



# Influence of anticancer agents on sexual function: An in vivo study based on the US FDA Adverse Event Reporting System

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## Abstract

**Background:** Patients with cancer are treated with chemotherapeutics that cause adverse effects, including erectile dysfunction (ED).

**Objectives:** We investigated erectile function in rats after the administration of anticancer agents based on data retrieved through mining of the US Food and Drug Administration (FDA) Adverse Event Reporting System (AERS) database.

**Materials and methods:** The statistical signal strength for the association between anticancer drugs and ED was calculated using the reporting odds ratio (ROR). A drug–event combination was detected when the lower limit of the 95% confidence interval (CI) of the ROR exceeded 1.00. Rats were administered anticancer agents detected in the FDA AERS analysis. Erectile function was assessed using intracavernous pressure (ICP) and mean arterial pressure (MAP) analysis after electrical stimulation of the cavernous nerve. Statistical significance was determined using Welch's *t*-test or two-way ANOVA.

**Results:** Melphalan (L-PAM; ROR = 4.72, 95% CI = 2.78–8.00), vincristine (VCR; ROR = 2.47, 95% CI = 1.54–3.97), docetaxel (DTX; ROR = 2.25, 95% CI = 1.28–3.95), methotrexate (MTX; ROR = 1.96, 95% CI = 1.39–2.75), and doxorubicin (DOX; ROR = 1.82, 95% CI = 1.07–3.19) enhanced ED risk. L-PAM and MTX decreased the ICP/MAP ratio 1 week after administration. VCR and DOX decreased erectile function 4 weeks after administration. DTX decreased erectile function at all assessed time points.

**Discussion and conclusion:** Certain anticancer agents should be considered risk factors for ED.

Our results provide possible treatment strategies for maintaining erectile function in cancer survivors, including careful erectile function monitoring after treatment.

## KEYWORDS

anticancer agent, erectile dysfunction, risk factor, US Food and Drug Administration Adverse Event Reporting System

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## 1 | INTRODUCTION

Numerous patients with cancer are treated with various chemotherapeutic agents. The National Cancer Institute has reported over 13.7 million cancer survivors in the United States (nearly 5% of the total US population), and this is projected to substantially increase by 31%, up to 20.3 million, by 2026.<sup>1</sup> However, the consequences of cancer and its treatment, including the risk of recurrence, other concomitant chronic diseases, and persistent adverse effects, significantly affect the physical function and quality of life of patients.<sup>2–5</sup> Only a few studies have investigated the effect of anticancer agents on erectile function. For instance, Mols et al.<sup>6</sup> reported that 42% of adult male patients treated with oxaliplatin (L-OHP) complain of erectile issues. It is, therefore, necessary to identify the anticancer agents that negatively affect erectile function in cancer survivors.

Data mining, a useful method for identifying novel and potential drugs that may cause adverse events (AEs), is used by regulatory agencies and the pharmaceutical industry to screen large databases and select drug–AE pairs for further clinical review.<sup>7,8</sup> The US Food and Drug Administration (FDA) Adverse Event Reporting System (AERS) database is a rich resource of such data that serves as a powerful platform for identifying risk signals of potential association between drugs and AEs.<sup>9,10</sup> In this study, we investigated erectile function following the administration of anticancer agents to rats using data retrieved by mining the FDA AERS database. We investigated the influence of anticancer agents *in vivo* through sex hormone and intracavernous pressure (ICP) measurement to identify potential target agents, namely, melphalan (L-PAM), methotrexate (MTX), vincristine (VCR), docetaxel (DTX), and doxorubicin (DOX).

## 2 | MATERIALS AND METHODS

### 2.1 | Data sources

Data were retrieved from the FDA AERS database.<sup>9</sup> Duplicate case report forms and missing data on drug names or AEs were omitted by applying the FDA recommendation of adopting the most recent case number.<sup>11</sup>

### 2.2 | Reorganizing drug names

The FDA AERS database accurately identifies and aggregates all case reports for each marketed drug. All drug name variants (including generic names, names outside the United States, misspellings, and dosage descriptions as originally entered in the FDA AERS database) were consolidated into the common name. Spelling errors were detected and carefully confirmed by experienced pharmacists. The target drugs comprised 33 cytotoxic anticancer drugs, which were searchable in the medical database DrugBank.<sup>12</sup>

### 2.3 | Definition of AEs

AEs in the AERS database were coded using preferred terms (PTs) among the Medical Dictionary for Regulatory Activities (MedDRA) terms.<sup>13</sup> The PT “Erectile dysfunction (ED)” was selected as the target AE.

### 2.4 | Signal detection

A statistically significant association with an AE was detected as a signal.<sup>9,14</sup> The statistical signal strength of the association between anticancer drugs and ED was calculated using the reporting odds ratio (ROR) as the indicator. The ROR was calculated by identifying case reports in the FDA AERS database. A signal for a drug–event combination was detected when the lower limit of the 95% confidence interval (CI) of the ROR exceeded 1.00. We added a single count to all cells in the corresponding  $2 \times 2$  table. SAS 9.4 software was used to analyze the data (SAS Institute, Inc., Cary, NC, USA).

### 2.5 | Animals and treatment protocol

Twelve-week-old male Wistar/ST rats (Japan SLC Inc., Hamamatsu, Japan) were used in all experiments (total 314 rats). All experimental protocols were approved by the ethics review board of Nagoya City University and conducted in accordance with institutional standards for the care and use of animals (H25-P-09). The rats ( $n = 8–12$ /group; total 168 rats) were administered anticancer agents at doses used to treat rat models in previous studies.<sup>15–20</sup> L-PAM (3 mg/kg, intravenously, *i.v.*) and MTX (20 mg/kg, intraperitoneally, *i.p.*) were injected on day 1 (Figure 3A) while VCR (0.1 mg/kg, *i.v.*), DTX (5 mg/kg, *i.v.*), DOX (3 mg/kg, *i.v.*), and saline (Control, *i.v.*) were injected on days 1, 8, 15, and 22 (Figure 4A). Erectile function was measured following 1-, 2-, and 4-week treatment periods. In the Control, VCR, DTX, and DOX groups, erectile function was also assessed after 4 weeks of treatment as well as after a subsequent 4-week rest period in which no treatments were administered ( $n = 8$ /group, total 32 rats). Additionally, endothelial function was measured following a 4-week treatment period in the Control, VCR, DTX, and DOX groups ( $n = 8–10$ /group; total 34 rats). Other rats ( $n = 4$ /group; total 24 rats) were administered anticancer agents at the same doses. Finally, rats in the Control, L-PAM and MTX groups following 1 week of treatment, as well as those in the Control, VCR, DTX and DOX groups following 4 weeks of treatment, were administered tadalafil (a phosphodiesterase 5 (PDE-5) inhibitor; 10 mg/kg, *p.o.*)<sup>21</sup> at least 1 h before assessment of erectile function ( $n = 8$ /group, total 56 rats). Blood was collected weekly from the tail vein before administration of anticancer agents. After coagulation and centrifugal separation at  $800 \times g$  for 20 min at 4°C, the serum samples were stored at  $-80^\circ\text{C}$  until analysis.

## 2.6 | Measurement of biological parameters

The testosterone level was measured using a testosterone ELISA kit (Enzo Biochem, Inc., Farmingdale, NY, USA). The luteinizing hormone (LH) level was measured using a luteinizing hormone EIA Kit (Cayman Chemical, Ann Arbor, MI, USA). All measurements were performed according to the recommended protocols. The absorbance was measured using a multifunctional plate reader (Nivo 3S; PerkinElmer, Waltham, MA, USA). All biological parameters were analyzed by Fujifilm Vet Systems Co., Ltd. (Chofu, Tokyo, Japan).

## 2.7 | Examination of erectile function

ICP was measured using electrical stimulation, as previously reported.<sup>22–24</sup> Briefly, the rats from each group ( $n = 6–12$ ) were anesthetized using isoflurane (Mylan, Canonsburg, PA, USA). The carotid artery was cannulated for the continuous monitoring of the mean arterial pressure (MAP), and the left crus of the corpus cavernosum was cannulated using a 23-G needle for continuous ICP monitoring. The pressure transducer was connected via an amplifier to a data acquisition board (PowerLab 2/26; ADInstruments Pty. Ltd., Bella Vista, Australia). Stainless steel bipolar wire electrodes (Unique Medical, Osaka, Japan) and a pulse generator (Nihon Kohden, Tokyo, Japan) were used for penile stimulation under the following conditions: 1 min at 5 V, 1–16 Hz, and a square wave duration of 5 ms. Erectile function was evaluated using the maximum ICP/MAP ratio, as ICP is influenced by systemic arterial pressure.<sup>25</sup>

## 2.8 | Endothelial function analysis

Endothelial function was measured using isometric tension, as previously reported ( $n = 11$ ).<sup>22–24</sup> Briefly, the CC strips were equilibrated for a minimum of 60 min in an aerated organ bath containing Krebs's solution (119 mM NaCl, 4.6 mM KCl, 1.5 mM CaCl<sub>2</sub>, 1.2 mM MgCl<sub>2</sub>, 15 mM NaHCO<sub>3</sub>, 11 mM D-glucose, and 1.2 mM NaH<sub>2</sub>PO<sub>4</sub>) at 37°C with 5% CO<sub>2</sub>. The resting force for tissues was set to 500 mg, and changes in isometric tension were recorded using a force transducer (Nihon Kohden) connected to a data acquisition board (PowerLab 4/26). Relaxant experiments were conducted using strips pretreated with 10 μM noradrenaline (NA; Sigma Aldrich, MO, USA); the relaxant effect was induced by acetylcholine (ACh; Wako Pure Chemical Industries) and sodium nitroprusside (SNP; Sigma Aldrich). Cumulative dose (10<sup>-10</sup> to 10<sup>-4</sup> M) response curves were obtained for ACh and SNP using different tissue specimens.

## 2.9 | Real-time quantitative polymerase chain reaction (qRT-PCR)

qRT-PCR analysis was performed as previously reported.<sup>20</sup> Total RNA was extracted from CC samples ( $n = 6$ ) after ICP measurement using

RNAiso Plus (TaKaRa, Shiga, Japan) according to the manufacturer's instructions. Using a ReverTra Ace-α kit (Toyobo, Osaka, Japan), 1 μg of total RNA was reverse-transcribed into cDNA, which served as the template for qRT-PCR performed using the KAPA SYBR Fast qPCR Kit (Roche, Pleasanton, CA, USA). The primer sequences are shown in Table S1. Amplification and detection were performed using the ABI 7300 system (Applied Biosystems). The thermal cycler conditions were as follows: 50°C for 2 min; 95°C for 10 min; 40 cycles each at 95°C for 15 s and 60°C for 1 min; 15 s for 95°C, 15 s for 60°C, and 15 s for 95°C to analyze the dissociation curve. Primer specificity was verified via analysis of the dissociation curve. Target gene expression was quantified relative to β-actin expression using the comparative C<sub>T</sub> method. All measurements were performed in triplicate.

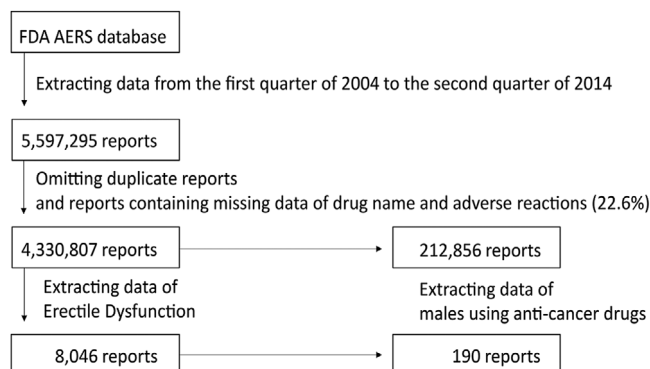
## 2.10 | Statistical analyses

Results are expressed as box-and-whisker plots. Statistical significance was determined using *F*-test and Welch's *t*-test in EZR on R commander ver 1.41.<sup>26</sup> Statistical significance in the dose-response curve for ACh and SNP was determined using two-way analysis of variance (two-way ANOVA). Results with a  $p < 0.05$  were considered statistically significant.

## 3 | RESULTS

### 3.1 | Data collection and cleaning

Figure 1 presents the flowchart for data collection and cleaning. The FDA AERS database stores 5,597,295 case reports received by the FDA from the first quarter of 2004 through the first quarter of 2014. After omitting duplicate case report forms, as well as missing data for drug names or adverse reactions, 4,330,807 case reports were included in analysis. The number of case reports with ED events during the study period was 8046. After narrowing down the cases to men



**FIGURE 1** Flowchart of data collection and cleaning. The US Food and Drug Administration (FDA) Adverse Events Reporting System (AERS) database stores 5,597,295 reports received by the FDA from the first quarter of 2004 through the first quarter of 2014

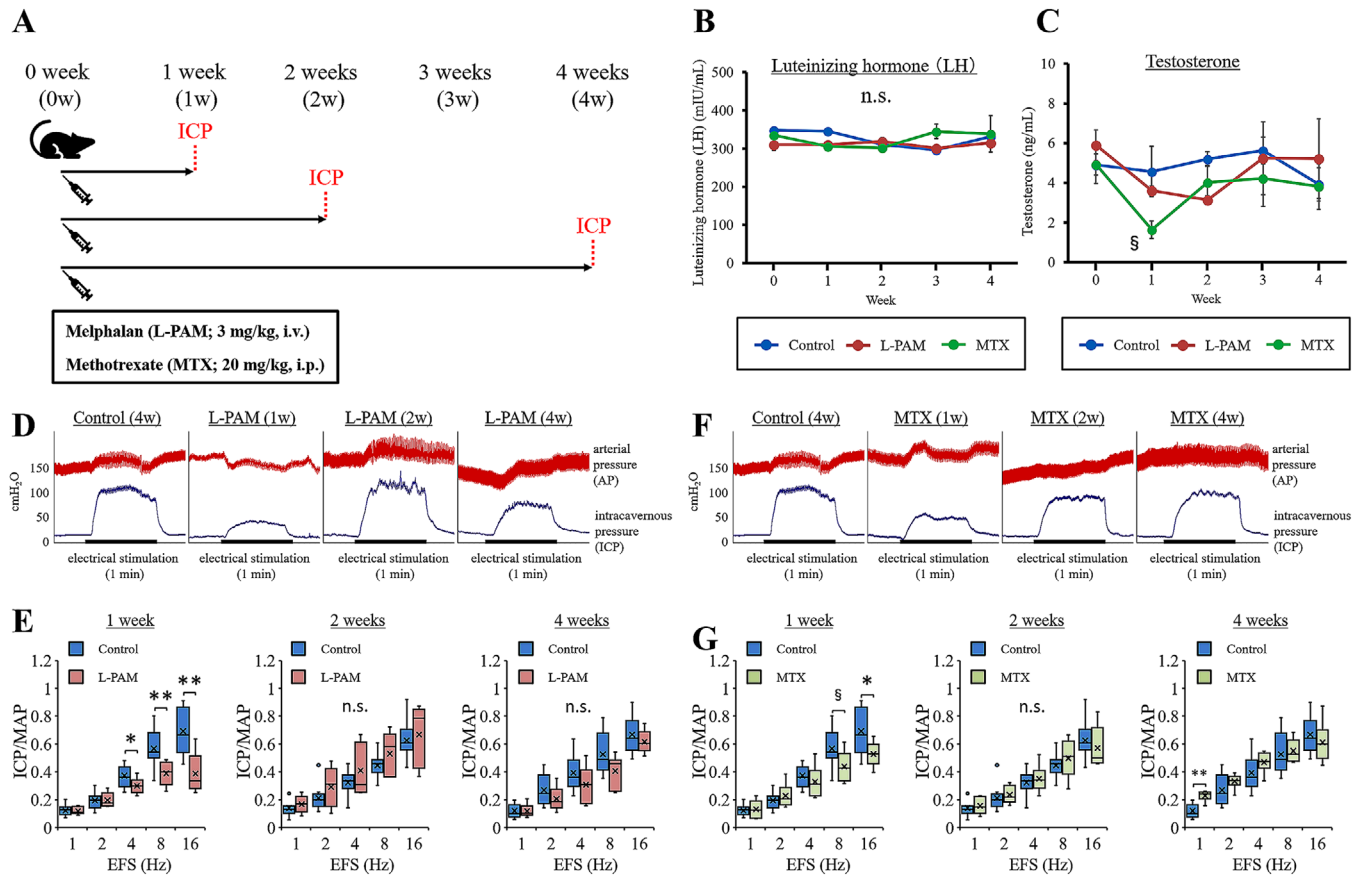


TABLE 1 Biological parameter data for rats

Group	Time	TP (g/dL)	Albumin (g/dL)	BUN (mg/dL)	Creatinine (mg/dL)	AST (IU/L)	ALT (IU/L)	LDH (IU/L)	T-CHO (mg/dL)	Triglyceride (mg/dL)	HDL-C (mg/dL)
Control	1 week	3.8 ± 0.2	2.3 ± 0.1	17.1 ± 1.7	0.32 ± 0.05	62.7 ± 7.9	26.7 ± 2.2	145 ± 18	51.0 ± 3.1	3.3 ± 0.7	20.7 ± 1.3
	2 weeks	2.8 ± 0.2	2.0 ± 0.2	13.2 ± 2.0	0.21 ± 0.02	51.0 ± 7.1	26.7 ± 3.8	167 ± 34	34.7 ± 6.8	5.3 ± 1.9	14.7 ± 2.7
	4 weeks	3.0 ± 0.3	2.0 ± 0.2	16.9 ± 2.2	0.28 ± 0.02	53.7 ± 4.4	20.3 ± 7.6	255 ± 29	32.7 ± 3.8	10.7 ± 6.7	14.3 ± 1.5
L-PAM	1 week	3.7 ± 0.4	2.5 ± 0.3	15.3 ± 1.9	0.29 ± 0.04	55.3 ± 7.0	27.3 ± 3.3	167 ± 19	35.7 ± 3.8*	4.0 ± 1.5	16.0 ± 2.3
	2 weeks	3.3 ± 0.2	2.3 ± 0.1	16.0 ± 0.9	0.27 ± 0.02	56.3 ± 9.6	27.0 ± 4.0	121 ± 11	32.7 ± 1.2	4.3 ± 0.3	14.7 ± 0.3
	4 weeks	3.4 ± 0.3	2.4 ± 0.2	16.2 ± 2.6	0.29 ± 0.02	56.7 ± 8.4	25.0 ± 2.0	361 ± 167	38.0 ± 5.9	7.3 ± 2.6	16.0 ± 2.1
MTX	1 week	3.0 ± 0.7	2.2 ± 0.5	12.5 ± 1.9	0.23 ± 0.05	49.3 ± 14.0	20.3 ± 6.4	178 ± 71	39.3 ± 9.4	4.3 ± 1.5	17.0 ± 3.6
	2 weeks	2.9 ± 0.2	2.0 ± 0.1	14.7 ± 0.9	0.22 ± 0.01	51.3 ± 2.3	23.7 ± 2.6	119 ± 7	32.3 ± 3.2	9.7 ± 2.4	15.7 ± 1.3
	4 weeks	2.0 ± 0.2	1.3 ± 0.1	8.7 ± 0.8	0.16 ± 0.02	31.0 ± 4.0	16.0 ± 2.1	134 ± 47	24.7 ± 2.2	4.5 ± 2.0	10.0 ± 0.6
VCR	1 week	3.4 ± 0.3	2.3 ± 0.3	15.6 ± 0.9	0.32 ± 0.02	48.3 ± 8.4	14.7 ± 2.4*	244 ± 104	43.7 ± 5.5	5.3 ± 1.5	17.3 ± 1.8
	2 weeks	4.2 ± 0.4*	3.0 ± 0.2*	18.7 ± 4.3	0.28 ± 0.03	79.3 ± 16.9	35.3 ± 4.8	249 ± 96	72.7 ± 15.2	7.0 ± 1.7	29.7 ± 5.0
	4 weeks	3.9 ± 0.1*	2.8 ± 0.1	17.6 ± 0.6	0.34 ± 0.03	62.0 ± 9.6	28.3 ± 0.9	314 ± 203	41.3 ± 3.8	4.3 ± 0.7	18.7 ± 0.9
DTX	1 week	2.7 ± 0.1	2.0 ± 0.1	10.4 ± 0.6*	0.23 ± 0.01	45.7 ± 3.4	16.3 ± 0.7*	138 ± 30	32.3 ± 2.0*	2.3 ± 0.33	12.7 ± 0.3*
	2 weeks	2.1 ± 0.2	1.5 ± 0.2	8.2 ± 0.9	0.25 ± 0.05	41.0 ± 4.7	17.0 ± 2.1*	76 ± 6	30.7 ± 5.8	3.7 ± 0.7	11.0 ± 2.1
	4 weeks	3.9 ± 0.2	2.6 ± 0.1*	18.8 ± 1.8	0.32 ± 0.02	53.7 ± 1.2	28.3 ± 3.3	180 ± 49	66.0 ± 3.2**	7.3 ± 0.7	28.0 ± 1.2
DOX	1 week	2.5 ± 0.2*	1.8 ± 0.1**	12.3 ± 1.7	0.19 ± 0.02	36.3 ± 1.2	17.0 ± 1.5	107 ± 25	28.7 ± 0.3*	4.7 ± 2.2	13.3 ± 0.3*
	2 weeks	3.0 ± 0.3	2.1 ± 0.2	15.2 ± 0.9	0.33 ± 0.08	52.0 ± 7.2	20.3 ± 4.4	196 ± 59	56.3 ± 8.0	5.7 ± 2.7	21.7 ± 2.2
	4 weeks	4.6 ± 0.2*	1.4 ± 0.1	34.8 ± 1.9**	0.65 ± 0.04**	31.0 ± 11.0	20.3 ± 5.0	181 ± 21	499.7 ± 8.7**	474.0 ± 10.4**	46.7 ± 8.3**

L-PAM: melphalan; MTX: methotrexate; VCR: vincristine; DTX: docetaxel; DOX: doxorubicin; TP: total protein; BUN: blood urea nitrogen; AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase; T-CHO: Total cholesterol; HDL-C: high density lipoprotein cholesterol.

Data have been reported in terms of mean ± standard deviation (n = 6 per group). \*p < 0.10, \*\*p < 0.05, and \*\*\*p < 0.01 vs. each group using Welch's t-test.



**FIGURE 3** Study of the effects of melphalan (L-PAM) or methotrexate (MTX) administration in rats. (A) Experimental design: in the Control group, rats were injected with saline. In the L-PAM group, rats were injected with L-PAM (3 mg/kg, intravenously [i.v.]; day 1). In the MTX group, rats were injected with MTX (20 mg/kg, intraperitoneally [i.p.]; day 1). Rats were submitted to erectile function testing *in vivo* at the predetermined timepoints. (B) Luteinizing hormone (LH) and (C) testosterone levels after L-PAM or MTX administration. Erectile function of rats after L-PAM administration. (D) Representative tracings of the changes in intracavernous pressure (ICP) and arterial pressure (AP) during electrical stimulation (16 Hz) of the cavernous nerve in the Control and L-PAM rats at 1, 2, and 4 weeks. (E) Erectile function according to the ICP/mean AP (MAP) ratio. Erectile function of rats after MTX administration. (F) Representative tracings of the changes in ICP and AP during electrical stimulation (16 Hz) of the cavernous nerve in the Control and MTX rats at 1, 2, and 4 weeks. (G) Erectile function according to the ICP/MAP ratio. Data are presented as box-and-whisker plot ( $n = 6-12$ );  $\$p < 0.10$ ,  $*p < 0.05$ , and  $**p < 0.01$  vs. each group using Welch's *t*-test

changes in ICP and arterial pressure (AP) during electrical stimulation of the cavernous nerve in the L-PAM group. The ICP in the L-PAM group at 1 week appeared to be significantly lower than that in the Control group, whereas it was similar in both groups at 2 and 4 weeks. The L-PAM group at 1 week exhibited a significantly lower ICP/MAP ratio than the Control group at 8 and 16 Hz ( $p < 0.05$ ; Figure 3E). Additionally, the MTX group exhibited a significantly lower ICP/MAP ratio at 1 week compared to that of the Control group at 16 Hz ( $p < 0.05$ ; Figure 3G).

Meanwhile, the VCR group exhibited a significantly lower ICP/MAP ratio at 4 weeks compared to the Control group at 8 and 16 Hz ( $p < 0.05$ ; Figure 4E). Additionally, the ICP/MAP ratio in the VCR group following 4 weeks of treatment and subsequent 4 weeks of rest, without injections, was significantly lower than that of the Control group at 2, 4, 8, and 16 Hz ( $p < 0.05$ ).

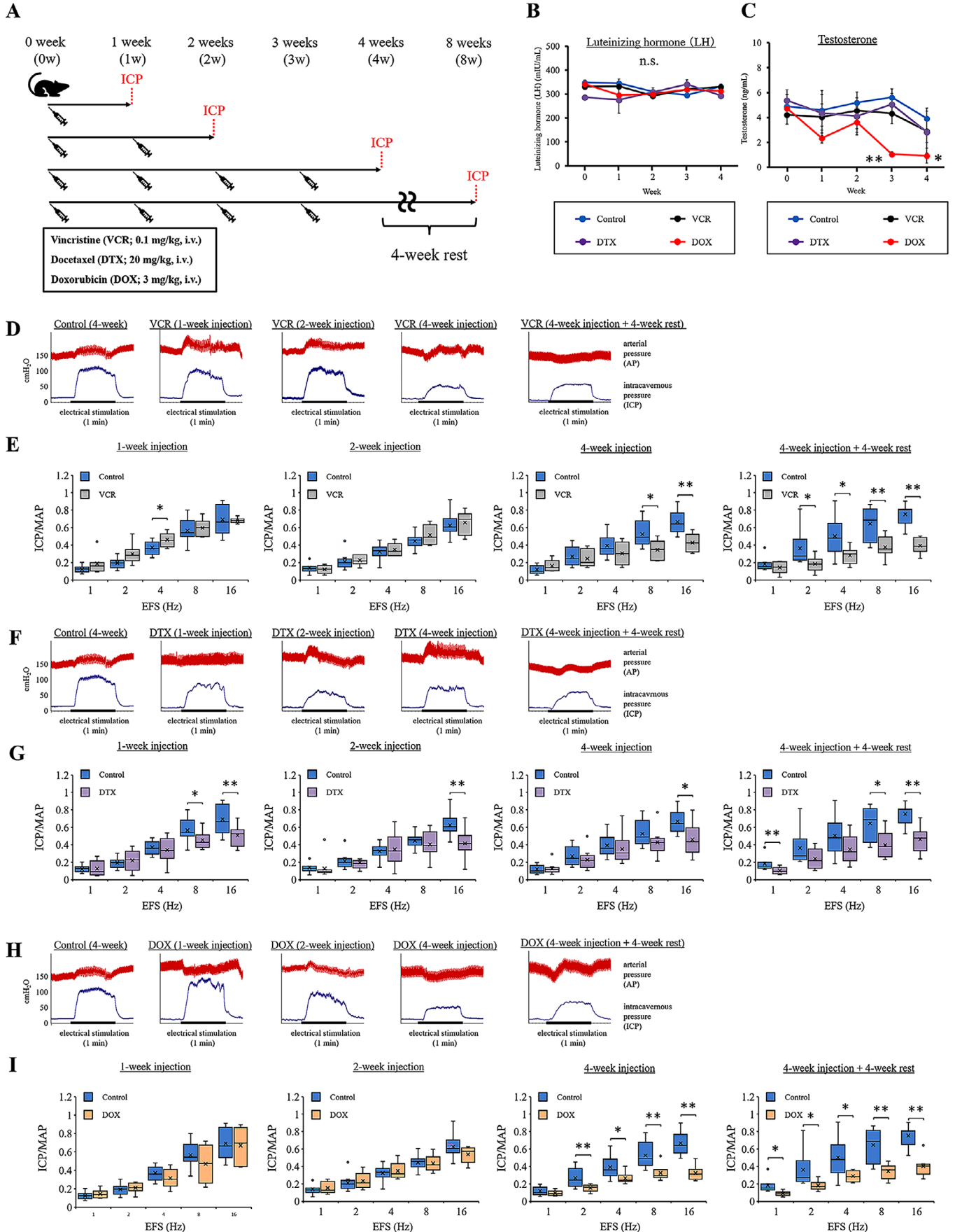
The DTX group showed a decrease in the ICP/MAP ratio at all periods compared to the Control group at 16 Hz ( $p < 0.05$ ; Figure 4G). Similarly, the ICP/MAP ratio in the DTX group following 4 weeks of

injection and 4 weeks of rest was significantly lower than that of the Control group at 8 and 16 Hz ( $p < 0.05$ ; Figure 4G).

The DOX group exhibited a significantly lower ICP/MAP ratio at 4 weeks than the Control group at all stimulation levels ( $p < 0.05$ ; Figure 4I). The ICP/MAP ratio in the DOX group following 4 weeks of treatment and 4 weeks of rest was significantly lower than that of the Control group in all Hz ( $p < 0.05$ ; Figure 4I).

Figure 5 shows erectile function in rats treated with tadalafil. The ICP/MAP ratio in the Control + Tad tended to increase compared to the Control group at 1, 2, and 16 Hz ( $p < 0.10$ ). Similarly, the ICP/MAP ratio in the L-PAM + Tad rats was significantly increased compared to the L-PAM group at 16 Hz ( $p < 0.05$ ). The ICP/MAP ratio in the MTX + Tad also tended to increase compared to the MTX group at 1 and 16 Hz, however these results did not reach statistical significance ( $p < 0.10$ ) (Figure 5D).

The ICP/MAP ratio in the Control + Tad was significantly increased compared to the Control group at 2, 8, and 16 Hz ( $p < 0.05$ ). The ICP/MAP ratio in the VCR + Tad did not differ compared to the VCR



group ( $p > 0.05$ ). The ICP/MAP ratio in the DTX + Tad tended to increase compared to the DTX group at 16 Hz, however this result was not significant ( $p < 0.10$ ). The ICP/MAP ratio in the DOX + Tad did not differ compared to the DOX group ( $p > 0.05$ ) (Figure 5F).

### 3.6 | Endothelial function

Figure 6 shows the relaxation responses of the NA-pretreated rat CC strips to increasing concentrations of ACh and SNP in the Control, VCR, DTX, and DOX group at 4 weeks. The VCR, DTX, and DOX groups exhibited significantly lower responses to ACh than the Control group ( $p < 0.01$ ). The SNP responses at  $10^{-10}$  to  $10^{-4}$  M did not significantly differ between the groups ( $p > 0.05$ ).

### 3.7 | mRNA expression analysis

eNOS and nNOS mRNA expressions were not altered in the L-PAM group ( $p > 0.05$ ; Figure 7A); however, the NADPH oxidase mRNA expression was significantly increased in this group ( $p < 0.05$ ; Figure 7A). Although eNOS and nNOS mRNA expressions were significantly decreased in the MTX group ( $p < 0.01$ ; Figure 7B), the NADPH oxidase-4 mRNA expression significantly increased ( $p < 0.05$ ). Similarly, eNOS and nNOS mRNA expressions were significantly decreased in the VCR group at 4 weeks ( $p < 0.01$ ; Figure 7C), while NADPH oxidase-2 and -4 expression was significantly increased at the same time point ( $p < 0.01$ ). eNOS and nNOS mRNA expressions were significantly decreased in the DTX group at all periods ( $p < 0.01$ ; Figure 7D), however, the expression of NADPH oxidase-2 and -4 mRNA was only significantly increased at 4 weeks ( $p < 0.01$ ). Finally, eNOS and nNOS mRNA expressions were significantly decreased in the DOX group at 1 and 4 weeks ( $p < 0.01$ ; Figure 7E), whereas the expression of NADPH oxidase -1 and -2 mRNA was significantly increased ( $p < 0.05$ ).

## 4 | DISCUSSION

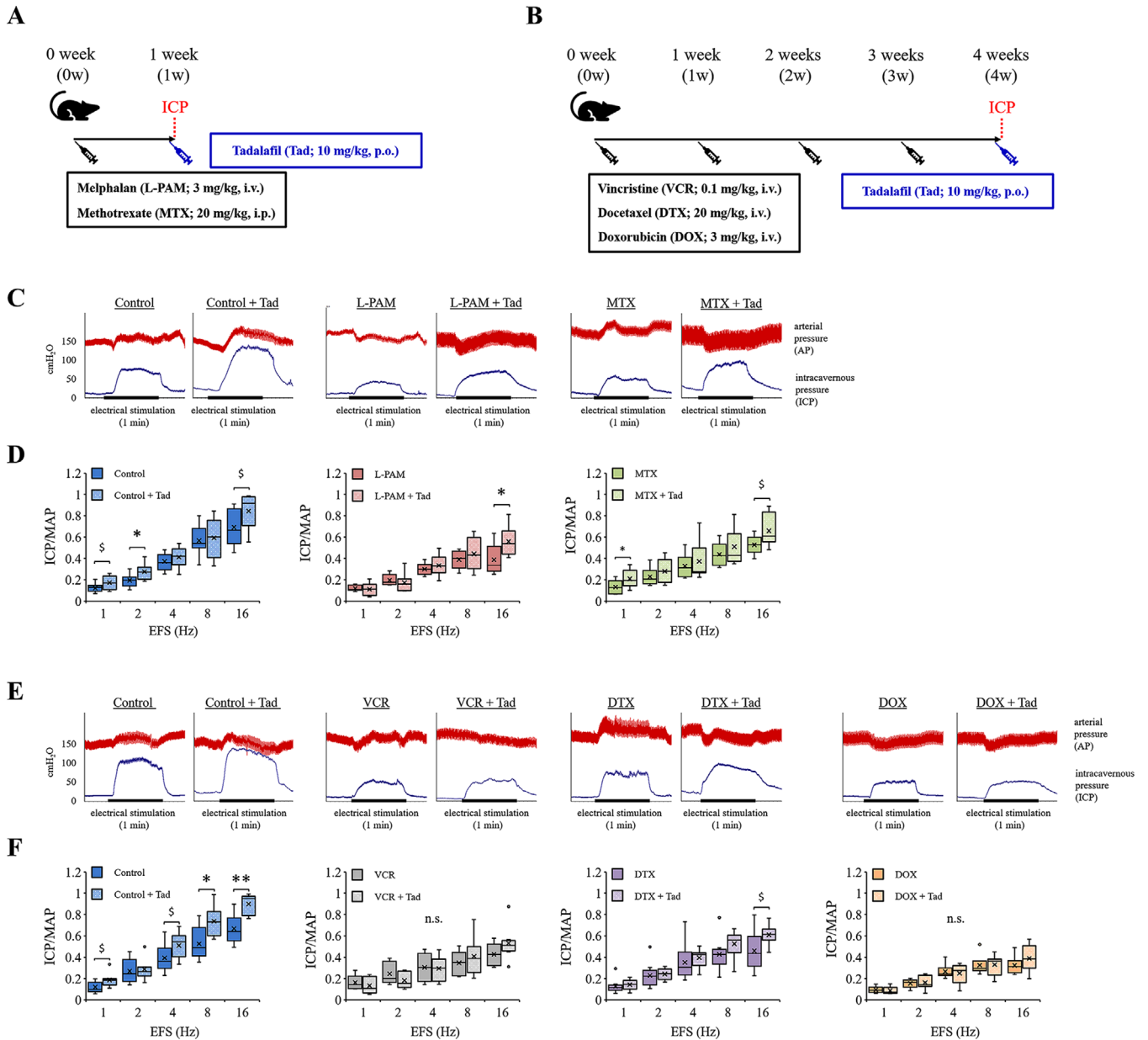
In this study, by analyzing information retrieved from the FDA AERS database we identified five cytotoxic anticancer agents (L-PAM, VCR, DTX, MTX, and DOX) that significantly increase the risk of ED. Moreover, we found that these anticancer agents affect erectile function in rats. L-PAM and MTX decrease the ICP/MAP ratio 1 week after

administration, whereas no change was observed after 2 and 4 weeks of treatment. These two drugs are used to treat patients with multiple myeloma undergoing hematopoietic cell transplantation. Following administration of L-PAM and MTX to rats on day 1, high doses of both temporarily decreased erectile function and testosterone levels.<sup>27,28</sup> However, L-PAM also injures Leydig cells and decreases testosterone level, which are essential for erectile function.<sup>29</sup> Therefore, it may be necessary to carefully monitor erectile function during treatment with L-PAM. To further investigate the mechanism of the observed ED, we also measured the mRNA expression of specific enzymes associated with oxidative stress in the corpus cavernosum. Both L-PAM and MTX were found to upregulate oxidative stress through increasing NADPH oxidase in the corpus cavernosa. Additionally, the expression of eNOS and nNOS mRNA decreased in MTX rats. These factors have all been reported to cause tissue damage through increasing inflammation.<sup>30</sup> However, further analysis of the protein levels is required to verify these findings. Nevertheless, changes in NO production should be monitored in patients administered L-PAM and MTX. Moreover, in this study, administration of tadalafil, a 5 PDE-5 inhibitor, effectively increased the erectile function in rats and, thus, may represent an effective treatment option to counter ED in patients administered these anticancer agents.

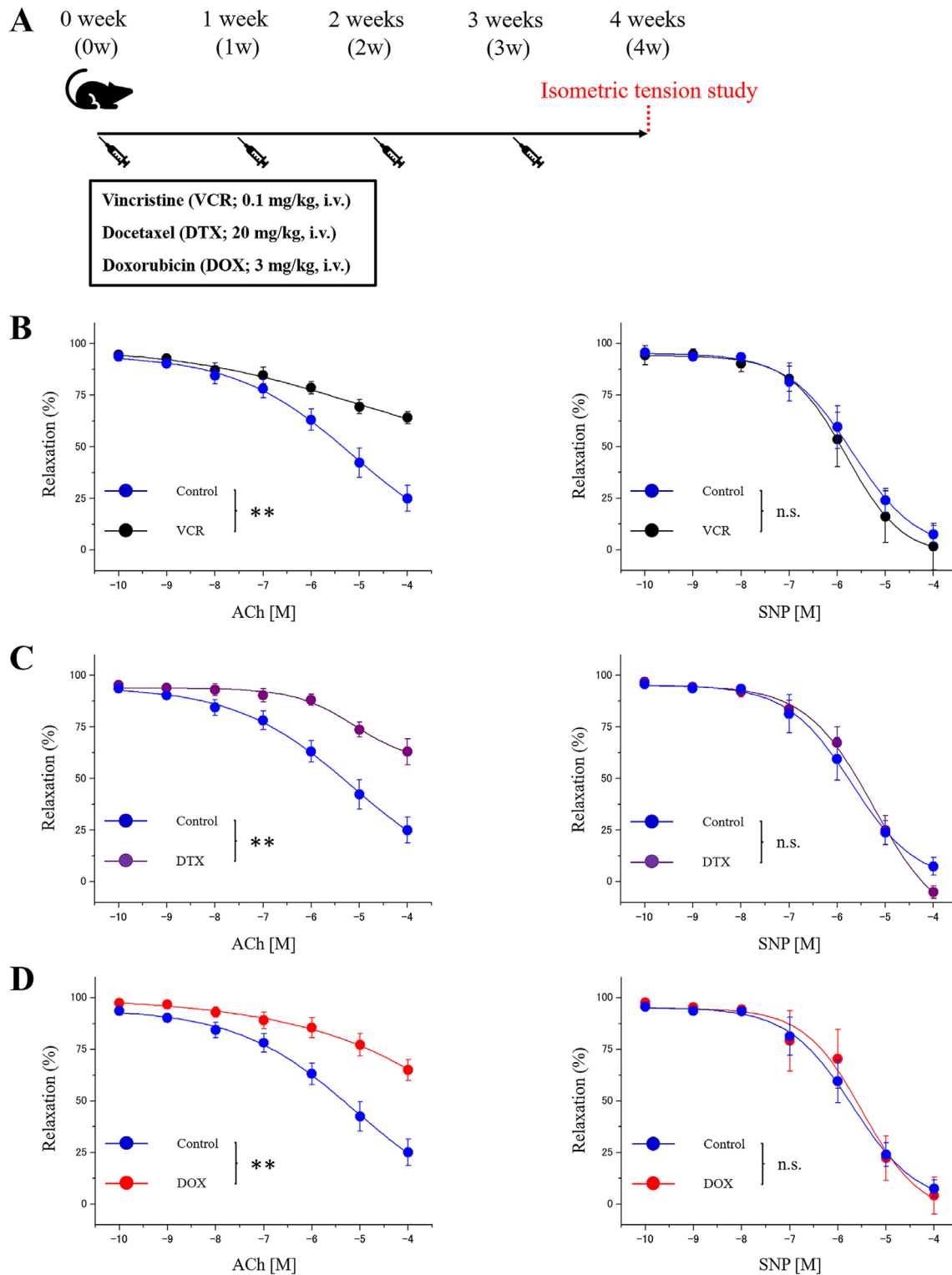
Both VCR and DOX are used in the cyclophosphamide, hydroxydaunorubicin (DOX), oncovin (vincristine), and prednisone or prednisolone (CHOP) chemotherapeutic protocol for malignant lymphoma and pediatric tumors.<sup>31</sup> VCR and DOX did not affect erectile function until after 2 weeks of treatment, and significantly decreased erectile function by 4 weeks. An infamous adverse effect of VCR is peripheral neuropathy,<sup>32</sup> which can damage the cavernous nerve. Meanwhile, DOX induces cardiotoxicity,<sup>33</sup> which is accelerated in androgen receptor knock-out mice.<sup>34</sup> In our study, both VCR and DOX increased oxidative stress and decreased eNOS and nNOS mRNA expression. Moreover, assessment of endothelial function using isometric tension analysis revealed that VCR and DOX administration for 4 weeks decreased the response to ACh; however, no significant change was noted in the response to SNP. These results indicate that VCR and DOX do not affect smooth muscle relaxation of the corpus cavernosum but rather increased relaxation via endothelial NO, causing endothelial dysfunction. Hence, these functional disorders may alter the manifestations of ED. Moreover, considering that DOX also decreased testosterone levels, it is expected to also decrease libido in patients. Further, DOX caused renal dysfunction and

**FIGURE 4** Study of the effects of vincristine (VCR), docetaxel (DTX), or doxorubicin (DOX) administration in rats. (A) Experimental design: in the Control group, rats were injected with saline. In the VCR group, rats were injected with VCR (0.1 mg/kg, intravenously [i.v.]; days 1, 8, 15, and 22). In the DTX group, rats were injected with DTX (5 mg/kg, i.v.; days 1, 8, 15, and 22). In the DOX group, rats were injected with DOX (3 mg/kg, i.v.; days 1, 8, 15, and 22). Rats were submitted to erectile function testing in vivo at the predetermined timepoints. (B) Luteinizing hormone (LH) and (C) testosterone levels after VCR, DTX, or DOX administration. Erectile function of rats after VCR administration. (D) Representative tracings of the changes in intracavernous pressure (ICP) and arterial pressure (AP) during electrical stimulation (16 Hz) of the cavernous nerve in the Control and VCR rats at 1, 2, and 4 weeks. (E) Erectile function according to the ICP/mean AP (MAP) ratio. (F–G) Erectile function of rats after DTX administration. (F) Representative tracings of the changes in ICP and AP during electrical stimulation (16 Hz) of the cavernous nerve in the Control and DTX rats at 1, 2, and 4 weeks. (G) Erectile function according to the ICP/MAP ratio. Erectile function of rats after DOX administration. (H) Representative tracings of the changes in ICP and AP during electrical stimulation (16 Hz) of the cavernous nerve in the Control and DOX rats at 1, 2, and 4 weeks. (I) Erectile function according to the ICP/MAP ratio. Data are presented as box-and-whisker plot ( $n = 6-12$ );  $\$p < 0.10$ ,  $*p < 0.05$ , and  $**p < 0.01$  vs. each group using Welch's *t*-test

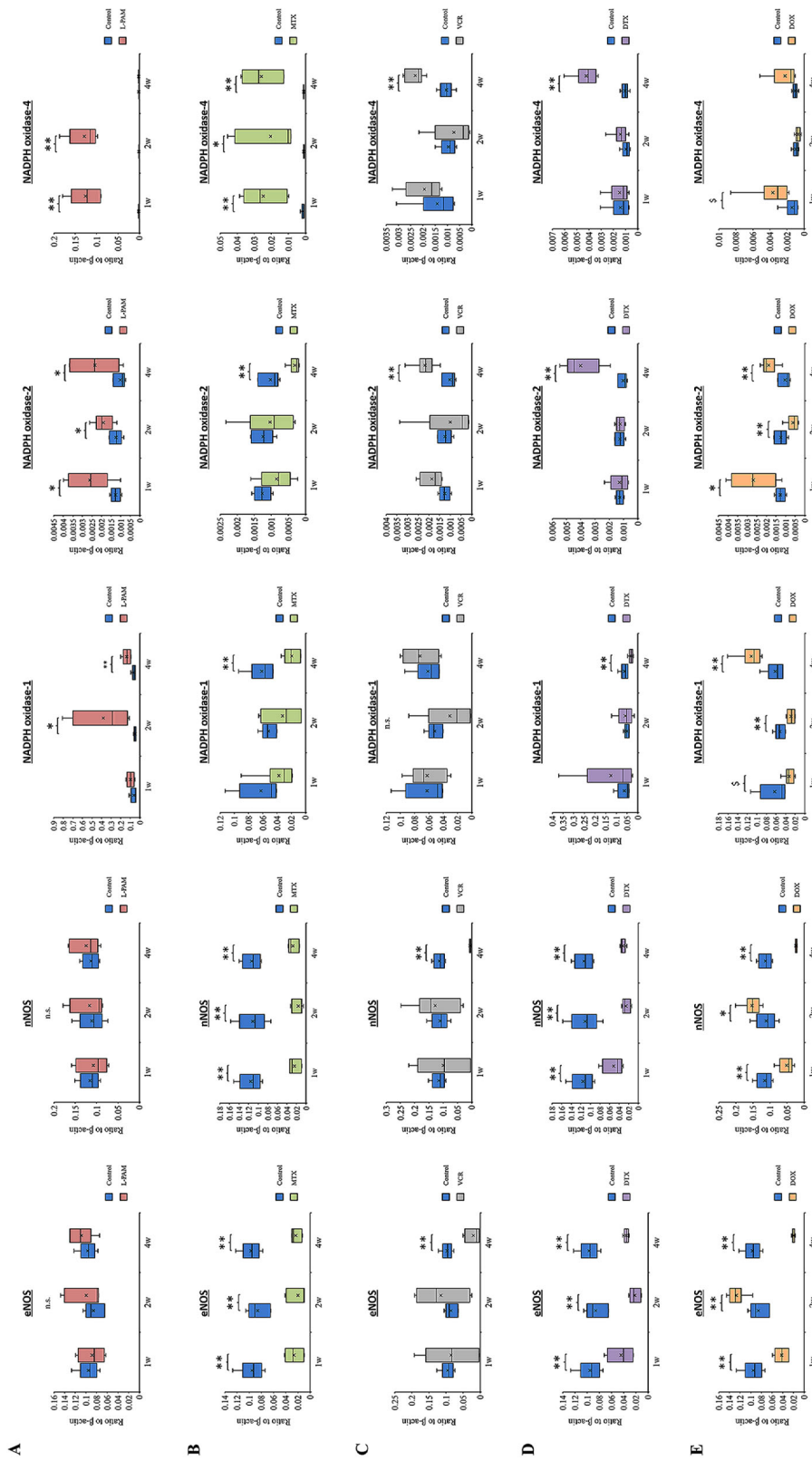




**FIGURE 5** Study of the effects of tadalafil (Tad) following administration of melphalan (L-PAM), methotrexate (MTX), vincristine (VCR), docetaxel (DTX), or doxorubicin (DOX) to rats. (A), (B) Experimental design: Control group, rats were injected with saline; L-PAM and L-PAM + Tad groups, injected with L-PAM (3 mg/kg, intravenously [i.v.]; day 1); MTX and MTX + Tad groups, injected with MTX (20 mg/kg, intraperitoneally [i.p.]; day 1); VCR and VCR + Tad groups, injected with VCR (0.1 mg/kg, intravenously [i.v.]; days 1, 8, 15, and 22); DTX and DTX + Tad groups, injected with DTX (5 mg/kg, i.v.; days 1, 8, 15, and 22); DOX and DOX + Tad groups, injected with DOX (3 mg/kg, i.v.; days 1, 8, 15, and 22). Tadalafil (10 mg/kg) was administered at least 1 h before erectile function measurements. Rats were subjected to erectile function testing in vivo at the predetermined timepoints. Erectile function of rats after L-PAM and MTX administration and Control with tadalafil. (C) Representative tracings of the changes in intracavernous pressure (ICP) and arterial pressure (AP) during electrical stimulation (16 Hz) of the cavernous nerve in rats. (D) Erectile function according to the ICP/mean AP (MAP) ratio. Erectile function after VCR, DTX, and DOX administration and Control with tadalafil. (E) Representative tracings of the changes in ICP and AP during electrical stimulation (16 Hz) of the cavernous nerve. (G) Erectile function according to the ICP/MAP ratio. Data are presented as box-and-whisker plots ( $n = 8$ );  $\$p < 0.10$ ,  $*p < 0.05$ , and  $**p < 0.01$  vs. each group using Welch's  $t$ -test



**FIGURE 6** Analysis of isometric tension in vincristine (VCR), docetaxel (DTX), or doxorubicin (DOX) administered rats. (A) Experimental design: Control group, injected with saline; VCR group, injected with VCR (0.1 mg/kg, intravenously [i.v.]; days 1, 8, 15, and 22); DTX group, injected with DTX (5 mg/kg, i.v.; days 1, 8, 15, and 22); DOX group, injected with DOX (3 mg/kg, i.v.; days 1, 8, 15, and 22). Rats were subjected to isometric tension analysis after 4 weeks of treatment. (B–D) Acetylcholine (ACh)-induced relaxation in rat corpus cavernosum strips. The relaxant effect of increasing concentrations of ACh ( $10^{-10}$  to  $10^{-4}$  M) on corpus cavernosum strips. The strips were precontracted using  $10^{-5}$  M noradrenaline (NA). Sodium nitroprusside (SNP)-induced relaxation curve for rat CC strips. The relaxant effect of increasing concentrations of SNP ( $10^{-10}$  to  $10^{-4}$  M) on CC strips. The strips were preexposed to  $10^{-5}$  M NA. Data have been reported in terms of mean  $\pm$  standard deviation ( $n = 7$ –11 per group). \*\* $p < 0.01$  vs. the control group using two-way analysis of variance (two-way ANOVA)



**FIGURE 7** Expression of endothelial nitric oxide (eNOS), neuronal NOS (nNOS), and oxidative stress-related genes in the corpus cavernosum of rats. Target gene expression was quantified relative to the expression of  $\beta$ -actin using the comparative  $C_T$  method. L-PAM, Melphalan; MTX, methotrexate; VCR, vincristine; DTX, docetaxel; DOX, doxorubicin; NADPH, neuronal nitric oxide synthase. Data are presented as box-and-whisker plot ( $n = 6$ );  $\$p < 0.10$ ,  $*p < 0.05$ , and  $**p < 0.01$  vs. each group using Welch's t-test

dyslipidemia. Thus, unfortunately, ED caused by VCR or DOX would be prolonged. Although the time required for rats cannot be directly applied to humans, Sengupta reports that 16.4–16.7 days for a rat equates to approximately one human year.<sup>35,36</sup> Thus, the 4-week rest period applied in the current study, would approximate two human years. Further, since administration of PDE-5 inhibitor before ICP measurement did not impact the ED caused by VCR or DOX, we believe that continuous administration of PDE-5 inhibitors, or other preventive treatments, will be needed as well as careful monitoring of erectile function during the administration of these drugs.

DTX is widely used for treating cancers, such as lung, gastric, and prostate cancers.<sup>37</sup> However, it has also been shown to cause peripheral neuropathy and could, thus, injure the cavernous nerve.<sup>38</sup> In the current study, DTX decreased erectile function at all examined time points and also decreased nNOS and eNOS mRNA expression. Moreover, DTX upregulated oxidative stress and decreased endothelial function, causing prolonged ED. However, administration of tadalafil increased the erectile function in rats and may, thus, be useful as a treatment option for ED caused by DTX.

Although L-OHP has been reported to cause ED,<sup>39</sup> we did not observe this effect. This discrepancy in findings is likely due to the FDA AERS database contributing spontaneous reports to the FDA. Thus, the number of ED reports is limited, particularly in patients with cancer. In the future, we plan to evaluate the influence of L-OHP on erectile function in rats.

Certain limitations were noted in this study. First, the FDA requires further evaluation using real-world evidence, such as through the Sentinel System. Although we attempted to evaluate the results of FDA AERS using data from a hospital information system, the number of reported AEs related to erectile function owing to anticancer agents was small. Therefore, we elected to evaluate the results using an animal model instead. Second, although we found that the anticancer agents caused ED in rats, we did not fully elucidate the underlying mechanisms. To address this in future studies, histological evaluation and quantification of protein levels will be necessary.

With an increase in the number of cancer survivors, it is increasingly important for health care professionals to report ED cases to the FDA, which will serve to enhance the accuracy of analyses. As the current study only incorporated a portion of reported ED cases following administration of anticancer agents in the FDA database, we plan to continue our investigation on the influence of these medications on erectile function. Since these anticancer agents are used in combination with multiple drugs depending on the regimens, it is necessary to investigate the influence of multiple drugs. Additionally, age differences also need to be considered. The drugs detected to have a significant risk for ED have different mechanisms of action; thus, further studies are needed in this regard.

## 5 | CONCLUSIONS

The investigated anticancer agents, retrieved after data mining of the FDA AERES database, affected erectile function in an animal model.

Our results should be corroborated by additional investigations; however, the identified anticancer agents should be considered a risk factor for ED. Furthermore, it may be necessary to carefully monitor erectile function in patients after treatment. Our study sheds light on possible treatment strategies for improving the quality of life for cancer survivors, including maintaining erectile function.

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## CONFLICT OF INTEREST

The authors have no conflicts of interest to declare that are relevant to the content of this article.

## AUTHOR CONTRIBUTIONS

TK, AS, and KK conceptualized the study. TK, AS, and KK contributed to the methodology. TK, AS, JS, TM, YH, and YM contributed to the investigation. TK, AS, and KK wrote the original draft. YK, MT, and KK reviewed and edited the draft. TK and KK acquired funding. TK, AS, JS, TM, YH, and YM provided resources. MT and KK supervised the research.

## RESEARCH INVOLVING ANIMALS

All experimental protocols were approved by the ethics review board of Nagoya City University and conducted in accordance with institutional standards for the care and use of animals (H25-P-09).

## DATA AVAILABILITY STATEMENT

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

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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