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Original article

Venlafaxine's effect on human genetic material: *in vitro* study

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Abstract

Introduction: Venlafaxine is a prescription drug approved for the treatment of major depressive disorder, generalized anxiety disorder, panic disorder and social anxiety disorder. It is a serotonin and norepinephrine reuptake inhibitor and a weak dopamine reuptake inhibitor. The aim of the present study is to investigate the in vitro effect of venlafaxine on human genetic material, by estimating sensitive cytogenetic indices.

Methods: Five venlafaxine solutions (A=15μg/ml, B=30μg/ml, C=45μg/ml, D=60μg/ml, E=75μg/ml) were added to cultures of peripheral blood lymphocytes of six healthy donors. After 72 hours of incubation, the cultured lymphocytes were plated on glass slides, stained with the Fluorescence plus Giemsa method and Sister Chromatid Exchanges (SCEs), a sensitive marker of genotoxicity, Proliferation Rate Index (PRI), a reliable marker of cytostatic activity and Mitotic Index (MI), a marker which shows precisely the ability of a cell to proliferate were measured with the optical microscope.

Results: Result analysis revealed t: a) a statistically significant (p=0.001) dose-dependent increase of SCEs and b) a statistically significant (p=0.001) reduction of PRI and MI in all concentrations. Furthermore, a correlation was observed between a) SCE and PRI index variations, b) MI and SCE index variations and c) PRI and MI index variations.

Conclusions: Venlafaxine exhibited dose-dependent cytogenetic activity in vitro, increasing SCE frequencies and diminishing PRI and MI levels in healthy human cultured lymphocytes. Venlafaxine as other antidepressants seems to affect human T lymphocytes by modifying epigenetic and DNA replication procedures. This may provide additional information about the mechanism of action of the drug. Considering that the use of venlafaxine has rapidly increased with many off label indications, further studies in other cell lines and in vivo experimental settings are needed in order to evaluate its potential effects on human genetic material.

Keywords: Venlafaxine, Cytogenetic Activity, Sister Chromatid Exchanges, Proliferation Rate Index, Mitotic Index.

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Abbreviations:

SCEs: Sister Chromatid Exchanges

PRI: Proliferation Rate Index

MI: Mitotic Index

SNRI: Serotonin–Noradrenaline Reuptake Inhibitor

Introduction

Nowadays, only a few new antidepressant drugs have been approved for mood disorders treatment. This situation leads to a challenging personalize treatment approach, concerning their use in order to improve efficacy and safety (van Westrhenen et al, 2020). Differences in genetic variability, environmental, physiological, psychological factors and comorbidities may alter drug treatment response and drug safety parameters (Lauschke et al, 2019). Furthermore, drugs like antidepressants, which are administered to a large amount of patients and are intended for long term use, should be evaluated for their cytogenetic behavior. Recent studies indicate antidepressants' interesting cytogenetic behavior (Demirtzoglou et al. 2016, Cobanoglu et al. 2018). Venlafaxine is one of the most widely used antidepressive drugs, it is approved for the treatment of major depressive disorder, generalized anxiety disorder, panic disorder and social anxiety disorder (Warren, 2016). It has also been used successfully for chronic headaches, fibromyalgia, and neuropathic pain (Ayabaktı et al. 2020). It is a serotonin–noradrenaline reuptake inhibitor (SNRI) and selectively inhibits the reuptake of both serotonin and noradrenaline blockers. This action increases the levels of these two neurotransmitters in the synapse and improves depressive symptoms. Despite its extended use, cytogenetic behavior of venlafaxine has not been studied extensively (Ayabaktı et al. 2020).

The aim of the present study was to investigate the in vitro effect of venlafaxine on human cultured lymphocytes, by estimating sensitive cytogenetic indices, as sister chromatid exchanges (SCEs), proliferation rate index (PRI) and mitotic index (MI). SCEs is the exchange of genetic material between two identical sister chromatids and they appear to be the result of DNA-replication mistakes on a damaged template, probably at the replication fork. SCEs have been identified as one of the most sensitive indices among sensitive biomarkers of genotoxicity, such as chromosomal aberrations, comet assay and micronuclei. PRI and MI have been used as sensitive indicators for the evaluation of the cytostatic activity of various environmental hazards or therapeutic agents (Mourelatos, 2016).

Materials and Methods

Ethics statement

The study was approved by the ethics committee of Aristotle University of Thessaloniki School of Medicine. Written informed consent was obtained from all individuals.

Healthy Blood Donors

Six healthy young people (3 male and 3 female, mean age 20±2 years) were the blood donors. They were non-smokers, not receiving any drugs, not consuming considerable quantities of alcohol, or not having suffered any kind of infection for the last 15 days. No pharmacological treatment was taken before blood sampling. Venous blood collected from all above mentioned individuals was used immediately after proper preparation for culturing lymphocytes for the SCE, PRI and MI assays.

In vitro SCE, PRI and MI assays.

Human lymphocyte cultures were prepared by adding in 5 ml chromosome medium (RPMI-1640, Biochrome, supplemented with 20% fetal calf serum, 0,63% L-glutamine, 0,63% penicillin/streptomycin and 2% phytohaemagglutinin) at the beginning of the culture life the following:

• 11-12 drops of heparinized whole peripheral blood
• 5-Bromodeoxyuridine (BrdU) solution (5μg/ml/culture) and
• Venlafaxine solution (A=15μg/ml or B=30μg/ml or C=45μg/ml or D=60 μg/ml or E=75μg/ml)

T lymphocyte cultures were incubated at 37o C for 72 hours in a dark incubator to minimize photolysis of 5 Bromodeoxyuridine. Colchicine was added 2h before the end of the incubation. T lymphocytes were then collected by centrifugation and exposed to 0,075M KCl (potassium chloride) for 12 minutes. The hypotonic solution spreads chromosomes and causes hemolysis of red blood cells. Pellet was fixed three times with methanol: acetic acid (3:1). Drops of concentrated suspension of cells were placed on microslides and allowed to air dry. For SCE, PRI and MI analysis, the slides were stained by a modification of the fluorescence plus Giemsa procedure to obtain harlequin chromosomes (Karapidaki et al. 2011).

Statistical Analysis.

For SCE estimation, 30 suitably spread second division cells from each culture were blindly scored. For PRI calculation 100 cells in the first, second, third and higher divisions from each culture were blindly scored. PRI=M1+2M2+3M3/100, where M1, M2 and M3+ are the percent values of cells in the first, second, third and higher divisions, respectively. For MI analysis, all cell divisions that are present in an optical field of 1000 nuclei were scored. MI=number of cells in mitosis/total number of nuclei (1000). The statistical analysis was carried out using the SPSS, vs 22.0 statistical package. All values were expressed as mean ± standard error of the mean (SEM). Comparison of values between the different groups and subgroups was accomplished by the nonparametric Kruskal-Wallis test. It was also used for the evaluation of the dosage effect of venlafaxine on cytogenetic indices. The Spearman's rank correlation coefficient was applied for calculating the correlation between SCEs, PRI and MI.

Results

Table 1 summarizes the effect of various venlafaxine concentrations on the SCE index variations of T cultured lymphocytes (Figure 1). A statistically significant (p=0.001) dose-dependent increase on SCEs frequency due to venlafaxine's effect was observed. Furthermore, the concentration of 75μg/ml duplicates the SCE rate in cultured lymphocytes of all blood donors.

Table 2 summarizes the effect of various venlafaxine concentrations on PRI index variations on T lymphocyte cultures (Figure 2). A significant PRI reduction is observed with escalating concentrations of venlafaxine (p=0.001).

Table 3 summarizes the effect of various venlafaxine concentrations on MI index variations on T cultured lymphocytes (Figure 3). A significant MI reduction is observed with escalating concentrations of venlafaxine (p=0.001).

Table 4 illustrates the values and statistical significance of correlation rates between SCEs, PRI and MI.

Table 1: Effect of venlafaxine on SCE frequency (Mean SCE/cell ±SE) in T lymphocyte cultures of healthy donors

Venlafaxine (μg/ml)	Healthy donors (n=6)					
	1	2	3	4	5	6
Control	5.1±0.12	6.23±0.22	7.31±0.23	5.86±0.09	7.9±0.33	6.48±0.24
15	6.8±0.20 [a]	7.45±0.17 [a]	8.77±0.36 [a]	7.21±0.51 [a]	7.05±0.26 [a]	8.21±0.21 [a]
30	8.05±0.09 [a]	9.27±0.11 [a]	10.07±0.13 [a]	8.72±0.35 [a]	8.48±0.10 [a]	9.56±0.31 [a]
45	9.38±0.17 [a]	10.1±0.41 [a]	11.23±0.17 [a]	9.89±0.31 [a]	11.03±0.12 [a]	10.65±0.10 [a]
60	10.75±0.31 [a]	11.22±0.26 [a]	12.06±0.08 [a]	11.03±0.11 [a]	12.22±0.30 [a]	11.92±0.36 [a]
75	12.02±0.19 [a]	12.5±0.03 [a]	13.87±0.24 [a]	12.88±0.22 [a]	13.76±0.10 [a]	13.44±0.15 [a]

[a] statistically significant (p=0.001) increase over the corresponding control (Kruskall Wallis test).

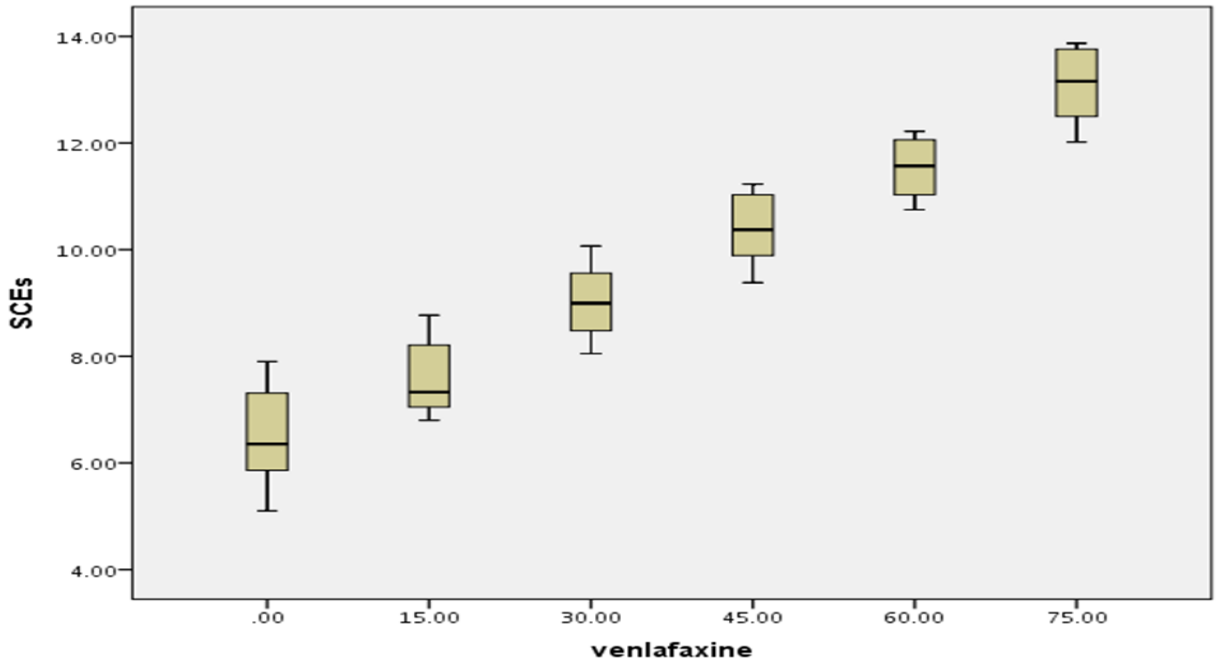

Figure 1: Effect of Venlafaxine on SCE mean frequency in human lymphocyte cultures from six healthy donors

Table 2: Effect of venlafaxine on PRI frequency in T lymphocyte cultures of healthy donors

Venlafaxine (μg/ml)	Healthy donors (n=6)					
	1	2	3	4	5	6
Control	2,83±0.03	2,92±0.02	2,77±0.10	2,61±0.02	2,57±0.03	2,84±0.13
15	2,32±0.07 [b]	2,41±0.02 [b]	2,22±0.03 [b]	2,12±0.12 [b]	2,07±0.14 [b]	2,31±0.08 [b]
30	2,01±0.05 [b]	2,09±0.06 [b]	1,96±0.01 [b]	1,88±0.04 [b]	1,81±0.05 [b]	2±0.05 [b]
45	1,8±0.02 [b]	1,86±0.03 [b]	1,79±0.03 [b]	1,62±0.06 [b]	1,6±0.05 [b]	1,72±0.11 [b]
60	1,41±0.06 [b]	1,52±0.05 [b]	1,4±0.02 [b]	1,31±0.02 [b]	1,29±0.04 [b]	1,44±0.07 [b]
p[1]	1,12±0.10 [b]	1,16±0.05 [b]	1,21±0.05 [b]	1,09±0.09 [b]	1,02±0.11 [b]	1,15±0.05 [b]

[b] statistically significant (p=0.001) decrease over the corresponding control (Kruskall Wallis test).

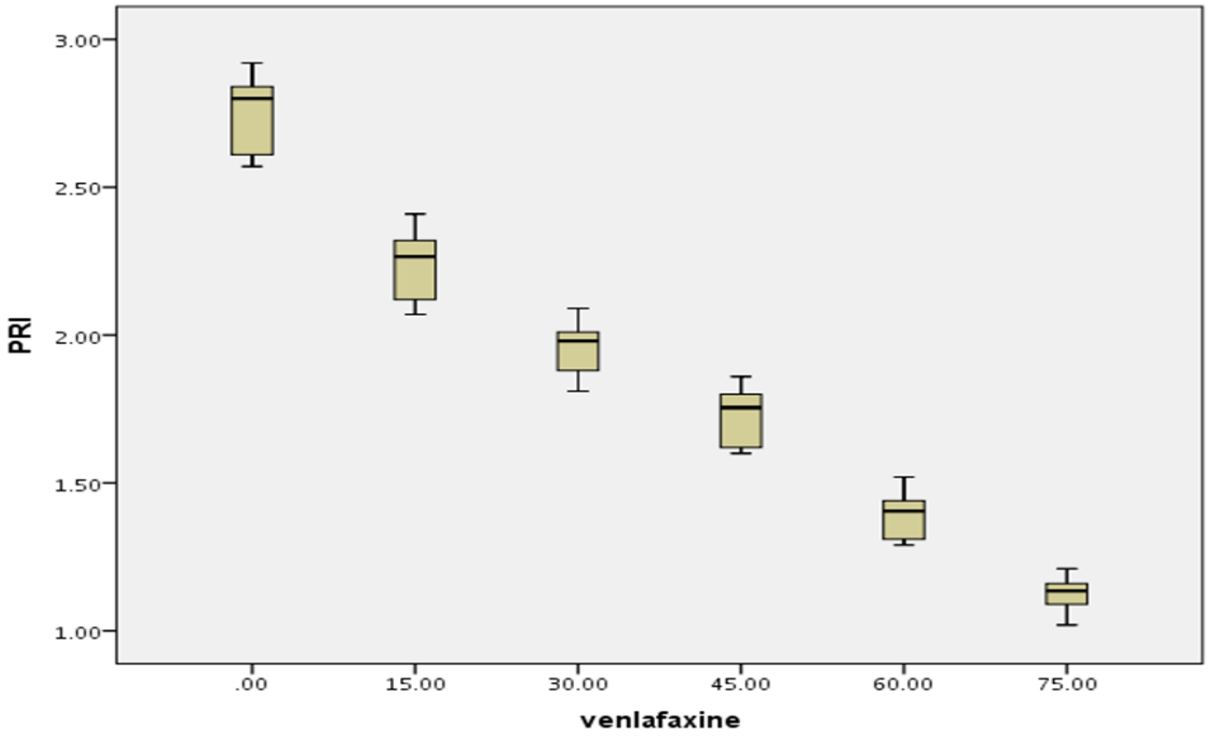

Figure 2: Effect of Venlafaxine on PRI mean frequency in human lymphocyte cultures from six healthy donors

Table 3: Effect of venlafaxine on MI frequency in T lymphocyte cultures of healthy donors

Venlafaxine (μg/ml)	Healthy donors (n=6)					
	1	2	3	4	5	6
Control	41±0.89	44±0.75	48±1.2	40±1.3	42±1.1	39±1.21
15	38±0.97 [c]	39±1.01 [c]	41±0.38 [c]	37±1.04 [c]	37±0.88 [c]	37±0.45 [c]
30	37±0.85 [c]	37±0.35 [c]	37±0.82 [c]	35±0.25 [c]	34±1.02 [c]	34±0.10 [c]
45	36±0.50 [c]	35±1.2 [c]	33±0.25 [c]	34±0.12 [c]	33±0.10 [c]	32±0.26 [c]
60	32±1.02 [c]	34±0.35 [c]	31±0.5 [c]	32±0.23 [c]	32±0.22 [c]	29±0.84 [c]
75	30±1.41 [c]	32±0.65 [c]	29±0.89 [c]	30±0.45 [c]	31±0.11 [c]	28±0.09 [c]

[c] statistically significant (p=0.001) decrease over the corresponding control (Kruskall Wallis test).

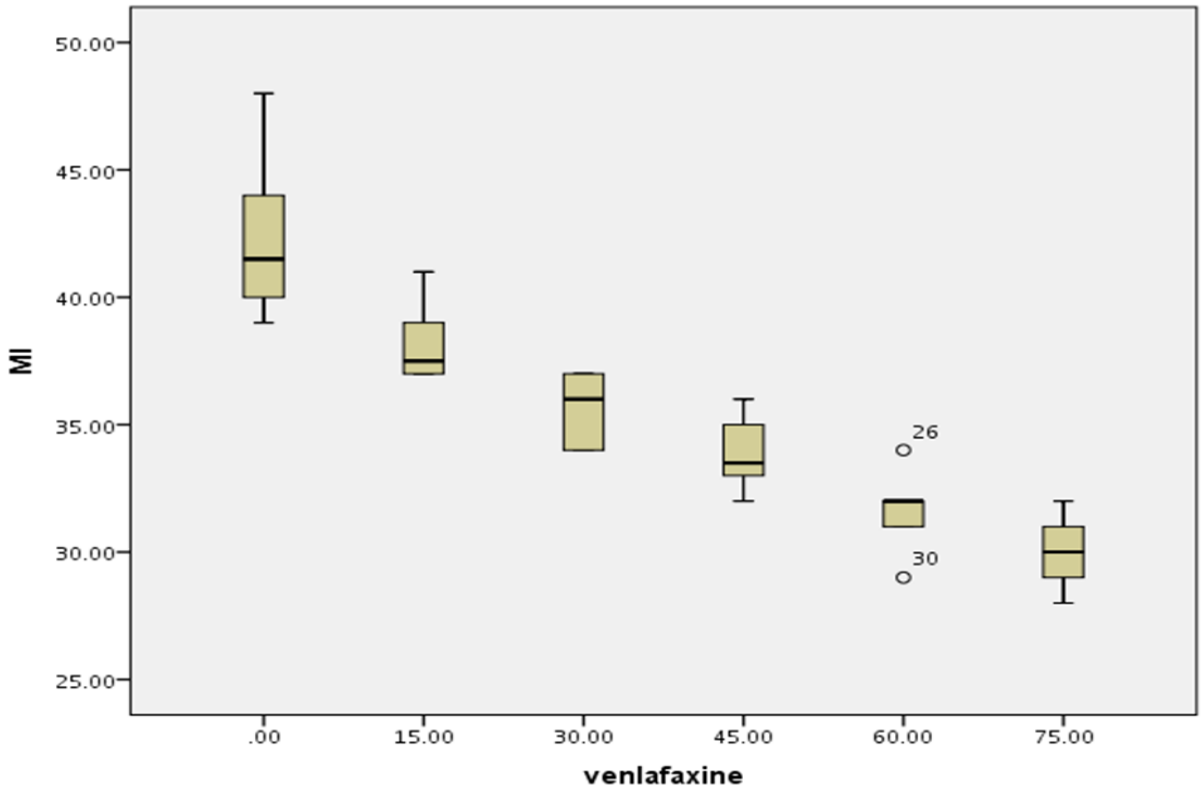

Figure 3: Effect of Venlafaxine on MI mean frequency in human lymphocyte cultures from six healthy donors

Table 4: Correlation rates

Correlation	Value	p[1]
SCEs -PRI	-0.944	0.01
SCEs -MI	-0.908	0.01
PRI -MI	0.949	0.01

[1]Spearman's rho

Discussion

Antidepressive drugs offer symptomatic relief in mood, anxiety and other psychiatric disorders not only by acting with a single biological target, but also interacting with multiple receptors and signaling pathways. Venlafaxine and other antidepressants require a treatment regimen of several weeks in order to achieve clinical response (Witkin et al, 2019). During this period, a lot of genetic and epigenetic molecular adaptations are taking place. However, human brain is difficult to access and study them. Evidence show that the hypothalamic-pituitary-adrenal axis is often severely disrupted in psychiatric disorders and inflammation has a crucial role in their pathophysiology (Leonard, 2018). Taking into consideration the role of T lymphocytes in inflammatory process (Rackaityte et al, 2020) and pathophysiology of major depressive disorder (Haapakoski et al, 2016), they can serve as an excellent in vitro model of genetic alterations in neuropsychiatric conditions.

Evidence show that venlafaxine seem to effect T lymphocytes by alter expression of genes involving cellular plasticity, cytoskeleton organization and lymphocyte migration (Kalman et al, 2005). Furthermore, venlafaxine seems to modulate gene expression in ionic transport that may participate in the Ca2+-signal required for activation of lymphocytes (Kalman et al, 2005). Furthermore, venlafaxine seems to modify T lymphocyte subpopulations in patients with major depressive disorder and regulate immune system function (Başterzi et al, 2010).

In the present study, the potential genotoxic and cytostatic effect of venlafaxine were evaluated using SCEs, PRI and MI assays in human cultured T lymphocytes *in vitro*. In addition, this method also has the advantage of testing the indices mentioned above in human CD4+ and CD8+ T lymphocyte populations. Dose dependent statistically significant SCEs induction, PRI and MI reduction due to venlafaxine's effect were observed. A literature review shows that our results come in agreement with results of Ayabaktı et al, 2020. They concluded that venlafaxine has a potential genotoxic behavior in human peripheral blood lymphocytes using other cytogenetic indices like chromosome aberrations and cytokinesis-block micronucleus assays. This genotoxic behavior of venlafaxine does not seem to be expressed in other cell lines *in vitro* (Brambilla et al, 2009). Other SNRIs like milnacipran (Yielmaz et al, 2017) and duloxetine (Madrigal-Bujaidar et al, 2015) also seem to induce DNA damage and express the same cytogenetic behavior as venlafaxine. This indicates a common mechanism of DNA damage induction by SNRIs. Mechanism of DNA damage expressed by SCEs index induction is not yet clear, but it

may be related to venlafaxine's chemical structure (Ayabaktı et al, 2020). Venlafaxine contains an N-dialkyl group which have an intercalative ability. Another possible mechanism of SCEs induction is an insufficiency in DNA damage repairing by venlafaxine's effect. Both DNA repair enzymes and epigenetic mechanisms that regulate DNA repair system may be involved. For instance, histone acetylation, one of histone post-translational modifications plays an important role in DNA damage response system and histone acetylation alterations may affect DNA integrity (Kim et al, 2019). Recent evidence indicates that venlafaxine alters histone acetylation (Qiao et al, 2019) and this can result in DNA damage response failure. Regarding the effects of venlafaxine on cell cycle kinetics, PRI and MI are diminished significantly and a strong correlation is observed between SCEs- PRI , SCEs-MI and PRI-MI alterations. These findings come to agreement with CD4+ T lymphocytes level decrease after exposure to venlafaxine (Fazzino et al, 2009). Another possible mechanism of cytostatic induction by venlafaxine is epigenetic alterations that lead to apoptosis. For example, dysregulation of histone deacetylases can lead to cell apoptosis (Patra et al, 2019). Finally, reactive oxygen species generation can induce DNA damage and apoptosis (Kocyigit et al, 2017), but venlafaxine seem to be protective against stress-induced oxidative DNA damage.

Conclusions

Taking into consideration the results of this study, venlafaxine shows a genotoxic and cytostatic cytogenetic behavior *in vitro.* In order to explicate these observations further studies are needed as cytogenetic studies in other cell lines (B lymphocytes, mononuclear cells etc) in vitro, or/and in vivo from patients with major depressive disorder as well as in population monitoring

Disclosure statement

No potential conflict of interest was reported by the authors.

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