Toxicology Reports 13 (2024) 101814

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Cilostazol geno-protective effects mitigate carbamazepine-induced genotoxicity in human cultured blood lymphocytes

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ARTICLE INFO

Keywords:
Carbamazepine
Cilostazol
Sister chromatid exchange
Cell kinetic assays
Genotoxic
Cytotoxic

ABSTRACT

Background: Carbamazepine is one of the most widely used antiepileptic drugs. Carbamazepine has been shown to be toxic to cells. Cilostazol, an antiplatelet agent, has known antioxidant, antiproliferative, anti-inflammatory, and anti-tumor effects.
Objective: This study aimed to explore whether carbamazepine and cilostazol exert genotoxic and/or cytotoxic effects in human cultured blood lymphocytes and the impact of combining both drugs on such effects.
Methods: Genotoxicity was examined using sister chromatid exchange (SCE) assay, while cytotoxicity was evaluated by cell kinetic assays (mitotic and proliferative indices).
Results: Study findings have revealed that carbamazepine markedly increased SCEs ($p<0.01$), while cilostazol significantly decreased their frequencies ($p<0.01$). In addition, the frequency of SCEs of the combination of both drugs was similar to that of the control group ($p>0.05$). Carbamazepine increased the cell proliferative index ($p<0.01$) while cilostazol decreased it ($p<0.01$). The proliferative index was normalized to the control level when both drugs were combined.
Conclusion: We suggest that cilostazol has the potential to protect human lymphocytes from carbamazepine-induced toxic effects.

1. Introduction

Carbamazepine is one of the most widely prescribed antiepileptic medications approved by Food and Drug Administration (FDA) for the management of several disorders including partial, generalized, and mixed seizures, trigeminal neuralgia, and bipolar I disorder [1,2]. Carbamazepine acts by inhibiting voltage-gated sodium channels leading to inhibition of action potential generation and synaptic transmission [2]. Previous research studies have indicated that carbamazepine is genotoxic in various systems including cultured human blood lymphocytes, human embryonic stem cells, hepatocytes, and other systems [3–10]. Moreover, some of the available evidence has demonstrated carbamazepine-induced cytotoxicity, while others have not [6–8].

Cilostazol, an antiplatelet agent and a vasodilator, is FDA approved for treating intermittent claudication of peripheral vascular disease [11]. It is also indicated for the secondary prevention of stroke in patients with a history of transient ischemic attacks or non-cardioembolic ischemic stroke [11,12]. It increases intracellular cyclic adenosine monophosphate (cAMP) by selectively inhibiting type-III phosphodiesterase enzyme (PDE3). Its clinical uses depend on its antiplatelet, anti-inflammatory, antioxidants, antiproliferative and vasodilatory effects [12–14]. By inhibiting PDE3 and the subsequent cAMP rise in platelets and blood vessels, cilostazol increases activated protein kinase A (PKA) which inhibits platelets aggregation and inactivates myosin light-chain kinase leading to vascular smooth muscle cells dilation [11]. Cilostazol has been demonstrated to suppress oxidative stress and exert protective properties in many models and systems [13,15–19]. In addition, cilostazol has been shown to have protective properties against genotoxicity caused by the anticancer drug methotrexate *in vitro* [20].

In this study, we have investigated the geno- and cyto-toxic effects of the combination of the antiepileptic agent carbamazepine and the

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https://doi.org/10.1016/j.toxrep.2024.101814
Received 26 July 2024; Received in revised form 28 October 2024; Accepted 12 November 2024
Available online 17 November 2024

cardiovascular medication cilostazol. Studying this combination is reasonable because people with epilepsy are at higher risk of cardiovascular disease (CVD) such as myocardial infarction, stroke, ischemic heart disease, and thromboembolism and have a higher mortality rate from these conditions compared to the general population. They also have an increased CV risk factors including higher risk of diabetes mellitus which significantly increases the risk of CVD and stroke [21–23]. This means that it is expected to have comorbid patients taking both carbamazepine as the antiepileptic agent and cilostazol as the antiplatelet/vasodilator medication for CVD prevention/treatment. Furthermore, cytochrome P450 enzyme-inducing antiepileptic agents (such as carbamazepine) have known to increase the CV risk [21,24]. Thus, it is rational to study carbamazepine as a commonly and widely prescribed antiepileptic medication with the antiplatelet agent/vasodilator cilostazol that is indicated for CVD prevention/treatment. Of note, cilostazol is a promising antiplatelet agent as it has demonstrated favorable clinical outcomes in atherosclerotic vascular disease like coronary and cerebral artery disease. For ischemic vascular protection, it has been used as adjunctive therapy with other antiplatelet medications such as clopidogrel (cilostazol has a synergistic antiplatelet mechanism of action when combined with clopidogrel) without increasing bleeding risk in high thrombotic risk patients [25,26].

Here, we hypothesized that cilostazol has beneficial effects that mitigate carbamazepine-induced genotoxicity. Thus, we investigated the genotoxic and cell survival effects of carbamazepine, cilostazol, and the combination of both drugs using human cultured blood lymphocytes, sister chromatids exchange (SCE) and mitotic/proliferative assays.

2. Materials and methods

2.1. Blood samples

Blood samples were withdrawn peripherally under aseptic conditions from 5 healthy adult male volunteers and were placed in heparinized test tubes. The donor ages ranged from 20 to 28 years. Exclusion criteria were subjects with medical conditions, on medications, drinking alcohol, or smoking tobacco. Venous blood samples were taken in the morning on the same test day to avoid diet interference. Participation in the study was voluntary and written informed consent was obtained from each subject after explaining the aims and procedure of the study. The study was approved by the Institutional Review Board (IRB approval no. 27/132/2020; Dated: 31/3/2020) at Jordan University of Science and Technology, and the study was implemented according to the Code of Ethics of the World Medical Association (Declaration of Helsinki) for Research Studies involving human.

2.2. Cell culture and treatment

Each freshly obtained whole blood sample was cultured immediately and was considered as one set of experiments. Lymphocyte cultures were prepared in 50 mL tissue-culture flasks by adding 1 mL of fresh heparinized blood to 9 mL optimized RPMI 1640 medium (PB-MAX™ Karyotyping Medium, Gibco-Invitrogen, UK) supplemented with 15 % fetal bovine serum, L-glutamine, gentamicin sulfate, and phytohemagglutinin [27]. Cultures were incubated in darkness in 5 % CO2 at 37 ºC for 72 hours.

Drug treatment of lymphocytes culture involved a stoke solution of carbamazepine (95 %, Sigma-Aldrich, USA) that was dissolved in ethanol (95 %, Sigma-Aldrich, USA) with a working concentration in the culture flask of 12 μg/mL corresponding to the therapeutic plasma carbamazepine concentration at steady stat [6,28,29]. Carbamazepine was added to the culture 24 hours before cell harvesting. Cilostazol (98 % pure, Santa Cruz Biotechnology, USA) stock solution (1.2 mg/mL) was prepared in dimethyl sulfoxide (DMSO) with a final working concentration (1.2 μg/mL) that complied with the serum cilostazol level of the standard dosage of this drug in human [30]. Cilostazol was added to the cell culture media at the start of culturing. Control cultures were treated with vehicle (ethanol) in equivalent quantity to that used in the drug treated groups.

The study groups were divided into four: one control group and three experimental groups. The experimental groups were carbamazepine, cilostazol, and a combination of both drugs.

2.3. Sister chromatid exchange (SCE) assay

Before incubating the cells, 25 μL of the light sensitive bromodeoxyuridine (0.01 g/mL) (BrdUrd, Sigma-Aldrich, USA) were added to the cultured cells in darkness to avoid photolysis [31]. Two hours before harvesting the culture cells, they were treated with the spindle inhibitor Colcemid (Gibco-Invitrogen, UK) with a final concentration of 0.1 μg/mL for cells' metaphase arresting. After the incubation period, cultured cells were transferred to 15 mL tubes which were then centrifuged at 1000 rpm for 10 minutes. Then, the cellular pellet was resuspended in pre-warmed hypotonic solution (0.56 % KCl) before being incubated for 30 minutes at 37 ºC. Following that, cells centrifugation at 1000 rpm for 10 minutes was done before fixing the resultant cellular pellet with a freshly prepared ice-cold fixative solution of methanol and acetic acid (3:1) and was left in darkness at room temperature for 20 minutes. The pellet was then rinsed 3 times with the fixative solution before being suspended in 1 mL of this same solution. The fixed cells were then dropped on pre-chilled slides for metaphases spreading after which they were allowed to air dry. Next, the cells were stained with Hoechst 32285 dye solution (10 μg/mL) for 20 minutes. After rinsing the slides with distilled water, they were mounted in McIlvaine buffer (pH 8.0). Then, slides were UV irradiated (350 nm) at 7 cm and 40 ºC for 30 minutes. Following that, slides were rinsed with distilled water and then dried at room temperature before being stained with 5 % Giemsa stain in Gurr buffer (pH 6.8) for 4 minutes. Lastly, slides were washed with distilled water and were allowed for air-dry at room temperature [32].

To analyze SCE, 1000x oil magnification of a high-resolution light microscope (Nikon, Japan) was used to score the second division metaphase (M2) cells per group/donor. The M2 (42–46 chromosomes) appeared with two differentially stained sister chromatids of light and dark stains. For the first division metaphase (M1), stained chromosomes appeared as two dark sister chromatids. When chromosomes appeared light, dark, and differentially stained, then this represented the third/fourth division metaphase (M3/M4) [33].

2.4. Cell kinetic analysis

For the evaluation of cytotoxicity on human cultured blood lymphocytes, the mitotic index (MI) and proliferative index (PI) were determined. The MI of blood lymphocytes was determined by the random analysis of 1000 cells per culture and by counting the metaphase cells. MI is the ratio of the number of cells undergoing mitosis per high power field of view divided by the total number of cells in the examined cell population*100 [34].

PI was calculated to assess the drugs' effect on the rate of the cell division process by scoring the first-, second-, and third-division mitoses (M1, M2, and M3, respectively) in 100 metaphases from each donor according to the following equation [35]

$$1*M1+ 2*M2+ 3* \geq M3)/100 \quad (1)$$

2.5. Health and safety

All mandatory laboratory health and safety procedures have been complied with all occupational health and safety instructions, and

university policies and procedures.

2.6. Statistical analysis

The data of the study were analyzed statistically using GraphPad Prism 8.4.3 (686). Data were represented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and Tukey's post hoc test were used for statistical analysis. The threshold for statistical significance was fixed at a $p<0.05$.

3. Results

3.1. Carbamazepine-induced genotoxicity and cilostazol impact

We evaluated the genotoxic effects of the two drugs cilostazol, and carbamazepine expressed as the mean frequency of sister chromatid exchanges (SCEs) on human cultured blood lymphocytes. The four study groups of untreated human cultured blood lymphocytes (control), cilostazol-treated (1.2 μg/mL), carbamazepine-treated (12 μg/mL), and carbamazepine- and cilostazol-treated cells have showed significant changes in the mean frequency of SCEs as it is shown in Fig. 1. The mean frequency of SCEs in the cilostazol-treated cells has been significantly reduced (1.73 ± 0.55, $p < 0.0001$) contrary to the carbamazepine-treated cells which has been significantly increased (4.90 ± 0.17, $p < 0.0001$) when both were compared to the control (3.38 ± 0.39). In addition, SCEs have been significantly different between these two drugs in which carbamazepine caused more SCEs than cilostazol (4.90 ± 0.17 versus 1.73 ± 0.55, respectively, $p < 0.0001$). However, when the two drugs were combined, the demonstrated cilostazol geno-protective effect and carbamazepine genotoxic effect of each agent alone were eliminated and were similar to the control (3.47 ± 0.51 versus 3.38 ± 0.39, respectively, $p > 0.05$). Moreover, the two-drug combination significantly increased SCE frequency mean compared to cilostazol alone (3.47 ± 0.51 versus 1.73 ± 0.55, respectively, $p<0.0001$) while reduced it in regard to carbamazepine only (3.47 ± 0.51 versus 4.90 ± 0.17, respectively, $p<0.001$).

3.2. Cilostazol antimitogenic effects and the impact of carbamazepine

The cytotoxic effects of cilostazol and carbamazepine on human cultured blood lymphocytes were evaluated using MI and PI. MI determines the mitotic activity of blood lymphocytes, and it is the ratio of the number of cells undergoing mitosis per high power field of view divided by the total number of cells in the examined cell population. Our results have demonstrated a statistically significant difference for the cilostazol-treated group compared with the others (Fig. 2). The MI of cilostazol-treated group (3.35 ± 0.89) was lower than that of the untreated control (4.33 ± 0.56, $p<0.05$) or carbamazepine-treated group (4.62 ± 1.05, $p<0.05$). However, there were no statistically significant differences ($p > 0.05$) in the cells' mitotic activity between carbamazepine-treated (4.62 ± 1.05), and carbamazepine- and cilostazol-treated cells (3.77 ± 0.48). In addition, the mitotic activity of these two groups was similar to the basal activity of the control group (4.33 ± 0.56, $p > 0.05$). This suggests that treating cells with carbamazepine has not caused cytostatic action, but on the contrary, it mitigated the cytotoxic effects of cilostazol to the baseline level. ANOVA and Tukey's post hoc test were used for statistical analysis.

The evaluation of human cultured blood lymphocytes proliferative activity upon treatment with cilostazol, carbamazepine, or both agents was determined by the PI. It was calculated to assess the drugs' effect on the rate of cell division process by scoring the first-, second-, and third-division mitoses (M1, M2, and M3, respectively) in 100 metaphases according to the previous equation (Section 2.4). Results (Fig. 3) have shown that the mean PI of the carbamazepine-treated group (2.53 ± 0.09) has shown significantly higher PI than the control (2.20 ± 0.11, $p < 0.001$), the cilostazol-treated cells (1.94 ± 0.14, $p < 0.001$), and the cells treated with combined carbamazepine and cilostazol (1.81 ± 0.14, $p < 0.0001$). In addition, the reduction in the mean PI of cells treated with cilostazol was statistically different from the control (1.94 ± 0.14 versus 2.20 ± 0.11, respectively, $p<0.001$). However, when cilostazol was combined with carbamazepine, it reduced the carbamazepine-induced increase in PI to the level of cilostazol-treated cells (1.81 ± 0.14 versus 1.94 ± 0.14, respectively, $p>0.05$). Notably, combining cilostazol and carbamazepine has significantly reduced the mean PI when compared to the control (1.81 ± 0.14 versus 2.20 ± 0.11, respectively, $p < 0.0001$).

4. Discussion

Despite being a commonly prescribed antiseizure medication, carbamazepine can cause genotoxicity. Consequently, reducing this toxicity is a necessity. Research studies investigating the potential geno- and cyto-toxic effects of carbamazepine on human cultured blood lymphocytes are limited. Furthermore, the effects of the antioxidant and antiproliferative agent cilostazol on potential geno- and cyto-toxicities of carbamazepine are scarce. Therefore, this study aimed to address these

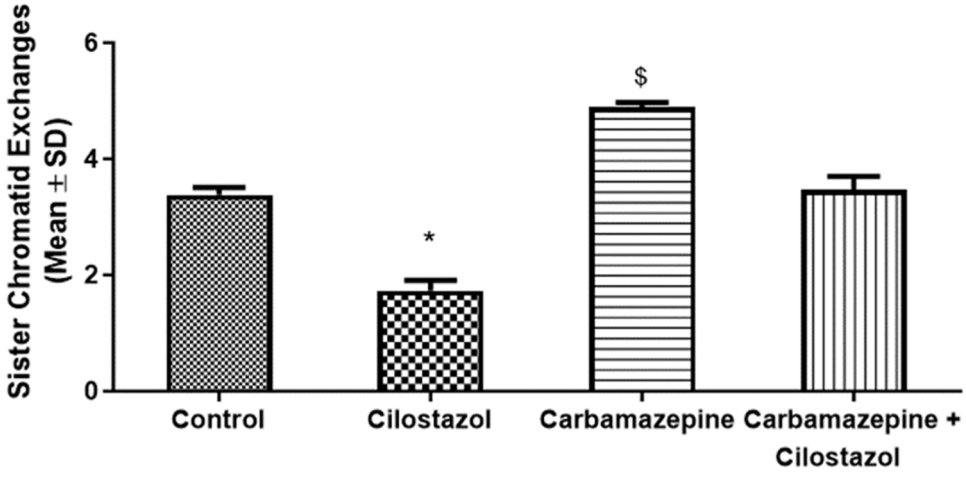

Fig. 1. Quantitative comparison of the means of sister chromatid exchange (SCE) frequency in human cultured blood lymphocytes upon treatment with cilostazol and/or carbamazepine. ANOVA and Tukey's post hoc test were used for statistical analysis. *: Denotes the statistical significance of each pairwise comparisons of cilostazol with the control ($p<0.0001$), carbamazepine ($p<0.0001$), and carbamazepine+ cilostazol ($p<0.0001$) groups. $: Denotes the statistical significance of each pairwise comparisons of carbamazepine with the control ($p<0.0001$), cilostazol ($p<0.0001$), and carbamazepine+ cilostazol ($p<0.001$) groups.

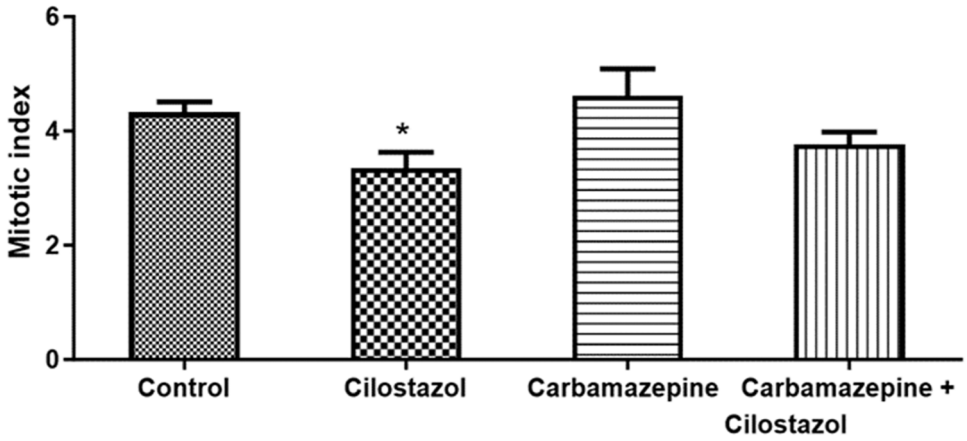

Fig. 2. Quantitative comparison of the ratio means of mitotic index (MI) in human cultured blood lymphocytes following treatment with cilostazol, carbamazepine or both. Error bars indicate standard deviation (n = 5–10). *: Denotes the statistical significance of each pairwise comparisons of cilostazol with the control ($p<0.05$), and carbamazepine ($p<0.05$) groups.

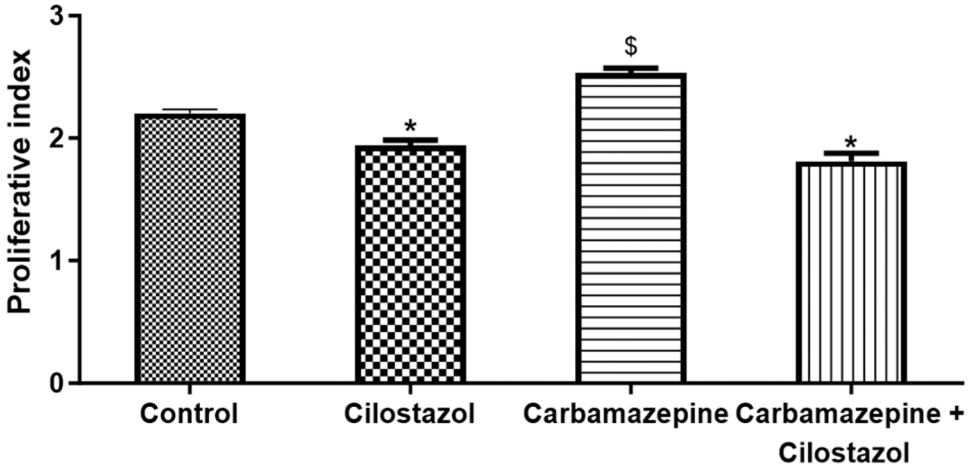

Fig. 3. Quantitative comparison of the means of cell proliferative index (PI) in human cultured blood lymphocytes upon treatment with cilostazol and/or carbamazepine. Error bars indicate standard deviation (n = 5–10). *: Denotes the statistical significance of each pairwise comparisons of cilostazol with the control ($p<0.001$), carbamazepine ($p<0.0001$), and carbamazepine+ cilostazol ($p>0.05$) groups. $: Denotes the statistical significance of each pairwise comparisons of carbamazepine with the control ($p<0.001$), cilostazol ($p<0.0001$), and carbamazepine+ cilostazol ($p<0.0001$) groups.

effects of both drugs on human cultured blood lymphocytes and examined cilostazol ability to avoid, stop or reverse the potential geno- or cyto-toxic effects induced by carbamazepine. Our findings bring to light cilostazol ameliorative effects of carbamazepine-induced genotoxicity under the experimental conditions, offering a promising agent to prevent this adverse effect. However, cilostazol exerted antimitogenic properties compared to carbamazepine.

4.1. Carbamazepine- induced genotoxicity and cilostazol impact

Although carbamazepine is one of the oldest antiepileptic drugs, it is still one of the common agents used to manage epilepsy. The demonstrated genotoxicity of the carbamazepine in our findings is in alignment with previous research results using the same research technique in the same model [6]. Moreover, it is also in line with other studies' results using various techniques and/or different systems [5,9]. This consistency between various methods and systems confirms that carbamazepine is genotoxic to cells. It has been debated whether there is an association between antiepileptic drugs and cancer with evidence showing many agents demonstrating this risk [36]. For carbamazepine, the available evidence is limited, and it is summarized into the risk of hepatocellular and testes tumors in rats [36]. Carbamazepine's genotoxic impact on blood lymphocytes could stem from its potential direct binding to DNA. Alternatively, it might exert immunomodulatory effects. The concept of localized drug metabolism beyond the liver adds an intriguing dimension to our understanding. It has been documented that the mRNA and protein of cytochrome P450s and Phase 2 hepatic metabolizing enzymes are expressed in white blood cells including peripheral blood lymphocytes [37]. The products of this localized metabolism can contribute to oxidative stress and toxicities which can lead to activating antigen presenting cells and an immune response [38]. Furthermore, carbamazepine is an enzyme inducer, and the potential induction of local metabolizing enzymes can generate reactive metabolites which can bind to macromolecules in leukocytes and contribute to toxicities or immune-medicated reactions [39,40]. The closely related drug to carbamazepine and relatively newer antiepileptic agent oxcarbazepine was also found to be genotoxic [36,41]. In addition, its metabolites which are common to those of carbamazepine were also reported to be genotoxic [41]. However, oxcarbazepine has been increasingly used due to its weak enzyme-inducing properties [36]. The manifested genotoxic effect of carbamazepine was mitigated by the protective effects of the well-documented antioxidant and anti-inflammatory agent cilostazol, a finding that matches well with previous literature [13,15–20]. There are several mechanistic pathways

that have been proposed for cilostazol pleiotropic protective roles including prevention of oxidative stress. These includes (i) preventing mitochondrial reactive oxygen species (ROS) production and depolarization; thus, preventing oxidative stress-induced mitochondrial dysfunction, (ii) in a dose-dependent manner, ameliorating the expressions of the inflammatory mediators tumor necrosis factor-alpha (TNF-α) and nuclear factor-kappa β (NF-κB) (partly by increasing cAMP levels) and the apoptotic marker caspase-3 (by activating cAMP-dependent protein kinase), and lowering the lipid peroxidation marker malondialdehyde and nitrite levels with a rise in glutathione concentration; thus, targeting the inflammatory and cell death signaling pathways and restoring an unbalanced oxidative status, (iii) suppressing oxidative DNA breakage induced by hydroxyl radicals, (iv) reducing oxidative stress biomarkers and increased antioxidant capacity regardless of cilostazol used dose. [13,15–17,19,42] The broad-spectrum antiepileptic medication valproic acid is also widely used for treating most forms of epilepsy and bipolar disorders [43]. It shares the mechanism of action of carbamazepine on voltage-gated sodium channels and histone deacetylase [43,44]. It is unclear if this antiepileptic drug has also geno- or cyto-toxic effects on blood lymphocytes similar to carbamazepine particularly as both agents can cause hematological abnormalities [45,46]. Valproic acid can cause coagulopathy and hematological abnormalities including thrombocytopenia and predispose patients treated with it to hemorrhagic risks [47,48]. The mechanisms of valproic acid hematological effects are still unclear; nonetheless, studies have suggested that valproate coagulopathy and thrombocytopenia risks are dose-dependent and may result from inhibiting cyclooxygenase and thromboxane A2 synthesis, reductions in the levels and activities of factors VII, VIII, XIII, protein C, fibrinogen, von Willebrand factor antigen, and von Willebrand factor ristocetin cofactor [45,47–55]. On the other hand, carbamazepine-induced thrombocytopenia is dose-independent and is an immune-mediated reaction [46]. Studies on valproic acid toxicities have indicated that oxidative stress and production of ROS, and inhibiting mitochondrial respiration, have contributed to its germ cell and hepatocytes damage [56–58]. Further research is needed to investigate valproic acid effects and other related antiepileptic medications to carbamazepine on blood lymphocytes and whether cilostazol will have the ability to mitigate any toxicities that may result.

4.2. *Cilostazol antimitogenic effects and the impact of carbamazepine*

The cytotoxic properties of cilostazol as they were demonstrated in our study come in agreement with previous evidence [59–61]. Cilostazol inhibited cell viability and exerted anticancer effects in colon and breast cancer cells [59,60]. In addition, it induced apoptosis leading to anti-proliferative and -tumor effects in hepatocellular carcinoma cells [61]. In our study, carbamazepine has not adversely affected lymphocytes viability and proliferation, on the contrary, it enhanced cell kinetics. Previous studies have indicated that carbamazepine did not show antiproliferative and antitumor effects on cells in malignant glioma [62]. Moreover, it did not affect cell viability in concentrations up to 80 μM but not in concentrations above 100 μM when cells' survival rates were reduced [63]. Carbamazepine's feature of autoinduction through inducing hepatic metabolizing enzymes may have contributed to its absent effects on inhibiting cell kinetics at the utilized dose [29]. However, carbamazepine, as a histone deacetylase inhibitor, generated ROS and caspase 3 in human colon adenocarcinoma cell line HT-29 and caused marked cytotoxicity and anticancer effect [64]. This discrepancy with our results can be related to the different doses used in which much higher doses (36 and 76 μg/mL; 152 and 322 μM) than our dose of carbamazepine (12 μg/mL; 50.8 μM) were utilized [64]. Furthermore, valproate is an antiepileptic agent that seems to exert antiproliferative and anticancer effects *in vitro* and *in vivo* through inhibiting histone deacetylase, a mechanism that is also a characteristic of carbamazepine [36,58,65,66]. Thus, the combined cilostazol and carbamazepine treatment abolished the later effects on cell kinetics and preserved the former antiproliferative properties.

This study was conducted in human cells under controlled experimental conditions rather than in the natural setting of a human being; thus, it is unclear if its findings can be extrapolated to the human *in vivo* sitting, particularly in human clinical studies. Some researchers have suggested that the *in vitro* to *in vivo* concordance may be established by "humanized" *in vitro* systems; using human cells [67,68]. However, it is still challenging to generalize and translate the *in vitro* outcomes to *in vivo* owing to contextual variations. Nonetheless, important insights into the *in vivo* system can be gained for potential translatability.

5. Conclusion

Our study has indicated that carbamazepine is genotoxic to human cultured blood lymphocytes and is a promoter of cell proliferation. On the other hand, our findings have illustrated that cilostazol is not only geno-protective and prevents carbamazepine-induced genotoxicity but also exerts antiproliferative effects. Therefore, cilostazol may be beneficial in terms of ameliorating oxidative stress and inflammation, presenting a promising agent for the treatment of many oxidative stress-related conditions. However, the clinical utility of this combination has not been examined. Future experimental and clinical studies are required for a possible risk and benefit assessment of patients receiving carbamazepine and cilostazol. In addition, further research studies are required to understand the exact mechanisms of cilostazol protective effects in human subjects for its practical implications as a new treatment strategy. It would also be valuable to extend and compare the results of this study to other related antiepileptic medications.

Abbreviation

ANOVA: analysis of variance; cAMP: cyclic adenosine monophosphate; CVD: cardiovascular disease; DMSO, dimethyl sulfoxide; FDA, food and drug administration; IRB, institutional review board; M1, first division metaphase; M2, second division metaphase; M3, third division metaphase; M4, fourth division metaphase; MI, mitotic index; NF-κB, nuclear factor-kappa β; PDE3, phosphodiesterase enzyme type 3; PI, proliferative index; PKA, protein kinase A; ROS, reactive oxygen species; SCE, sister chromatids exchange; SD, standard deviation ; TNF-α, tumor necrosis factor-alpha

Ethics approval and consent to participate

The study was ethically approved by the Deanship of Research at the Jordan University of Science and Technology (IRB approval number: 27/132/2020; Dated: 31/3/2020). Subjects who agreed to participate in the study were asked to sign an informed consent form.

Research involving humans

All human procedures were followed in accordance with the principles of the Declaration of Helsinki.

CRediT authorship contribution statement

Enaam M. Al Momany: Writing – original draft, Formal analysis, Data curation. **Abeer M. Rababa'h:** Writing – review & editing. **Karem H. Alzoubi:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization. **Omar F. Khabour:** Writing – review & editing, Supervision, Resources, Project-administration, Formal analysis, Data curation, Conceptualization.

Funding

This work was supported by the Deanship of Research at Jordan

University of Science and Technology under Grant no. (252/2020).

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.

Acknowledgements

No assistance in the preparation of this article is to be declared.

Author agreement statement

We the undersigned declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We understand that the Corresponding Author is the sole contact for the Editorial process. He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs

Data Availability

Data will be made available on request.

References

[1] A. Beydoun, S. DuPont, D. Zhou, M. Matta, V. Nagire, L. Lagae, Current role of carbamazepine and oxcarbazepine in the management of epilepsy, Seizure 83 (2020) 251–263, https://doi.org/10.1016/j.seizure.2020.10.018.

[2] C.S. Jasdave Maan, T.H. vi Duong, A. Saadabadi, Continuing Education Activity, n. d. ⟨https://www.ncbi.nlm.nih.gov/books/NBK482455/⟩.

[3] S. García-Medina, M. Galar-Martínez, L.M. Gómez-Oliván, R.M. del C. Torres-Bezaury, H. Islas-Flores, E. Gasca-Pérez, The relationship between cyto-genotoxic damage and oxidative stress produced by emerging pollutants on a bioindicator organism (Allium cepa): The carbamazepine case, Chemosphere 253 (2020) 126675, https://doi.org/10.1016/j.chemosphere.2020.126675.

[4] P.U. Ofoegbu, J. Lourenço, S. Mendo, A.M.V.M. Soares, J.L.T. Pestana, Effects of low concentrations of psychiatric drugs (carbamazepine and fluoxetine) on the freshwater planarian, Schmidtea mediterranea, Chemosphere 217 (2019) 542–549, https://doi.org/10.1016/j.chemosphere.2018.10.198.

[5] A. Celik, The assessment of genotoxicity of carbamazepine using cytokinesis-block (CB) micronucleus assay in cultured human blood lymphocytes, Drug Chem. Toxicol. 29 (2006) 227–236, https://doi.org/10.1080/01480540600566832.

[6] E.K. Hendawi, O.F. Khabour, L.N. Al-Eitan, K.H. Alzoubi, Reduction of Genotoxicity of Carbamazepine to Human Lymphocytes by Pre-treatment with Vitamin B12, Curr. Mol. Pharm. 16 (2023) 228–233, https://doi.org/10.2174/1874467215666220420135924.

[7] M. Witczak, I. Kociszewska, J. Wilczyński, D. Łopaczyńska, T. Ferenc, Evaluation of chromosome aberrations, sister chromatid exchange and micronuclei in cultured cord-blood lymphocytes of newborns of women treated for epilepsy during pregnancy, Mutat. Res. /Genet. Toxicol. Environ. Mutagen. 701 (2010) 111–117, https://doi.org/10.1016/j.mrgentox.2010.05.003.

[8] W.M. Awara, M. El-Gohary, S.H. El-Nabi, W.A. Fadel, In vivo and in vitro evaluation of the mutagenic potential of carbamazepine: does melatonin have anti-mutagenic activity? Toxicology 125 (1998) 45–52, https://doi.org/10.1016/S0300-483X(97)00160-1.

[9] M. Kardoost, E. Hajizadeh-Saffar, M.T. Ghorbanian, Z. Ghezelayagh, K. Pooshang Bagheri, M. Behdani, M. Habibi-Anbouhi, Genotoxicity assessment of antiepileptic drugs (AEDs) in human embryonic stem cells, Epilepsy Res 158 (2019) 106232, https://doi.org/10.1016/j.eplepsyres.2019.106232.

[10] S. Yan, R. Chen, M. Wang, J. Zha, Carbamazepine at environmentally relevant concentrations caused DNA damage and apoptosis in the liver of Chinese rare minnows (Gobiocypris rarus) by the Ras/Raf/ERK/p53 signaling pathway, Environ. Pollut. 270 (2021) 116245, https://doi.org/10.1016/j.envpol.2020.116245.

[11] A.M. Balinski, C.V. Preuss, Cilostazol (2024).

[12] R.Y. Kherallah, M. Khawaja, M. Olson, D. Angiolillo, Y. Birnbaum, Cilostazol: a Review of Basic Mechanisms and Clinical Uses, Cardiovasc Drugs Ther. 36 (2022) 777–792, https://doi.org/10.1007/s10557-021-07187-x.

[13] A.M. Rababa'h, A.N. Mardini, K.H. Alzoubi, M.A. Ababneh, R.Y. Athamneh, The effect of cilostazol on hippocampal memory and oxidative stress biomarkers in rat model of diabetes mellitus, Brain Res 1715 (2019) 182–187, https://doi.org/10.1016/j.brainres.2019.03.025.

[14] N.A.V. Motta, L.J. Autran, S.C. Brazão, R. de, O. Lopes, C.B.V. Scaramello, G. F. Lima, F.C.F. de Brito, Could cilostazol be beneficial in COVID-19 treatment? Thinking about phosphodiesterase-3 as a therapeutic target, Int Immunopharmacol. 92 (2021) 107336, https://doi.org/10.1016/j.intimp.2020.107336.

[15] S.C. Chattipakorn, S. Thummasorn, J. Sanit, N. Chattipakorn, Phosphodiesterase-3 inhibitor (cilostazol) attenuates oxidative stress-induced mitochondrial dysfunction in the heart, J. Geriatr. Cardiol. 11 (2014) 151–157, https://doi.org/10.3969/j.issn.1671-5411.2014.02.014.

[16] E.M. Othman, H.A. Habib, M.E. Zahran, A. Amin, G.H. Heeba, Mechanistic Protective Effect of Cilostazol in Cisplatin-Induced Testicular Damage via Regulation of Oxidative Stress and TNF-α/NF-κB/Caspase-3 Pathways, Int J. Mol. Sci. 24 (2023), https://doi.org/10.3390/ijms241612651.

[17] N.K. Agrawal, R. Maiti, D. Dash, B.L. Pandey, Cilostazol reduces inflammatory burden and oxidative stress in hypertensive type 2 diabetes mellitus patients, Pharm. Res 56 (2007) 118–123, https://doi.org/10.1016/j.phrs.2007.04.007.

[18] T. Oguchi, R. Ono, M. Tsuji, H. Shozawa, M. Somei, M. Inagaki, Y. Mori, T. Yasumoto, K. Ono, Y. Kiuchi, Cilostazol Suppresses Aβ-induced Neurotoxicity in SH-SY5Y Cells through Inhibition of Oxidative Stress and MAPK Signaling Pathway, Front Aging Neurosci. 9 (2017) 337, https://doi.org/10.3389/fnagi.2017.00337.

[19] X. Xie, X. Xu, C. Sun, Z. Yu, Protective effects of cilostazol on ethanol-induced damage in primary cultured hepatocytes, Cell Stress Chaperon-.-. 23 (2018) 203–211, https://doi.org/10.1007/s12192-017-0828-3.

[20] A.M. Rababa'h, S.A. Hussein, O.F. Khabour, K.H. Alzoubi, The Protective Effect of Cilostazol in Genotoxicity Induced by Methotrexate in Human Cultured Lymphocytes, Curr. Mol. Pharm. 13 (2020) 137–143, https://doi.org/10.2174/1874467212666191023120118.

[21] M.L. Gaertner, S. Mintzer, C.M. DeGiorgio, Increased cardiovascular risk in epilepsy, Front Neurol. 15 (2024), https://doi.org/10.3389/FNEUR.2024.1339276.

[22] Z. Chen, D. Liew, P. Kwan, Excess mortality and hospitalized morbidity in newly treated epilepsy patients, Neurology 87 (2016) 718–725, https://doi.org/10.1212/WNL.0000000000002984.

[23] S.W. Terman, C.E. Aubert, C.E. Hill, J. Skvarce, J.F. Burke, S. Mintzer, Cardiovascular disease risk, awareness, and treatment in people with epilepsy, Epilepsy Behav. 117 (2021) 107878, https://doi.org/10.1016/j.yebeh.2021.107878.

[24] C.B. Josephson, S. Wiebe, G. Delgado-Garcia, A. Gonzalez-Izquierdo, S. Denaxas, T. T. Sajobi, M. Lamidi, M. Wang, M.R. Keezer, Association of Enzyme-Inducing Antiseizure Drug Use With Long-term Cardiovascular Disease, JAMA Neurol. 78 (2021) 1367–1374, https://doi.org/10.1001/jamaneurol.2021.3424.

[25] H. Hoshino, K. Toyoda, K. Omae, N. Ishida, S. Uchiyama, K. Kimura, N. Sakai, Y. Okada, K. Tanaka, H. Origasa, H. Naritomi, K. Houkin, K. Yamaguchi, M. Isobe, K. Minematsu, M. Matsumoto, T. Tominaga, H. Tomimoto, Y. Terayama, S. Yasuda, T. Yamaguchi, C.S.P.Scom Trial, Investigators, Dual Antiplatelet Therapy Using Cilostazol With Aspirin or Clopidogrel: Subanalysis of the CSPS.com Trial, Stroke 52 (2021) 3430–3439, https://doi.org/10.1161/STROKEAHA.121.034378.

[26] K. Kalantzi, N. Tentolouris, A.J. Melidonis, S. Papadaki, M. Peroulis, K.A. Amantos, G. Andreopoulos, G.I. Bellos, D. Boutel, M. Bristianou, D. Chrisis, N. A. Dimitsikoglou, J. Doupis, C. Georgopoulou, S.A. Gkintikas, S. Iraklianou, K Kanellas, K. Kotsa, T. Koufakis, M. Kouroglou, A.G. Koutsovasilis, L. Lanaras, E. Liouri, C. Lixouriotis, A. Lykoudi, E. Mandalaki, E. Papageorgiou, N. Papanas, S. Rigas, M.I. Stamatelatou, I. Triantafyllidis, A. Trikkalinou, A.N. Tsouka, O. Zacharopoulou, C. Zoupas, I. Tsolakis, A.D. Tselepis, Efficacy and Safety of Adjunctive Cilostazol to Clopidogrel-Treated Diabetic Patients With Symptomatic Lower Extremity Artery Disease in the Prevention of Ischemic Vascular Events, J. Am. Heart Assoc. 10 (2021) e018184, https://doi.org/10.1161/JAHA.120.018184.

[27] O.F. Khabour, F.M. Enaya, K. Alzoubi, S.I. Al-Azzam, Evaluation of DNA damage induced by norcantharidin in human cultured lymphocytes, Drug Chem. Toxicol. 39 (2016) 303–306, https://doi.org/10.3109/01480545.2015.1113988.

[28] G. Grześk, W. Stolarek, M. Kasprzak, E. Grześk, D. Rogowicz, M. Wiciński, M. Krzyżanowski, Therapeutic Drug Monitoring of Carbamazepine: A 20-Year Observational Study, J. Clin. Med 10 (2021), https://doi.org/10.3390/jcm10225396.

[29] G.K. Hiremath, P. Kotagal, W. Bingaman, C. Hovinga, E. Wyllie, H. Morris, D. Nelson, Risk factors for carbamazepine elevation and toxicity following epilepsy surgery, Seizure 14 (2005) 312–317, https://doi.org/10.1016/j.seizure.2005.04.003.

[30] C. Aoki, Y. Hattori, A. Tomizawa, T. Jojima, K. Kasai, Anti-inflammatory role of cilostazol in vascular smooth muscle cells in vitro and in vivo, J. Atheroscler. Thromb. 17 (2010) 503–509, https://doi.org/10.5551/jat.3392.

[31] S.A. Al-Sweedan, O. Khabour, R. Isam, Genotoxicity assessment in patients with thalassemia minor, Mutat. Res 744 (2012) 167–171, https://doi.org/10.1016/j.mrgentox.2012.02.010.

[32] M.A.Y. Alqudah, F.Y. Al-Ashwal, K.H. Alzoubi, M. Alkhatatbeh, O. Khabour, Vitamin E protects human lymphocytes from genotoxicity induced by oxaliplatin, Drug Chem. Toxicol. 41 (2018) 281–286, https://doi.org/10.1080/01480545.2017.1384835.

[33] S. Wolff, V. Afzal, Segregation of DNA polynucleotide strands into sister chromatids and the use of endoreduplicated cells to track sister chromatid exchanges induced by crosslinks, alkylations, or x-ray damage, Proc. Natl. Acad. Sci. USA 93 (1996) 5765–5769, https://doi.org/10.1073/pnas.93.12.5765.

[34] T.A. Kato, J.S. Haskins, Mitotic Index Analysis, Methods Mol. Biol. 2519 (2023) 17–26, https://doi.org/10.1007/978-1-0716-2433-3_3.
[35] K. Alzoubi, O. Khabour, M. Khader, N. Mhaidat, S. Al-Azzam, Evaluation of vitamin B12 effects on DNA damage induced by paclitaxel, Drug Chem. Toxicol. 37 (2014) 276–280, https://doi.org/10.3109/01480545.2013.851686.
[36] G. Singh, P.H. Driever, J.W. Sander, Cancer risk in people with epilepsy: The role of antiepileptic drugs, Brain 128 (2005) 7–17, https://doi.org/10.1093/brain/awh363.
[37] J.L. Raucy, M. Ingelman-Sundberg, S. Carpenter, A. Rannug, A. Rane, M. Franklin, M. Romkes, Drug metabolizing enzymes in lymphocytes, J. Biochem Mol. Toxicol. 13 (1999) 223–226, https://doi.org/10.1002/(sici)1099-0461(1999)13:3/4<223::aid-jbt14>3.0.co;2-z.
[38] S.N. Lavergne, B.K. Park, D.J. Naisbitt, The roles of drug metabolism in the pathogenesis of T-cell-mediated drug hypersensitivity, Curr. Opin. Allergy Clin. Immunol. 8 (2008) 299–307, https://doi.org/10.1097/ACI.0b013e3283079c64.
[39] J.P. Uetrecht, The role of leukocyte-generated reactive metabolites in the pathogenesis of idiosyncratic drug reactions, Drug Metab. Rev. 24 (1992) 299–366, https://doi.org/10.3109/03602539208996297.
[40] G. Grześk, W. Stolarek, M. Kasprzak, E. Grześk, D. Rogowicz, M. Wiciński, M. Krzyżanowski, Therapeutic Drug Monitoring of Carbamazepine: A 20-Year Observational Study, J. Clin. Med 10 (2021) 5396, https://doi.org/10.3390/jcm10225396.
[41] Z. Atlı Şekeroğlu, H. Kefelioğlu, S. Kontaş Yedier, V. Şekeroğlu, B. Delmecioğlu, Oxcarbazepine-induced cytotoxicity and genotoxicity in human lymphocyte cultures with or without metabolic activation, Toxicol. Mech. Methods 27 (2017) 201–206, https://doi.org/10.1080/15376516.2016.1273430.
[42] M. Etli, O. Karahan, Ö. Akkaya, H.B. Savaş, Cilostazol induces angiogenesis and regulates oxidative stress in a dose-dependent manner: A chorioallantoic membrane study, Turk. Gogus Kalp Damar Cerrah-.-. Derg. 29 (2021) 449–456, https://doi.org/10.5606/tgkdc.dergisi.2021.22212.
[43] W. Löscher, Basic pharmacology of valproate: a review after 35 years of clinical use for the treatment of epilepsy, CNS Drugs 16 (2002) 669–694, https://doi.org/10.2165/00023210-200216100-00003.
[44] T. Tomson, D. Battino, E. Perucca, The remarkable story of valproic acid, Lancet Neurol. 15 (2016) 141, https://doi.org/10.1016/S1474-4422(15)00398-1.
[45] A. Verrotti, A. Scaparrotta, S. Grosso, F. Chiarelli, G. Coppola, Anticonvulsant drugs and hematological disease, Neurol. Sci. 35 (2014) 983–993, https://doi.org/10.1007/s10072-014-1701-0.
[46] E. Tichy, S. Lam, U. Militano, O. Bessmertny, A case of severe thrombocytopenia and antiepileptic hypersensitivity syndrome, J. Pedia Pharm. Ther. 8 (2003) 29–33, https://doi.org/10.5863/1551-6776-8.1.29.
[47] E. Cannizzaro, M. Albisetti, G. Wohlrab, M. Schmugge, Severe bleeding complications during antiepileptic treatment with valproic acid in children, Neuropediatrics 38 (2007) 42–45, https://doi.org/10.1055/s-2007-981448.
[48] R. Zhu, Q. Wang, Y. Zhou, W. Shi, Y. Zhang, M. Wang, H. Li, R. Zhao, The prevalence and risk factors of coagulopathy in pediatric patients undergoing surgery for epilepsy, J. Neurosurg. Pedia 32 (2023) 527–534, https://doi.org/10.3171/2023.6.PEDS23196.
[49] S. Acharya, J.B. Bussel, Hematologic Toxicity of Sodium Valproate, J. Pedia Hematol. Oncol. 22 (2000) 62–65, https://doi.org/10.1097/00043426-200001000-00012.
[50] R. Kumar, J. Vidaurre, S. Gedela, Valproic Acid–Induced Coagulopathy, Pedia Neurol. 98 (2019) 25–30, https://doi.org/10.1016/j.pediatrneurol.2019.04.019.
[51] B. Kis, Z. Szupera, Z. Mezei, A. Gecse, G. Telegdy, L. Vécsei, Valproate treatment and platelet function: the role of arachidonate metabolites, Epilepsia 40 (1999) 307–310, https://doi.org/10.1111/j.1528-1157.1999.tb00709.x.
[52] S. Koenig, T. Gerstner, A. Keller, M. Teich, E. Longin, C.-E. Dempfle, High incidence of vaproate-induced coagulation disorders in children receiving valproic acid: a prospective study, Blood Coagul. Fibrinolysis 19 (2008) 375–382, https://doi.org/10.1097/MBC.0b013e3282f3f9ec.
[53] G. Köse, E. Arhan, B. Unal, E. Ozaydın, A. Guven, T.R. Saylı, Valproate-Associated Coagulopathies in Children During Short-Term Treatment, J. Child Neurol. 24 (2009) 1493–1498, https://doi.org/10.1177/0883073808331084.
[54] M. Buoli, M. Serati, A. Botturi, • A.Carlo Altamura, The Risk of Thrombocytopenia During Valproic Acid Therapy: A Critical Summary of Available Clinical Data, (2017). https://doi.org/10.1007/s40268-017-0224-6.
[55] I. Olaizola, M.F. Brodde, B.E. Kehrel, S. Evers, The Impact of Levetiracetam and Valproate on Platelet Functions—A Double-Blind, Placebo-Controlled Crossover Study, J. Clin. Med 12 (2023) 933, https://doi.org/10.3390/JCM12030933.
[56] S. Khan, T. Ahmad, C.V. Parekh, P.P. Trivedi, S. Kushwaha, G. Jena, Investigation on sodium valproate induced germ cell damage, oxidative stress and genotoxicity in male Swiss mice, Reprod. Toxicol. 32 (2011) 385–394, https://doi.org/10.1016/j.reprotox.2011.09.007.
[57] T. Komulainen, T. Lodge, R. Hinttala, M. Bolszak, M. Pietilä, P. Koivunen, J. Hakkola, J. Poulton, K.J. Morten, J. Uusimaa, Sodium valproate induces mitochondrial respiration dysfunction in HepG2 in vitro cell model, Toxicology 331 (2015) 47–56, https://doi.org/10.1016/j.tox.2015.03.001.
[58] A. Safdar, F. Ismail, A comprehensive review on pharmacological applications and drug-induced toxicity of valproic acid, Saudi Pharm. J. 31 (2023) 265–278, https://doi.org/10.1016/j.jsps.2022.12.001.
[59] K. Murata, M. Kameyama, F. Fukui, H. Ohigashi, M. Hiratsuka, Y. Sasaki, T. Kabuto, M. Mukai, T. Mammoto, H. Akedo, O. Ishikawa, S. Imaoka, Phosphodiesterase type III inhibitor, cilostazol, inhibits colon cancer cell motility, Clin. Exp. Metastas-.-. 17 (1999) 525–530, https://doi.org/10.1023/a:1006626529536.
[60] N. Hao, W. Shen, R. Du, S. Jiang, J. Zhu, Y. Chen, C. Huang, Y. Shi, R. Xiang, Y. Luo, Phosphodiesterase 3A Represents a Therapeutic Target that Drives Stem Cell-like Property and Metastasis in Breast Cancer, Mol. Cancer Ther. 19 (2020) 868–881, https://doi.org/10.1158/1535-7163.MCT-18-1233.
[61] K.H. Sim, M.-S. Shu, S. Kim, J.-Y. Kim, B.-H. Choi, Y.J. Lee, Cilostazol Induces Apoptosis and Inhibits Proliferation of Hepatocellular Carcinoma Cells by Activating AMPK, Biotechnol. Bioprocess Eng. 26 (2021) 776–785, https://doi.org/10.1007/s12257-021-0002-8.
[62] C. Yagi, J. Tatsuoka, E. Sano, Y. Hanashima, Y. Ozawa, S. Yoshimura, S. Yamamuro, K. Sumi, H. Hara, Y. Katayama, A. Yoshino, Anti-tumor effects of anti-epileptic drugs in malignant glioma cells, Oncol. Rep. 48 (2022) 216, https://doi.org/10.3892/or.2022.8431.
[63] Im, Kim, Chau, Um, Carbamazepine Enhances Adipogenesis by Inhibiting Wnt/β-catenin Expression, Cells 8 (2019) 1460, https://doi.org/10.3390/cells8111460.
[64] Sohaib MEzhilarasan D, Carbamazepine, a Histone Deacetylase Inhibitor Induces Apoptosis in Human Colon Adenocarcinoma Cell Line HT-29, J. Gastrointest. Cancer 51 (2020) 564–570, https://doi.org/10.1007/s12029-019-00286-x.
[65] A. Wawruszak, M. Halasa, E. Okon, W. Kukula-Koch, A. Stepulak, Valproic Acid and Breast Cancer: State of the Art in 2021, Cancers (Basel) 13 (2021), https://doi.org/10.3390/cancers13143409.
[66] M. Shokrzadeh, M. Modanloo, S. Samadi, I. kunter, H. Haghighi, The Effects of Valproic Acid on Cell Viability of Breast Cancer and Genotoxicity of Human Lymphocytes Blood Cell Line, J. Res Med Dent. Sci. 9 (2021) 341–345. ⟨https://www.jrmds.in/articles/the-effects-of-valproic-acid-on-cell-viability-of-breast-cancer-and-genotoxicity-of-human-lymphocytes-blood-cell-line-87416.html⟩ (accessed October 28, 2024).
[67] T. Steger-Hartmann, M. Raschke, Translating in vitro to in vivo and animal to human, Curr. Opin. Toxicol. 23–24 (2020) 6–10, https://doi.org/10.1016/J.COTOX.2020.02.003.
[68] W.B. Mattes, In vitro to in vivo translation, Curr. Opin. Toxicol. 23–24 (2020) 114–118, https://doi.org/10.1016/J.COTOX.2020.09.001.