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THE SAFETY AND EFFICACY OF A SILYMARIN AND SELENIUM COMBINATION IN MEN AFTER RADICAL PROSTATECTOMY – A SIX MONTH PLACEBO-CONTROLLED DOUBLE-BLIND CLINICAL TRIAL

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Background. Silymarin, a milk thistle flavonolignan mixture, has anti-proliferative and anti-angiogenic activities in xenografts of human prostate cancer (PCa). Low dietary selenium on the other hand has been associated with increased incidence of PCa. The purpose of the current trial was to determine whether a daily administration of a silymarin and selenium (SM-Se) combination for 6 months would alter basic clinical chemistry and oxidative stress markers, and improve the quality of life score (QoL) in men after radical prostatectomy (RP).

Methods. Thirty seven participants, 2–3 months after RP, were randomly assigned to receive 570 mg of silymarin and 240 μg of selenium as selenomethionine (n = 19, SM-Se group) or placebo (n = 18, Placebo group) daily for six months. Both groups had similar clinical and demographic characteristics. Physical examination, QoL score, haematology, basic clinical chemistry and oxidative stress markers, selenium and testosterone levels, antioxidant status were evaluated at baseline, at 3 and 6 months.

Results. The six months administration of silymarin and selenium improved the QoL score, decreased low density lipoproteins (LDL) and total cholesterol and, increased serum selenium levels. The combination had no effect on blood antioxidant status and no influence on testosterone level. No adverse events were recorded. No improvement was found in the placebo group.

Conclusions. The selected combination of silymarin and selenium significantly reduced two markers of lipid metabolism known to be associated with PCa progression, LDL and total cholesterol in the blood of men after RP. This suggests that this combination may be effective in reducing PCa progression.

INTRODUCTION

Prostate cancer (PCa) is the third leading cause of cancer death after colon/rectum and lung cancer in Europe[1]. The pathogenesis of PCa reflects hereditary, environmental and dietary components[2]. The latter are mainly dietary fats and calcium which may contribute to prostate cancer risk[3,4]. In recent years there has been an increasing interest in the potential chemopreventive properties of dietary components that may protect against prostate cancer[4]. Dietary factors proposed to decrease the risk of PCa are isothiocyanate sulforaphane and indole-3-carbinol in cruciferous vegetables, green tea polyphenols, particularly (-)-epigallocatechine-3-gallate, isoflavons genistein and daidzein found mainly in soybeans, long-chain n-3 polyunsaturated fatty acids, mixed tocotrienols and lycopene, the major carotenoid in tomatoes. In the literature, there are various opinions on the relation between the essential trace nutrient selenium acquired from normal dietary intake and the risk of PCa[5]. However, large studies have confirmed that selenium supplementation reduces the risk of PCa, particularly in men with low serum selenium levels[4,6]. The above-mentioned components are all nutraceuticals. Of phytoceuticals, only the extract from the seeds of milk thistle (*Silybum marianum*) silymarin and its major constituent, silibinin have shown efficacy in arresting human prostate carcinoma proliferation in a number of *in vitro* and *in vivo* preclinical models[7]. Silibinin blocks tumor cell proliferation[8] and angiogenesis. The latter is inhibited possibly by silibinin reduction of basic fibroblast growth factor and vascular endothelial growth factor[9].

Radical prostatectomy (RP) offers the best chance of curing prostate cancer. Unfortunately however, some patients have elevated levels of prostate-specific antigen (PSA) even after surgery. For this reason we hypothesized that a combination of silymarin and selenium (SM-Se) might be effective in reducing PCa progression. The aim of the present study was to evaluate the safety and tolerability of a 6 months daily consumption of 570 mg

silymarin and 240 μg selenium as a potential dietary supplement to be used in the tertiary prevention in patients after RP.

MATERIALS AND METHODS

Characteristics of study drug and placebo. The study preparations were manufactured according to Good Manufacturing Practise (GMP) by FAVEA (Kopřivnice, Czech Republic). The SM-Se tablet contained 190 mg silymarin (lot 040105, TEVA Pharmaceutical Company, Opava, Czech Republic) of the following composition (%; w/w): taxifolin 4.13, silychristin 17.00, silydianin 7.70, silibinin A 23.66, silibinin B 29.01, isosilibinin A+B 11.38, and undefined components 7.11; 80 μg selenium as selenomethionine (Lalmin® Se2000, Lallemand Human Nutrition A/S, Birkerød, Denmark); microcrystalline cellulose (250 mg), isomalt (60 mg), and hydroxypropyl cellulose (10 mg). The placebo tablet consisted of microcrystalline cellulose (250 mg), isomalt (250 mg), and hydroxypropyl cellulose (10 mg). The SM-Se and placebo tablets were coated with hypromellose, hydroxypropyl cellulose, talc, titanium dioxide, and caramel.

Study Design. A 6 months double blind placebo controlled trial was designed to assess the effect of a silymarin and selenium combination on patients after RP. The study protocol was approved by the Ethics Committee of the University Hospital and the Faculty of Medicine and Dentistry, Palacky University in Olomouc, Czech Republic. All of the participants signed an informed consent before any study procedures were initiated. The study took place from August 2007 to September 2008 at the Department of Urology, University Hospital in Olomouc, Czech Republic.

Study Subjects. Thirty seven men after RP, 51 to 72 years old, were invited to participate in the study. All subjects entering the study were 2 or 3 months after RP. Exclusion criteria included no nutrients, vitamins and minerals such as lycopene, vitamin E, selenium or herbal products with possible effects on prostate health, antibiotics, anti-inflammatory drugs, α-blockers and 5α-reductase inhibitors, intake of a vegetarian diet rich in isoflavonoids, history of food allergies, chronic liver or kidney diseases, gastrointestinal, or metabolic disorder or any other chronic health condition such as diabetes identified from the findings of the interview. Participants were randomly divided into two groups: Placebo (n = 18, aged 65.0 ± 3.9 years) and SM-Se (n = 19, aged 62.4 ± 6.4 years). They were instructed not to consume food rich in phenolics or make dietary or lifestyle changes during the study. In the SM-Se group, three tablets daily were given at approximately equal intervals throughout the day for a 6-month period. The placebo group received placebo tablets (3 tablets/day).

Health Investigation. During the health examination on the first day, after 3-months, and on the last day of study the following parameters were routinely assessed: (i) detailed medical history; (ii) assessment of all concurrent medical drug and therapies; (iii) dietary habits; (iv) quality of life score (QoL); (v) urinanalysis; (vi) kidney and bladder ultrasound; and (vii) a routine blood analysis.

Clinical Biochemistry and Hematology. Basic biochemical and hematological parameters were determined in all samples – sodium, potassium, chlorides, total cholesterol, low-density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, triacylglycerols (TAG), apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB), C-reactive protein (CRP), lactate dehydrogenase (LD), alanine transaminase (ALT), aspartate transaminase (AST), gamma glutamyl transferase (GMT), alkaline phosphatase (ALP), urea, creatinine, bilirubin, and testosterone (TST) using a HITACHI Modular Evo P analyzer (Hitachi, Japan). Prostate specific antigen (PSA) in serum was determined using an Architect type LEIA analyzer (Abbott Laboratories, Abbott Park, IL, USA). Selected parameters for evaluation of oxidative stress were determined as total antioxidant capacity (TAC) and SH groups in plasma (SHG_{tot}), lipid peroxidation products such as malondialdehyde in plasma (PMDA) and erythrocytes (MDA), advanced oxidation protein products (AOPP) in plasma; glutathione (GSH); glutathione peroxidase (GPX); catalase (CAT); glutathione reductase (GSR); glutathione transferase (GST); superoxide dismutase (SOD) in erythrocytes as described by Vidlar et al.[10] Selenium in plasma was determined by atomic absorption spectrometry using the AA6300 instrument (Shimadzu, Japan). Hemoglobin (Hb), hematocrit (Htc), erythrocytes (RBC), thrombocytes (PLT) and leukocytes (WBC) were measured in Na_2EDTA blood. *Urinanalysis.* Urine samples were collected from a midstream clean catch and analyzed using the IQ200 Automated Urinanalysis System (IRIS International, USA).

Statistics. Data were analyzed using software R. Nonparametric Wilcoxon two-tailed tests (paired-sample and two-sample) were used to determine any statistically significant differences between values of parameters on day 0 and after 6 months for the two groups. The level of significance was set at 5%. Values are presented as 1st quartile/median/3rd quartile or mean ± standard deviation. Box plots and graphs of empirical cumulative distribution functions are used as graphic illustration of significant differences in progression during 6 months between the Placebo and SM-Se groups.

RESULTS

Patients were recruited from August 2007 to March 2008, 37 men with a history of PCa and 2–3 months after radical prostatectomy participated in this randomized double-blind, placebo-controlled study. The baseline clinical and demographic characteristics are shown in Table 1 for all subjects and two randomization groups. The daily dose of silymarin and selenium was 570 mg and 240 μg respectively or placebo. Patients who received the combination of both components for 6 months (SM-Se group) had a better QoL score (Fig. 1) than those in the Placebo group. Haematology values were unchanged with the ex-

Table 1. Baseline demographics and clinical characteristics.

	Overall (*n* 37)	Placebo group (*n* 18)	SM-Se group (*n* 19)
Age (year)	63.8 ± 5.3	65.0 ± 3.9	62.4 ± 6.4
BMI	28.14 ± 2.48	27.9 ± 2.68	28.37 ± 2.33
QoL	2.19 ± 1.13	1.89 ± 0.96	2.47 ± 1.22
Selenium (µmol/l)	1.35 ± 0.48	1.24 ± 0.33	1.45 ± 0.58

Mean values and standard deviations.

Table 2. Markers of haematology in Placebo and SM-Se groups.

Parameter	Placebo group		SM-Se group	
	Day 0	Day 180	Day 0	Day 180
Hb (g/l)	145.5/150.5/155.5	142.0/152.5/158.8	135.0/145.0/148.5	**144/151/153***
RBC (10^{12}/l)	5.00/5.25/5.40	4.90/5.05/5.31	4.70/4.99/5.24	**4.86/5.12/5.33***
WBC (10^9/l)	5.89/7.27/8.68	6.01/6.68/7.67	5.53/7.01/7.92	5.42/6.59/7.45
Htc	0.42/0.45/0.47	0.42/0.44/0.47	0.41/0.42/0.44	0.42/0.44/0.45
PLT (10^9/l)	187/230/261	191/203/243	192/221/252	181/221/241

The values were expressed as 1st quartile/median/3rd quartile. The values are expressed as mean ± S.D. *$p < 0.05$ *vs* placebo.

Table 3. Markers of clinical chemistry in Placebo and SM-Se groups.

Parameter	Placebo group		SM-Se group	
	Day 0	Day 180	Day 0	Day 180
Na (mmol/l)	139/140/141	140/141/142	140/141/143	140/141/143
K (mmol/l)	4.25/4.43/4.58	4.18/4.44/4.62	4.12/4.30/4.47	4.17/4.35/4.56
Cl (mmol/l)	103/104/106	104/105/107	102/104/107	103/105/106
Urea (mmol/l)	4.95/5.80/6.18	5.60/6.05/6.47	4.70/5.30/6.70	5.10/5.80/6.75
Creatinine (µmol/l)	71.5/77.5/81.8	73.8/83.0/88.8	75.0/83.0/96.0	74.0/85.0/93.5
Bilirubin (µmol/l)	5.0/6.5/8.8	6.0/7.0/9.0	5.0/5.0/8.5	**5.0/7.0/11.0***
ALT (µkat/l)	0.34/0.40/0.50	0.38/0.46/0.66	0.39/0.44/0.71	0.43/0.53/0.67
AST (µkat/l)	0.42/0.46/0.50	0.39/0.44/0.56	0.40/0.47/0.56	0.46/0.53/0.57
ALP (µkat/l)	1.45/1.75/1.94	1.31/1.87/2.08	1.24/1.65/1.92	1.21/1.71/1.92
GMT (µkat/l)	0.38/0.45/0.75	**0.42/0.61/0.98***	0.29/0.48/0.54	0.33/0.45/0.53
LD (µkat/l)	2.44/2.72/3.07	2.62/2.74/3.05	2.71/2.82/3.04	2.67/2.85/3.05
CRP (mg/l)	1.0/1.0/2.0	1.0/1.5/2.8	1.0/1.0/2.5	1.0/1.0/2.0
Cholesterol (mmol/l)	4.86/5.23/6.03	4.53/4.93/5.87	5.06/5.55/7.08	**4.57/5.31/5.70***
TAG (mmol/l)	1.25/1.53/2.24	1.48/1.77/2.65	1.23/1.62/2.39	1.09/1.63/2.38
HDL (mmol/l)	1.12/1.31/1.58	**1.01/1.23/1.36***	1.10/1.19/1.64	1.12/1.23/1.44
LDL (mmol/l)	2.84/3.08/3.61	2.45/2.93/3.55	2.74/3.09/4.55	**2.51/2.98/3.70***
ApoA1 (g/l)	1.23/1.43/1.62	1.22/1.46/1.57	1.27/1.41/1.51	1.33/1.50/1.58
ApoB (g/l)	0.80/0.96/1.08	0.75/1.00/1.24	0.84/1.03/1.24	0.82/1.05/1.12
PSA_{tot} (µg/l)	0.00/0.00/0.02	0.00/0.01/0.10	0.00/0.00/0.01	0.00/0.00/0.04
Testosterone (nmol/l)	13.32/15.15/18.78	14.35/17.05/21.30	10.25/12.50/17.30	11.15/13.70/17.35
Selenium (µmol/l)	1.03/1.29/1.43	**0.68/0.86/1.13***	1.02/1.34/1.84	1.79/2.21/1.84*

The values are expressed as 1st quartile/median/3rd quartile. * $p < 0.05$ *vs* day 0.

Table 4. Markers of oxidative stress in Placebo and SM-Se Groups.

parameter	Placebo group		SM-Se group	
	Day 0	Day 180	Day 0	Day 180
PMDA (nmol/g)[a]	35.2/54.6/65.9	38.3/57.8/68.7	38.6/58.8/72.2	39.61/59.46/78.59
SHG_{tot} (μmol/g)[a]	2.37/3.16/3.81	2.44/3.14/3.49	2.37/3.19/4.14	2.16/3.40/4.08
AOPP (μmol/l)	123.4/159.8/268.1	140.0/168.6/221.5	159.0/214.9/240.5	152.0/168.8/213.9
TAC (nA)	5.49/6.82/7.17	5.4/7.0/7.7	5.61/6.24/7.45	5.63/6.58/7.27
MDA (nmol/g)[b]	0.34/0.39/0.43	0.36/0.40/0.49	0.39/0.44/0.46	0.38/0.43/0.48
GSH (μmol/g)[b]	12.16/12.96/14.15	**11.23/11.98/13.15***	10.62/11.78/13.57	10.60/11.04/12.41
SOD (U/g)[b]	2.33/2.57/3.05	**2.04/2.49/2.82***	2.34/2.73/2.99	2.19/2.55/3.08
GPX (μmol/min/g)[b]	17.59/22.16/31.52	**16.8/19.6/26.9***	20.56/27.31/35.54	18.75/26.95/37.56
CAT (μmol/min/g)[b]	117.3/129.3/153.7	119.9/132.1/151.5	101.9/117.2/151.3	99.62/124.83/154.28
GST (μmol/min/g)[b]	9.04/14.43/42.31	8.71/12.8/43.1	10.4/15.5/40.5	8.8/13.2/39.3
GSR (μmol/min/g)[b]	3.81/4.89/5.72	3.62/4.21/5.94	4.06/5.75/13.78	4.96/5.84/13.20

The values are expressed as 1st quartile/median/3rd quartile. * $p < 0.05$ *vs* day 0. [a]The value is expressed on g of protein. [b] The value is expressed on g of hemoglobin.

ception of a significant increase in erythrocyte count and hemoglobin in the SM-Se group, but the fluctuation was within normal physiological limits (Table 2). The effect of the SM-Se combination was further evaluated by monitoring liver function (ALT, AST, and GMT), lipid metabolism (cholesterol, HDL-cholesterol, LDL-cholesterol, TAG, ApoA1, and ApoB) and other laboratory parameters used for assessment of the safety and efficacy of the administrated preparation. In the SM-Se group, significant positive effects were found on plasma cholesterol and LDL-cholesterol levels (Table 3). On the other hand, a significant decrease in HDL-cholesterol level was found in the Placebo group. Long term consumption of the SM-Se combination led to raised levels of serum selenium (Fig. 2). Although change in bilirubin concentration was significantly different after six months for the SM-Se group, the fluctuation was within normal physiological limits. The oxidative stress markers are shown in Table 4. In the SM-Se group the silymarin and selenium consumption did not alter blood antioxidant status or other markers of oxidative stress. The antioxidant potential of the blood, measured as reduced glutathione and activity of antioxidant enzymes, superoxide dismutase and glutathione peroxidase differed significantly from baseline values in the Placebo group. After six months, PSA and testosterone levels were unchanged in both groups (Table 3). All participants finished the study and no subjective adverse effects were reported.

DISCUSSION

Factors that increase the risk of prostate cancer are (i) genetic disposition, (ii) age, (iii) ethnic background, (iv) chronic prostatitis, and (v) environmental factors including diet[2]. Finasteride and dutasteride, inhibitors of 5α-reductase, are recommended for the prevention of benign prostatic hyperplasia connected with chemoprotective effects on PCa[11]. The role of diet in prostate cancer development or PCa post-diagnosis is not fully understood[4]. Two studies on gene-enviroment interaction in relation to PCa showed the importance of dietary composition[12,13]. Of nutraceuticals, phytoceuticals and complex plant extracts or a combination of them, lycopene[14], soy flavonoids[15], silibinin[16] and preparations of soy and a combination of other supplements including vitamin E, selenium, lycopene and silymarin have been tested in human clinical trials (reviewed in ref.[4]). The data from these studies suggest that the phytochemicals tested retard the PSA double time increase in prostate cancer patients with relapsing disease and may delay progression of both hormone-refractory and hormone-sensitive PCa[17]. Silymarin is a complex of seven non-nutritive flavonolignans and one flavonoid[18]. It is one of the best pharmacologically characterized plant extracts. In mechanistic studies, silymarin displays antioxidant, anti-inflammatory, anti-proliferative, anti-fibrotic, anti-viral, and immunomodulatory activities[19]. It has clinical applications in alcoholic liver diseases, liver cirrhosis, *Amanita phalloides* poisoning, vi-

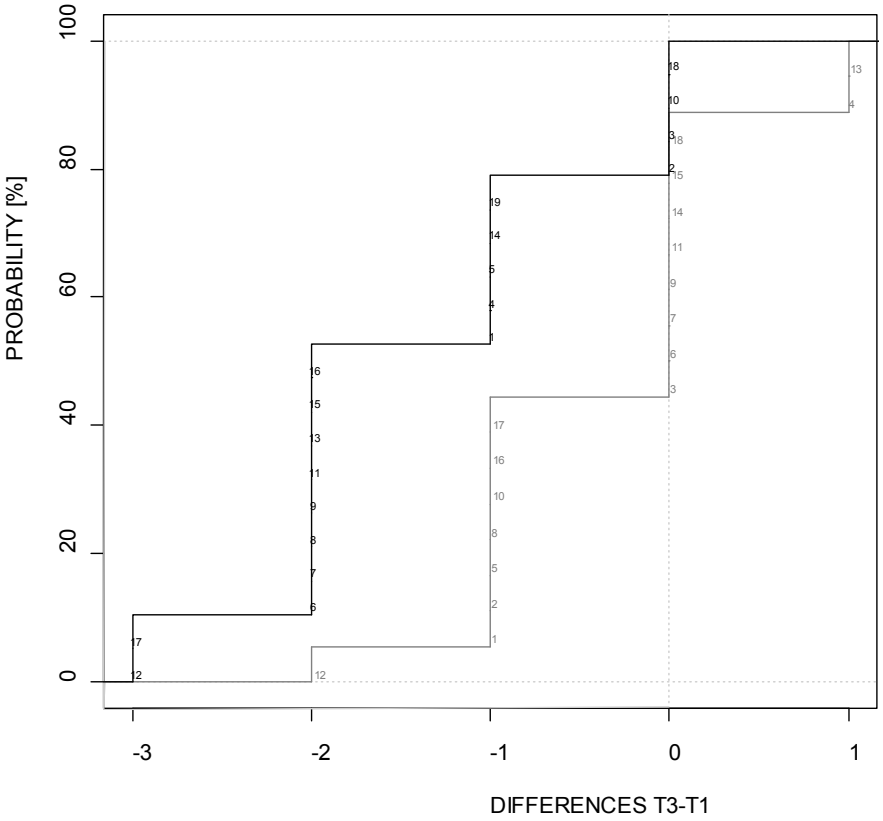

Fig. 1. Effect of silymarin and selenium combination on quality of life score during 6 month treatment. The values are expressed as differences value on day 180 and day 0 of study. SM-Se group, black line; Placebo group, grey line. *P < 0.05 vs Placebo group.

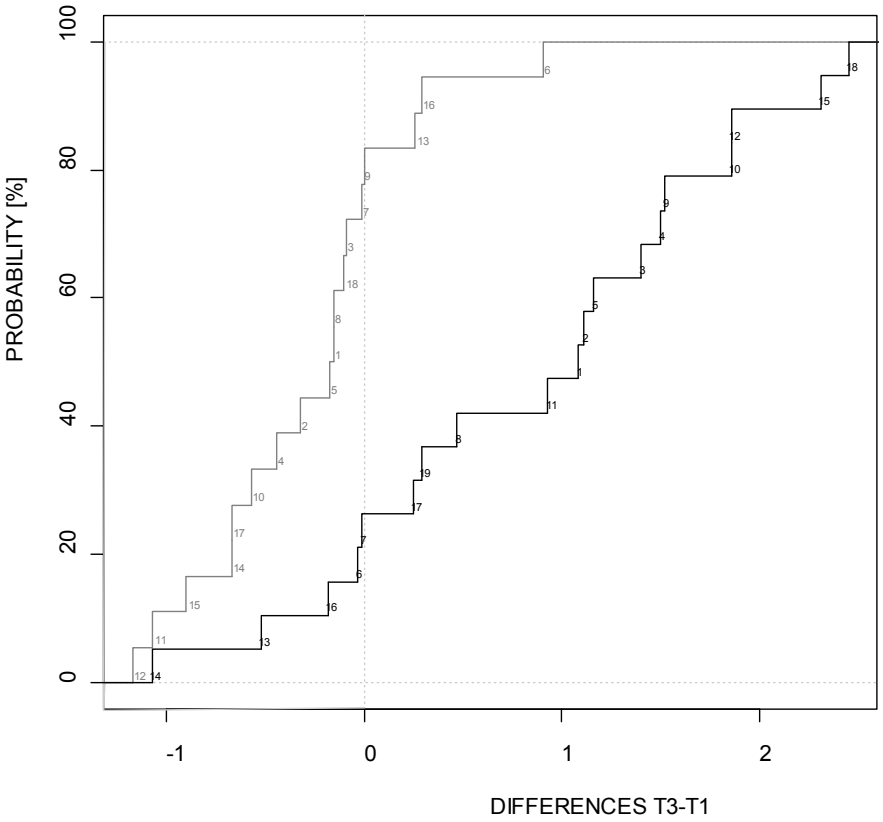

Fig. 2. Effect of silymarin and selenium combination on plasma selenium level during 6 month treatment. The values are expressed as differences value on day 180 and day 0 of study. SM-Se group, black line; Placebo group, grey line. *P < 0.05 vs Placebo group.

ral hepatitis C, and toxic and drug induced liver diseases[20]. Recently we reported that silymarin has a direct effect on LDL-cholesterol and total cholesterol in the blood of human volunteers[21,22]. In the study presented here, we selected patients after radical prostatectomy. For our pilot study a daily dose of 570 mg silymarin was chosen according to results from earlier study on healthy volunteers[21]. Our choice of 240 μg/day of selenium in the form of selenomethionine was based on the low level of selenium in our area. The 6 months administration of silymarin and selenium improved the QoL, decreased low density lipoproteins (LDL) and total cholesterol levels and increased the concentration of selenium in the blood. The combination had no effect on blood antioxidant status and had no influence on testosterone level. No adverse events were recorded. No improvement was found in the placebo group.

In summary, our study demonstrated that the orally administered silymarin and selenium had a positive effect on the organism of men after RP by (i) improving significantly the lipid parameters and (ii) increasing the blood selenium level. These findings suggest that a dietary intervention with a SM-Se combination could benefit patients after radical prostatectomy and who are at the risk of PCa progression.

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All authors declare having no conflict of interest.

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