RAASIs with high-frequency usage,
284 as shown above, we examined PC12 cells, which have been widely used in both

285 neurobiological and neurotoxicological studies as a model of neuronal differentiation.^{28, 29)}

286 Treatment with OXA dose-dependently reduced neurite outgrowth in differentiated PC12

287 cells; however, RAASIs, including candesartan, enalapril, aliskiren, and spironolactone,

288 significantly improved the decreased neurite outgrowth following OXA treatment. Therefore,

289 it is strongly suggested that these medications may afford protection against OXA-induced

290 neuronal damage. In the present *vitro* assay, non-protein binding OXA may induce neuronal

291 damage, because we used serum-free medium to examine neuroprotective effects of RAASIs

292 on OXA-induced neuronal damage. Although OXA has a high plasma protein binding rate,

293 this study revealed OXA significantly induced neuronal damage in PC12 cells, suggesting that

294 there is no effect of protein binding OXA under this condition. Moreover, the concentration

295 (0.1-10 μ M) of OXA we assessed in *vitro* assay is a similar level of patient plasma

296 concentration.³⁰⁾

297 It has been reported that OXA-induced neurotoxicity in PC12 cells may be associated with
298 oxidative stress and reduced intracellular calcium concentrations.^{28, 29)} Scuteri et al. have
299 reported that prolonged exposure to OXA inhibits ERK1/2 and c-Jun N-terminal
300 kinase/stress-activated protein kinase (JNK/SAPK) activity in dorsal root ganglia (DRG)
301 neurons, which mediate neuronal apoptosis, and that high dose NGF (100 μ M) increases
302 neuronal survival by restoring the phosphorylation of ERK1/2 and JNK/SAPK.³¹⁾ Our present
303 results are in accordance with this observation; however, this is the first report to document
304 that the neuroprotective mechanisms of candesartan and enalapril mediate a direct increased
305 activation of ERK1/2 and AC in PC12 cells with a significant decrease in neurite outgrowth
306 caused by OXA. In rodents, ARBs ameliorated stress-induced disorders, anxiety, and
307 depression, decreased brain inflammation, amyloid- β neurotoxicity, and traumatic brain injury.
308 ³²⁾ Direct anti-inflammatory and protective effects of ARBs, as demonstrated in cultured

309 microglia, cerebrovascular endothelial cells, neurons, and human circulating monocytes,
310 could be attributed to AT1 receptor (AT1R) blockade, as well as activation of
311 peroxisome-proliferator-activated receptor γ .³²⁾ Our previous research has demonstrated that
312 the treatment of type 2 diabetic rats with candesartan significantly ameliorates the decrease in
313 neurite elongation via AT2R in DRG cells.³³⁾ Endogenous AT2R, but not AT1R, is
314 predominantly expressed in PC12 cells.³⁴⁾ AT1R also activates ERK1/2 in rat vascular
315 smooth muscle cells and AT1R-transfected PC12 cells³⁵⁾. Therefore, in the present study, the
316 neuroprotective effects of candesartan and enalapril may be not involved in ATR stimulation.
317 Nostramo et. al confirmed the presence of (pro)renin receptor protein in PC12 cells, which
318 has been reported to be localized primarily in the Golgi and endoplasmic reticulum in
319 NGF-induced PC12 cells.³⁶⁾ However, there has been no definitive role of RAS system to
320 control the neurite outgrowth in PC12 cells.

321 On the other hand, the neuroprotective effects of aliskiren and spironolactone is not involved
322 in the increased activation of ERK1/2 and AC. Aliskiren reportedly confers protection against
323 the development and progression of diabetic neuropathy through antioxidant effects, including
324 enhanced superoxide dismutase and glutathione peroxidase activity.³⁷⁾ To our knowledge, the
325 neuroprotective effects of spironolactone on OXA-induced neuronal damage in PC12 cells
326 has not been elucidated yet. Hence, it is necessary to examine the neuroprotective
327 mechanisms of aliskiren and spironolactone, and whether RAS system changes caused by the
328 treatment with RAASIs are involved in the improvement of OXA-induced neurotoxicity
329 under the same conditions.

330 In conclusion, our findings indicate that RAASIs have preventive effects on both acute and
331 chronic OIPN and can strongly facilitate the development of drug repositioning research and
332 new drugs for OIPN.

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336

337 **AUTHORSHIP**

338 MU and ST wrote the manuscript. MU, ST, H. Kawazoe, and ST, H. Kawasaki designed the
339 observational study and *in vitro* assay, respectively. MU, SU, TN, HN, AT, HA, YZ, KI, YK,
340 TS, MT and MU, ST, KT, HN, KS contributed to data collection and interpretation, analyzing
341 the data, respectively. All authors have reviewed and approved the final version of the
342 manuscript.

343

344 **CONFLICT OF INTEREST**

345 The authors declare no conflict of interest.

346

347 **SUPPLEMENTARY MATERIALS**

348 This article contains supplementary materials.

349

REFERENCES

- 1) Cassidy J, Clarke S, Díaz-Rubio E, Scheithauer W, Figer A, Wong R, Koski S, Lichinitser M, Yang TS, Rivera F, Couture F, Sirzén F, Saltz L. Randomized phase III study of capecitabine plus oxaliplatin compared with fluorouracil/folinic acid plus oxaliplatin as first-line therapy for metastatic colorectal cancer. *J Clin Oncol.*, **26**, 2006-2012 (2008).
- 2) Yamada Y, Higuchi K, Nishikawa K, Gotoh M, Fuse N, Sugimoto N, Nishina T, Amagai K, Chin K, Niwa Y, Tsuji A, Imamura H, Tsuda M, Yasui H, Fujii H, Yamaguchi K, Yasui H, Hironaka S, Shimada K, Miwa H, Hamada C, Hyodo I. Phase III study comparing oxaliplatin plus S-1 with cisplatin plus S-1 in chemotherapy-naïve patients with advanced gastric cancer. *Annals Oncol.*, **26**, 141-148 (2015).
- 3) Conroy T, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, Adenis A, Raoul JL, Gourgou-Bourgade S, de la Fouchardière C, Bennouna J, Bachet JB, Khemissa-Akouz

362 F, Péré-Vergé D, Delbaldo C, Assenat E, Chauffert B, Michel P, Montoto-Grillot
 363 C, Ducreux M, Groupe Tumeurs Digestives of Unicancer; PRODIGE Intergroup.
 364 FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med.*, **364**,
 365 1817-1825 (2011).

366 4) Bennett BK, Park SB, Lin CS, Friedlander ML, Kiernan MC, Goldstein D. Impact of
 367 oxaliplatin-induced neuropathy: a patient perspective. *Support Care Cancer.*, **20**,
 368 2959-2967 (2012).

369 5) André T, Boni C, Mounedji-Boudiaf L, Navarro M, Tabernero J, Hickish T, Topham
 370 C, Zaninelli M, Clingan P, Bridgewater J, Tabah-Fisch I, de Gramont A, Multicenter
 371 International Study of Oxaliplatin/5-Fluorouracil/Leucovorin in the Adjuvant Treatment of
 372 Colon Cancer (MOSAIC) Investigators. Oxaliplatin, fluorouracil, and leucovorin as
 373 adjuvant treatment for colon cancer. *N Engl J Med.*, **350**, 2343-2351 (2004).

- 374 6) Saif MW, Syrigos K, Kaley K, Isufi I. Role of pregabalin in treatment of
375 oxaliplatin-induced sensory neuropathy. *Anticancer Res.*, **30**, 2927-2933 (2010).
- 376 7) Smith EML, Pang H, Cirrincione C, Fleishman S, Paskett ED, Ahles T, Bressler LR, Fadul
377 CE, Knox C, Le-Lindqwister N, Gilman PB, Shapiro CL. Effect of duloxetine on pain,
378 function, and quality of life among patients with chemotherapy-induced painful peripheral
379 neuropathy: a randomized clinical trial. *JAMA.*, **309**, 1359-1367 (2013).
- 380 8) Oki E, Emi Y, Kojima H, Higashijima J, Kato T, Miyake Y, Kon M, Ogata Y, Takahashi
381 K, Ishida H, Saeki H, Sakaguchi Y, Yamanaka T, Kono T, Tomita N, Baba H, Shirabe
382 K, Kakeji Y, Maehara Y. Preventive effect of Goshajinkigan on peripheral neurotoxicity of
383 FOLFOX therapy (GENIUS trial): a placebo-controlled, double-blind, randomized phase
384 III study. *Int J Clin Oncol.*, **20**, 767-775 (2015).

- 385 9) Schloss JM, Colosimo M, Airey C, Masci P, Linnane AW, Vitetta L. A randomised,
386 placebo-controlled trial assessing the efficacy of an oral B group vitamin in preventing the
387 development of chemotherapy-induced peripheral neuropathy (CIPN). *Support Care*
388 *Cancer.*, **25**, 195-204 (2017).
- 389 10) Loprinzi CL, Qin R, Dakhil SR, Fehrenbacher L, Flynn KA, Atherton P, Seisler D, Qamar
390 R, Lewis GC, Grothey A. Phase III randomized, placebo-controlled, double-blind study of
391 intravenous calcium and magnesium to prevent oxaliplatin-induced sensory neurotoxicity
392 (N08CB/Alliance). *J Clin Oncol.*, **32**, 997-1005 (2014).
- 393 11) de Andrade DC, Teixeira MJ, Galhardoni R, Ferreira KSL, Mileno PB,
394 Scisci N, Zandonai A, Teixeira WGJ, Saragiotto DF, Silva
395 V, Raicher I, Cury RG, Macareno R, Heise CO, Brotto MWI, de Mello AA, Megale
396 MZ, Dourado LHC, Bahia LM, Rodrigues AL, Parravano D, Fukushima JT, Lefaucheur

397 JP, Bouhassira D, Sobroza E, Riechelmann RP, Hoff PM, PreOx Workgroup; da Silva
398 FV, Chile T, Dale CS, Nebuloni D, Senna L, Brentani H, Pagano RL, de Souza ÂM.
399 Pregabalin for the prevention of oxaliplatin-induced painful neuropathy: A randomized,
400 double-blind trial. *Oncologist.*, **22**, 1154-e105 (2017).

401 12) Shinde SS, Seisler D, Soori G, Atherton PJ, Pachman DR, Lafky J, Ruddy KJ, Loprinzi
402 CL. Can pregabalin prevent paclitaxel-associated neuropathy? An ACCRU pilot trial.
403 *Support Care Cancer.*, **24**, 547-553 (2016).

404 13) Kautio AL, Haanpää M, Leminen A, Kalso E, Kautiainen H, Saarto T. Amitriptyline in the
405 prevention of chemotherapy-induced neuropathic symptoms. *Anticancer Res.*, **29**,
406 2601-2606 (2009).

- 407 14) Adelsberger H, Quasthoff S, Grosskreutz J, Lepier A, Eckel F, Lersch C. The
408 chemotherapeutic oxaliplatin alters voltage-gated Na (+) channel kinetics on rat sensory
409 neurons. *Eur J Pharmacol.*, **406**, 25-32 (2000).
- 410 15) Hobara N, Goda M, Yoshida N, Takatori S, Kitamura Y, Mio M, Kawasaki H. Angiotensin
411 II type 2 receptors facilitate reinnervation of phenol-lesioned vascular calcitonin
412 gene-related peptide-containing nerves in rat mesenteric arteries. *Neuroscience.*, **150**,
413 730-741 (2007).
- 414 16) Uchida M, Kawazoe H, Takatori, S, Namba H, Uozumi R, Tanaka A, Kawasaki H, Araki
415 H. Preventive effects of renin-angiotensin system inhibitors on oxaliplatin-induced
416 peripheral neuropathy: A retrospective observational study. *Clin Ther.*, **40**, 1214-1222
417 (2018).

- 418 17) Cersosimo RJ. Oxaliplatin-associated neuropathy: a review. *Ann Pharmacother* **39**,
419 128-135 (2005).
- 420 18) Inoue N, Ishida H, Sano M, Kishino T, Okada N, Kumamoto K, Ishibashi K. Discrepancy
421 between the NCI-CTCAE and DEB-NTC scales in the evaluation of oxaliplatin-related
422 neurotoxicity in patients with metastatic colorectal cancer. *Int J Clin Oncol.*, **17**, 341-347
423 (2012).
- 424 19) Grothey A, Nikcevich DA, Sloan JA, Kugler JW, Silberstein PT, Dentchev T, Wender DB,
425 Novotny PJ, Chitaley U, Alberts SR, Loprinzi CL. Intravenous calcium and magnesium
426 for oxaliplatin-induced sensory neurotoxicity in adjuvant colon cancer: NCCTG N04C7. *J*
427 *Clin Oncol.*, **29**, 421-427 (2011).
- 428 20) Argyriou AA, Cavaletti G, Briani C, Velasco R, Bruna J, Campagnolo M, Alberti
429 P, Bergamo F, Cortinovis D, Cazzaniga M, Santos C, Papadimitriou K, Kalofonos HP.

430 Clinical pattern and associations of oxaliplatin acute neurotoxicity: a prospective study in
431 170 patients with colorectal cancer. *Cancer*, **119**, 438-444 (2013).

432 21) Ewertz M, Qvortrup C, Eckhoff L. Chemotherapy-induced peripheral neuropathy in
433 patients treated with taxanes and platinum derivatives. *Acta Oncol.*, **54**, 587-591 (2015).

434 22) Pachman DR, Qin R, Seisler DK, Smith EML, Beutler AS, Ta LE, Lafky JM,
435 Wagner-Johnston ND, Ruddy KJ, Dakhil S, Staff NP, Grothey A, Loprinzi CL. Clinical
436 course of oxaliplatin-induced neuropathy: Results from the randomized phase III trial
437 N08CB (Alliance). *J Clin Oncol.*, **33**, 3416-3422 (2015).

438 23) Yoshida N, Hosokawa T, Ishikawa T, Yagi N, Kokura S, Naito Y, Nakanishi M, Kokuba Y,
439 Otsuji E, Kuroboshi H, Taniwaki M, Taguchi T, Hosoi H, Nakamura T, Miki T. Efficacy of
440 goshajinkigan for oxaliplatin-induced peripheral neuropathy in colorectal cancer patients.
441 *J Oncol.*, **2013**, 139740 (2013).

- 442 24) Selvy M, Pereira B, Kerckhove N, Gonneau C, Feydel G, Pétorin C, Vimal-Baguet A,
443 Melnikov S, Kullab S, Hebbar M, Bouché O, Slimano F, Bourgeois M, Lebrun-Ly V,
444 Thuillier F, Mazard T, Tavan D, Benmammer KE, Monange B, Ramdani M, Péré-Vergé
445 D, Huet-Penz F, Bedjaoui A, Genty F, Leyronnas C, Busserolles J, Trevis S, Pinon V,
446 Pezet D, Balayssac D. Long-term prevalence of sensory chemotherapy-induced peripheral
447 neuropathy for 5 years after adjuvant FOLFOX chemotherapy to treat colorectal cancer: A
448 multicenter cross-sectional study. *J Clin Med.*, **9**, 2400 (2020).
- 449 25) Gornet JM, Savier E, Lokiec F, Cvitkovic E, Misset JL, Goldwasser F. Exacerbation of
450 oxaliplatin neurosensory toxicity following surgery. *Ann Oncol.*, **13**, 1315-1318 (2002).
- 451 26) Alejandro LM, Behrendt CE, Chen K, Openshaw H, Shibata S. Predicting acute and
452 persistent neuropathy associated with oxaliplatin. *Am J Clin Oncol.*, **36**, 331-337 (2013).

- 453 27) Vincenzi B, Frezza MA, Schiavon G, Spoto C, Silvestris N, Addeo R, Catalano
454 V, Graziano F, Santini D, Tonini G. Identification of clinical predictive factors of
455 oxaliplatin-induced chronic peripheral neuropathy in colorectal cancer patients treated
456 with adjuvant Folfox IV. *Support Care Cancer.*, **21**, 1313-1319 (2013).
- 457 28) Kawashiri T, Shimizu S, Shigematsu N, Kobayashi D, Shimazoe T. Donepezil
458 ameliorates oxaliplatin-induced peripheral neuropathy via a neuroprotective effect. *J*
459 *Pharmacol Sci.*, **140**, 291-294 (2019).
- 460 29) Takeshita M, Banno Y, Nakamura M, Otsuka M, Teramachi H, Tsuchiya T, Itoh Y. The
461 pivotal role of intracellular calcium in oxaliplatin-induced inhibition of neurite outgrowth
462 but not cell death in differentiated PC12 cells. *Chem Res Toxicol.*, **24**, 1845-1852 (2011).
- 463 30) Ito H, Yamaguchi H, Fujikawa A, Tanaka N, Furugen A, Miyamori K, Takahashi N, Ogura
464 J, Kobayashi M, Yamada T, Mano N, Iseki K. A full validated hydrophilic interaction

465 liquid chromatography-tandem mass spectrometric method for the quantification of
466 oxaliplatin in human plasma ultrafiltrates. *J Pharm Biomed Anal.*, **71**, 99-103 (2012).

467 31) Scuteri A, Galimberti A, Ravasi M, Pasini S, Donzelli E, Cavaletti G, Tredici G. NGF
468 protects dorsal root ganglion neurons from oxaliplatin by modulating JNK/Sapk and
469 ERK1/2. *Neurosci Lett.*, **486**, 141-145 (2010).

470 32) Saavedra JM. Angiotensin II AT(1) receptor blockers as treatments for inflammatory brain
471 disorders. *Clin Sci (Lond.)*, **123**, 567-590 (2012).

472 33) Hashikawa-Hobara N, Hashikawa N, Inoue Y, Sanda H, Zamami Y, Takatori S, Kawasaki
473 H. Candesartan cilexetil improves angiotensin II type 2 receptor-mediated neurite
474 outgrowth via the PI3K-Akt pathway in fructose-induced insulin-resistance rats. *Diabetes.*,
475 **61**, 925-932 (2012).

476 34) Saito M, Shinohara Y, Sasaki H, Netsu Y, Yoshida M, Nakahata N. Type 1 angiotensin
477 receptor (AT1-R)-mediated decrease in type 2 angiotensin receptor mRNA level is
478 dependent on Gq and extracellular signal-regulated kinase 1//2 in AT1-R-transfected PC12
479 cells. *J Neuroendocrinol.*, **20**, 299-308 (2008).

480 35) Ishida M, Ishida T, Thomas SM, Berk BC. Activation of extracellular signal-regulated
481 kinases (ERK1/2) by angiotensin II is dependent on c-Src in vascular smooth muscle cells.
482 *Circ Res.*, **82**, 7-12 (1998).

483 36) Nostramo R, Serova L, Laukova M, Tillinger A, Peddu C, Sabban EL. Regulation of
484 nonclassical renin-angiotensin system receptor gene expression in the adrenal medulla by
485 acute and repeated immobilization stress. *Am J Physiol Regul Integr Comp Physiol.*, **308**,
486 R517-R529 (2015).

487

488 37) Alkhudhayri S, Sajini R, Alharbi B, Qabbani J, Al-Hindi Y, Fairaq A, Yousef A.

489 Investigating the beneficial effect of aliskiren in attenuating neuropathic pain in diabetic

490 Sprague-Dawley rats. *Endocrinol Diabetes Metab.*, **4**, e00209 (2020).

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					Univariate	Multivariate
					analysis	analysis
					Crude HR (95%	Adjusted HR
					CI)	(95% CI)
	n	Event	Censored			
RAASIs, n	Yes	131	32	99	0.50 (0.34-0.71)	0.64 (0.42-0.95)
	No	510	245	265		
Age (year)					0.81 (0.43-1.57)	1.13 (0.58-2.26)
Sex, n	Female	264	121	143	1.12 (0.88-1.42)	1.04 (0.81-1.32)
	Male	377	156	221		
BMI (kg/m ²)					0.54 (0.15-1.86)	
eGFR (mL/min/1.73 m ²)					0.75 (0.27-2.06)	

Diabetes	Yes	108	32	76	0.53 (0.36-0.76)	0.61 (0.41-0.89)
mellitus, n	No	533	245	288		
Supportive	Yes	133	78	55	1.54 (1.18-1.99)	1.50 (1.14-1.96)
drugs, n	No	508	199	309		
Calcium	Yes	133	40	93	0.57 (0.40-0.79)	0.76 (0.52-1.08)
channel	No	508	237	271		
blockers, n						
Analgesic	Yes	240	112	128	1.17 (0.92-1.49)	
drugs/analge	No	401	165	236		
sic adjuvant,						
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493 Abbreviations: BMI, body mass index; CI, confidence interval; eGFR, estimated glomerular

494 filtration rate; HR, hazard ratio; RAASIs, renin-angiotensin-aldosterone system inhibitors

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					Univariate	Multivariate	
					analysis	analysis	
		n	Even	Censored	Crude HR (95%	Adjusted	HR
			t		CI)	(95% CI)	
RAASIs, n	Yes	101	26	75	0.45 (0.29-0.67)	0.59 (0.36-0.91)	
	No	388	190	198			
Age (year)					0.51 (0.25-1.03)	0.80 (0.39-1.68)	
Sex, n	Female	201	98	103	1.23 (0.94-1.60)	1.15 (0.88-1.52)	
	Male	288	118	170			
BMI (kg/m ²)					0.44 (0.17-1.10)		
eGFR (mL/min/1.73 m ²)					1.74 (0.57-5.17)		

Diabetes	Yes	74	23	51	0.57 (0.36-0.86)	0.64 (0.40-0.99)
mellitus, n	No	415	193	222		
Supportive	Yes	101	64	37	1.69 (1.25-2.25)	1.76 (1.30-2.35)
drugs, n	No	388	152	236		
Calcium	Yes	94	29	65	0.54 (0.36-0.79)	0.71 (0.46-1.08)
channel	No	395	187	208		
blockers, n						
Analgesic	Yes	190	91	99	1.14 (0.86-1.49)	
drugs/analgesic	No	299	125	174		
adjuvant, n						

499 Abbreviations: BMI, body mass index; CI, confidence interval; eGFR, estimated glomerular
500 filtration rate; HR, hazard ratio; RAAS, renin-angiotensin-aldosterone system inhibitors;
501 OIPN, oxaliplatin-induced peripheral neuropathy.

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					Univariate	Multivariate
					analysis	analysis
		n	Event	Censored	Crude HR (95%	Adjusted HR
					CI)	(95% CI)
RAASIs, n	Yes	30	6	24	0.44 (0.17-0.94)	0.37 (0.14-0.81)
	No	122	55	67		
Age (year)					2.04 (0.53-8.62)	3.54 (0.86-16.02)
Sex, n	Female	63	23	40	0.83 (0.49-1.39)	0.73 (0.42-1.23)
	Male	89	38	51		
BMI (kg/m ²)					7.31	
					(0.83-41.97)	

eGFR (mL/min/1.73 m ²)					0.58 (0.08-3.92)
Diabetes	Yes	34	9	25	0.59 (0.27-1.13)
mellitus, n	No	118	52	66	
Supportive	Yes	32	14	18	1.19 (0.63-2.12)
drugs, n	No	120	47	73	
Calcium	Yes	39	11	28	0.77 (0.38-1.43)
channel	No	113	50	63	
blockers, n					
Analgesic	Yes	50	21	29	0.86 (0.50-1.44)
drugs/analgesi	No	102	40	62	
c adjuvant, n					

507 Abbreviations: BMI, body mass index; CI, confidence interval; eGFR, estimated glomerular
508 filtration rate; HR, hazard ratio; RAAS, renin-angiotensin-aldosterone system inhibitors;
509 OIPN, oxaliplatin-induced peripheral neuropathy.

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No at risk								
RAASIs group, n	131	117	107	102	100	99	99	99
Non-RAASIs group, n	510	418	342	308	290	273	267	265

521 **Figure 1** Effects of the co-administration of RAASIs and OXA-containing regimen on grade-2 or higher OIPN

522 OIPN, OXA-induced peripheral neuropathy; OXA, oxaliplatin; RAASIs, renin-angiotensin-aldosterone system inhibitors.

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531 **Figure 2** Effects of RAASIs (A. candesartan, B. enalapril, C. aliskiren, D. spironolactone) and duloxetine (E) on the decreased neurite length
532 induced by OXA treatment in PC12 cells. Each bar indicates the mean $\pm$ standard error of the mean (SEM) of 7-8 preparations. * P <0.01 and
533 ** P <0.001 vs. control. OXA, oxaliplatin; RAASIs, renin-angiotensin-aldosterone system inhibitors.

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540 **Figure 3** Effects of ERK1/2 inhibitor on RAASIs (A. candesartan, B. enalapril, C. aliskiren, D. spironolactone)-induced neuroprotective effects
541 in PC12 cells. Each bar indicates the mean $\pm$ standard error of the mean (SEM) of 7-8 preparations. * P <0.05 and ** P <0.001 vs. control. ERK1/2,
542 extracellular signal-regulated kinase 1/2; OXA, oxaliplatin; RAASIs, renin-angiotensin-aldosterone system inhibitors.

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549 **Figure 4** Effects of AC inhibitor on RAASIs (A. candesartan, B. enalapril, C. aliskiren, D. spironolactone)-induced neuroprotective effects in

550 PC12 cells. Each bar indicates the mean $\pm$ standard error of the mean (SEM) of 7-8 preparations. * P <0.05, ** P <0.01 and *** P <0.001 vs. control.

551 AC, adenylate cyclase; OXA, oxaliplatin; RAASIs, renin-angiotensin-aldosterone system inhibitors.

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