A succinate-based composition ”rejuvenates” aging mice and alleviates menopausal symptoms in women without sex hormone replacement therapy

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Running title: Succinate-based therapy for menopause

Abstract

Objective

Menopausal transition is often accompanied by a variety of adverse pathological symptoms, currently treated with hormone replacement therapy, which is associated with a number of health risks. This report investigated the role of a food supplement—a composition of energy-exchange metabolites, with succinate as the main component—for treating menopausal syndrome.

Design

We studied the impact of a 4-week succinate-based food composition Amberen treatment on the estral cycle, and bone mass and calcium content of aging mice. The impact of Amberen on hormone levels and on the progression of several neurovegetative and psycho-emotional symptoms was further investigated in a randomized, double-blind, placebo-controlled clinical study of early menopausal women. Data were collected from questionnaires, Kupperman index scores, Spielberger-Hanin tests, and blood analysis of hormone levels taken at baseline and throughout the 5-week study.

Results

A “rejuvenating” effect of Amberen on aging animals was observed, expressed as restoration of the estral cycle and an increase in the weight and calcium content of bone tissue. Furthermore, in the randomized, placebo-controlled clinical study in menopausal women, Amberen-based monotherapy significantly lowered most subjectively evaluated characteristics of menopausal syndrome and increased blood serum levels of estradiol fourfold. This monotherapy also alleviated symptoms of some neurovegetative and psycho-emotional disorders, such as hot flushes, headache, and anxiety.

Conclusion

Succinate-based therapy alleviated many biochemical symptoms of menopause in both aging mice and early menopausal women, as well as neurovegetative and psycho-emotional disorders in women. Succinate-based therapy appeared to be free of adverse side effects.

Key words: menopause, aging, food supplement, succinate, estradiol

Introduction

Age-related menopause is characterized by cessation of the menstrual cycle accompanied by hormonal changes, as well as neurovegetative, psychological and atrophic symptoms.[18] The neurovegetative symptoms of the menopausal transition include hot flashes and sleep disturbances; psychological symptoms range from depression, anxiety, and irritability to memory loss.[7]

As a stage of reproductive senescence, menopause is triggered by a complex interplay of hormone levels and changes in the neuro-endocrine system, with changes in the ovaries and the hypothalamus being the crucial factors.[3,4,6,24] The early stage of menopausal transition, perimenopause, is also accompanied by vasomotor and psychological symptoms, as well as erratic behavior of the menstrual cycle and estrogen levels. The dramatic swings of estrogen production in the ovaries greatly affect the response of the aging hypothalamus-pituitary-ovary (HPO) axis, including induction of an increase in the level of the follicle-stimulating hormone (FSH). With final cessation of the menstrual cycle, estrogen production in the ovaries ceases, releasing the HPO axis from negative feedback by the ovaries and alleviating most climacteric symptoms.[3,6,18,24]

In the course of menopausal transition, more than 20% of women seek professional help in managing adverse symptoms. Currently, the only effective treatment of vasomotor and psychological menopausal symptoms is available in various forms of hormone replacement therapy (HRT), which aims to balance hormone levels artificially.[13,18] HRT and topical hormone creams are effective treatments for several symptoms, including hot flashes and vaginal atrophy. However, HRT has several adverse effects, including coronary heart disease and breast cancer, and in general, the health risks outweigh the benefits.[9,26] To this end, the current recommendations for such treatment regimens advise that the lowest possible dose be administered for as short a time as possible.[19]

An active role of the HPO axis in menopausal transition suggests that alternative treatment of menopausal symptoms may involve stimulating organs of the neuro-endocrine system. An aging hypothalamus loses sensitivity to estrogens,[3,4,6,24] which may play a role in decreased estrogen production of the ovaries.

Estrogen sensitivity of the hypothalamus can be restored with catecholamines.[3] However, influence of catecholamines on the hypothalamus declines with aging.[23] The alpha-adrenergic

agonist clonidin can restore the pulsatile pattern of luteinizing hormone (LH) secretion in the pituitary gland.[8] Yet, direct use of catecholamines and alpha-adrenergic agonists is undesirable because they induce various side reactions in the cardiovascular system.

According to our previous findings, catecholamine production in the body can be stimulated and mediated with succinate, a natural metabolic substrate.[14,15,16] Succinate administration can restore sensitivity of the hypothalamus to estrogen in aging rats.[5] Here, we probe the connection between the HPO axis and menopausal transition by studying the effects of a succinate-based compound (SBC) on the estrous cycle and bone status in animal experiments, and on the severity of menopausal symptoms in a clinical study.

Materials and methods

Animal studies

Forty-two female SHK mice were purchased from the Laboratory of Biological Research Animal Clinic of M.M. Shemyakin and Yu.A. Ovchinnikov (Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Pushchino). The mice were housed in polypropylene cages, (5 mice per cage) at a temperature of 22 ± 2°C. A light-dark cycle of 12/12 was used. The animals received standard laboratory chow (PK-121-2; Informkorm, Ltd.) and tap water ad libitum. Mice were checked daily by animal care personnel and weekly by a veterinarian. The study was carried out in accordance with regulations ensuring the humane treatment of animals under the approval of the Animal Research Committee of the Institute of Theoretical and Experimental Biophysics RAS.

Experiments were performed with 3 groups of outbred SHK female mice: young pubescent (2-3 months), adult (6-7 months), and aging (9-10 months) animals. Each group was subdivided into control and experimental groups, which were treated with the same combination of succinate salts and other components (according to the SBC Amberen formulation) used in the human studies. The dosage used in a pilot 2-week intrastomach administration of SBC to mice (5000 mg/kg) greatly exceeded that used in our experiments and did not reveal any acute toxicity. In the experiments, mice received 0.1 mL of aqueous SBC suspension (25 mg/mL, corresponding to 85 mg/kg by body weight) into the stomach twice-daily for 4 weeks. For humans, this corresponds to a single dose of 9.44 mg/kg, according to the human-to-mouse recalculation factor of 9.[10]

Stages of the estrous cycle in mice were analyzed cytologically, once per day (at 9 a.m.), by identifying the cellular composition of a vaginal smear. Smears were stained with azure-eosine by Romanovsky-Giemsa, fixed in Canada balsam, and analyzed with an optical microscope at 40× magnification (Amplival, Germany). We estimated the number of estral cycles (ECs), their duration, duration of the fertile stage (estrus), and variability of EC duration.

The isolated femoral bone tissue samples were weighed under wet conditions and then after drying for 3 days at 80°C. The samples were further ashed in a muffle kiln at 1000°C for 8 h. The calcium and sodium contents were determined in the ashed bone samples using an atomic absorption spectrophotometer (Perkin Elmer 503).

Reagents

The SBC formulation (Amberen) was designed at the Institute of Theoretical and Experimental Biophysics of the Russian Academy of Sciences (ITEB RAS). The formulation contains 2.1 mmol of succinate (246 mg), 0.43 mmol of glycine (32 mg), 0.21 mmol of glutamate (30.7 mg), 0.05 mmol of fumarate (6 mg), 47.4 mg of NH_4, 13.43 mg of Ca, 1.47 mg of Mg, 4.9 mg of Na, 1.71 mg of Zn, 7.2 mg of tocopherol acetate, and 9.2 mg of H_2O.

The compound was produced in paired gelatin pills: yellow and green—the yellow pill contained 200 mg of ammonium succinate, and the green pill contained all other components. Succinate (99.99% purity), its salts, and fumarate were produced by Ecomed-Service, Ltd. (Tula, Russia) using technology and patented processes developed by Ecomed-Service, Ltd. (Tula, Russia) together with ITEB RAS, which synthesized succinic acid and its salts that have unique bioactivity, determined by 3 types of active conformers. The natural conformational structure of succinate was tested and confirmed using polarized-laser-radiowave conformational spectral analysis with double-beam laser spectroscopy (ITEB RAS, Russia). The molar portion of the succinate anion in Amberen amounted to 75.77%.

Participants and procedures

Volunteers selected for participation in this study were patients diagnosed with menopausal syndrome. Out of 150 original volunteers, 80 were excluded for various reasons. A randomized, placebo-controlled, double-blind experiment with control measurements was carried out on all volunteers that were not excluded from participation. The patient exclusion criteria were

evidence of severe extragenital somatic pathology, evidence of any oncological pathologies, psychological disorders, and previous use of any hormone medication or supplement for menopausal syndrome management. A total of 70 patients were grouped into the following age brackets: 40-45 years (9 patients), 46-50 years (28), 51-55 years (25), 56-61 years (8). Thus, in general, most participants in this distribution were in the stages of early menopause. Of the 70 women in the randomized trial, 50 women were assigned to the experimental group and 20 control women were given placebo. Patients were treated with SBC Amberen contained in paired gelatinous 400-mg yellow and green capsules twice-daily—morning and evening—with food. The single Amberen dose was 5.27-5.7 mg/kg, and the daily dose amounted to 10.54-11.4 mg/kg.

Clinical observation

Each patient was observed by physicians before, throughout (especially during the first week), and at the end of the 21-day treatment, as well as 14 days after the end of the study period. The following pre-treatment and post-treatment clinical tests were performed:

- Clinical evaluation with Kupperman Index[1] (modified by E.V. Uvarova) by a consulting physician, gynecologist, and psychologist.
- The Spielberger's State Anxiety Inventory test.[22]
- Blood and urine tests for hormone levels, and blood tests for several biochemical parameters including cholesterol, A-cholesterol, triglyceride content, beta-lipoprotein, low-density lipoprotein, very low-density lipoprotein, total protein content, and glucose. The tests were performed with a biochemical analyzer (Hitachi 902/ Roche Diagnostics, and CardioChek™ P•A, PTS). We also determined thrombosis index and measured blood levels of estradiol, FSH, and LH with immunoenzyme kits (Micropale Steroid Estradiol, Gonadotropin IEA–FSH, and Gonadotropin IEA–LH).
- Ultrasonography scans of the uterus, ovaries, and mammary glands (HS-2000, Honda Electronics).
- Electrocardiography (ECG-9010, Nihon Kohden) was performed only during the initial exam.
- Volunteers were asked to keep a personal questionnaire journal, in which they scored the following symptoms: cardiac pains, vertigo, hot flushes, impairment of the ability to

concentrate, headache, irritability, depression, anxiety, insomnia, asthenia, muscle and joint pains, excessive sweating, and reduction of sexual desire. The journal was updated at least once per week during SBC or placebo administration and also after discontinuation of SBC. The journal was organized as follows:

1. Beginning date of SBC administration
2. Ending date of SBC administration.
3. Do you feel any discomfort associated with SBC administration? Mark the answer that corresponds to your feelings; if Yes, describe feelings of discomfort in detail and specify their duration over the treatment course and after its ending.
    - I did not experience any discomfort.
    - I felt discomfort (description).
4. Do you suffer from any chronic diseases? If so, specify the diseases you suffer from (consult with your physician before answering this question).
5. Did you receive any drugs apart from SBC? If so, specify the drugs, doses, and timeframe when taken (consult with your physician before answering this question).
6. 1st week of SBC administration. If you feel changes in the state of your health, describe them in your own words.
7. 2nd week of SBC administration. If you feel changes in the state of your health, describe them in your own words.
8. 3rd week of SBC administration. If you feel changes in the state of your health, describe them in your own words.
9. 1st week after discontinuation of SBC. If you feel changes in the state of your health, describe them in your own words.
    - Dates of functional and laboratory analyses: general blood test; general urine test; biochemical blood tests; ECG; ultrasound scanning of uterus, ovaries, and breast
    - Evaluation by gynecologist, psychiatrist, and consulting physician
10. Date of journal closure. Personal signature of patient. Signature of consulting physician.

Each patient was informed and signed an agreement describing the conditions of the study. Permission to perform patient examination and clinical research was granted by the Commission at the 1st Municipal Clinical Hospital of Ulyanovsk.

Statistical analysis

All primary documentation—in the form of questionnaire journals, patient cards filled by consulting physicians, and protocols of laboratory and instrumental studies—was recorded in a Microsoft Access database. The data obtained were evaluated by parametric tests using Microsoft Excel and Statisitca-6 software. The results are given as mean ± standard errors.

Results

Animal study

The administration of SBC to aging mice had a favorable effect on the appearance and behavior of animals—they became more active, their lackluster eyes acquired brightness, and the pale, yellowish coat regained white color and shine, resembling the coat of young animals. The bald skin spots grew new hair.

In mice, we evaluated the impact of SBC Amberen on the duration and variability of EC and the duration of the fertile estrus phase. Healthy young pubescent mice have four EC phases: proestrus phase, fertile estrus phase, and non-fertile metestrus and diestrus phases.

The principal difference between EC in young prepubescent and aging mice is that in aging mice, the fertile estrus phase is shorter (Table 1).

In pubescent mice, administration of SBC did not affect these parameters, although EC duration became more variable. In adult mice, EC duration variability decreased slightly with SBC treatment.

The most pronounced differences were observed in aging mice. Amberen treatment did not affect EC duration but increased EC duration variability by 48 %. Total duration of the fertile estrus phase increased by 69%, and duration of the estrus phase relative to the entire cycle increased almost twofold (Table 1). Most importantly, duration of the absolute and relative estrus phase in SBC-treated mice became comparable to that of healthy young mice.

Table 1.

Age-related changes in the estrous cycle of mice treated with SBC or Placebo for 4 weeks/

Injected preparation	EC duration (days)	Variability (% of EC duration)	Duration of E-stage (days)	E/EC Ratio
1.Young females (2-3 months),				
Placebo (n=6)	7.4±0.56	18.9±3.3	2.5±0.28	0.35±0.06
Amberen (n=6)	9.0±0.92	25.6±4.5	2.4±0.12	0.27±0.05
2.Adult females (6-7 months)				
Placebo (n=12)	8.4±0.99	26.2±2.4	2.8±0.31	0.34±0.03
Amberen (n=11)	7.2±0.22	6.9±0.7 p_{2P}<0.001	2.7±0.27	0.38±0.04
3. Old females (9-10 months)				
Placebo (n=8)	8.3±0.47	13.2±1.8 p_{2P}<0.01	1.6±0.21 p_{1P}<0,05 p_{2P}<0,01	0.19±0.025 p_{1P}<0,05 p_{2P}<0,01
Amberen (n=9)	8.2±0.70	19.5±2.3 p_{3P}<0,05	2.7±0.22 p_{3P}<0.01	0.33±0.04 p_{3P}<0.01
Impact of Amberen treatment on old mice	0	↑ 1.48	↑ 1.69	↑ 1.74

The data are given as means ± standard errors, where n is the number of animals in the group. P_{1C}, P_{2C}, P_{3C} are significance levels when the current group is compared with the 1st, 2nd and 3rd control groups respectively. Animals in the control groups were administered 0.9% NaCl. Variability of EC duration is defined as a quotient of standard deviation to average EC duration (%). ↑ - increase, ↓ - decrease, n-fold. Control, a group of animals treated for 4 weeks with an aqueous solution. SBC, a group of animals treated for 4 weeks with the SBC suspension.

Next, we measured the impact of SBC treatment on total mass and bone composition in female mice. Changes were observed in control adult animals with the onset of aging. Old mice, compared with adult mice, exhibited a reduction in body weight (33.5 ± 1.7 g versus 28.7 ± 2.4 g) and a decrease in bone weight, as well as in water and organic content (Figure 1). In addition, control mice showed a net increase in the total amount of bone sodium and calcium (Figure 2). SBC treatment of adult mice showed no effect on these characteristics. In contrast, SBC

treatment of old mice substantially decreased total weight loss (33.0 ± 1.0 g. versus 31.4 ± 1.5 g in young and old mice, respectively) and also increased bone weight and the total amount of water, organic matter, calcium, and sodium in the bone tissue (Figures 1 and 2).

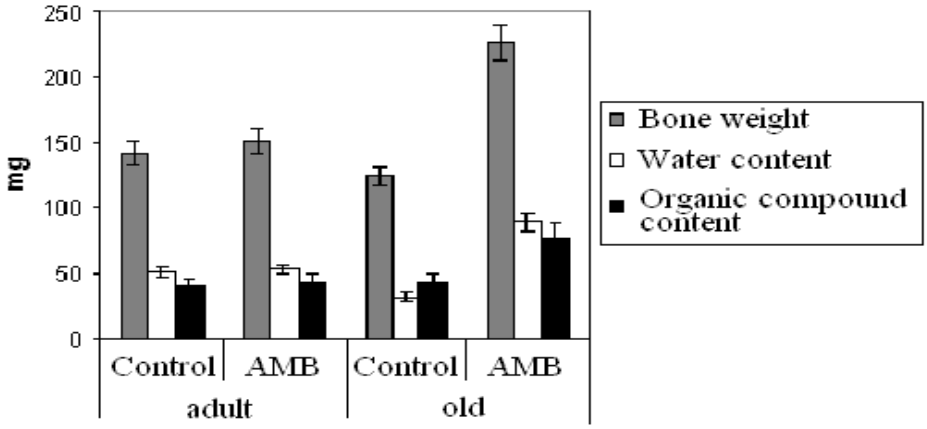

Figure 1. Femoral bone weight and the total amount of bone water and organic matter in adult (6-7 months) and old (9-11 months) mice treated for 4 weeks with a suspension of SBC or 0.9% NaCl.

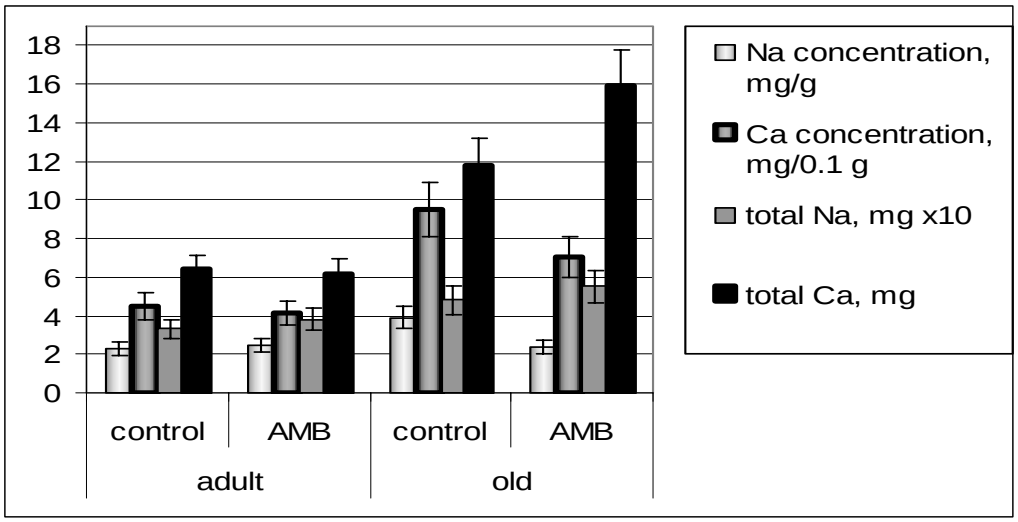

Figure 2. Concentrations and total amount of sodium and calcium in the femoral bones of adult (6-7 months) and old (9-11 months) mice treated for 4 weeks with suspension of SBC or 0.9% NaCl.

Clinical study

In both the experimental and control groups, menopausal women had lower average levels of estradiol (E2) than usually observed in healthy perimenopausal women at the beginning of follicle maturation, when the E2 level is minimal.[21] However, the levels of FSH and LH were much closer to those in perimenopausal women. E2 levels increased nearly fourfold with SBC, compared with pretreatment levels (Figure 3), while E2 levels were unaffected with placebo. FSH and LH levels did not change with either Amberen or placebo.

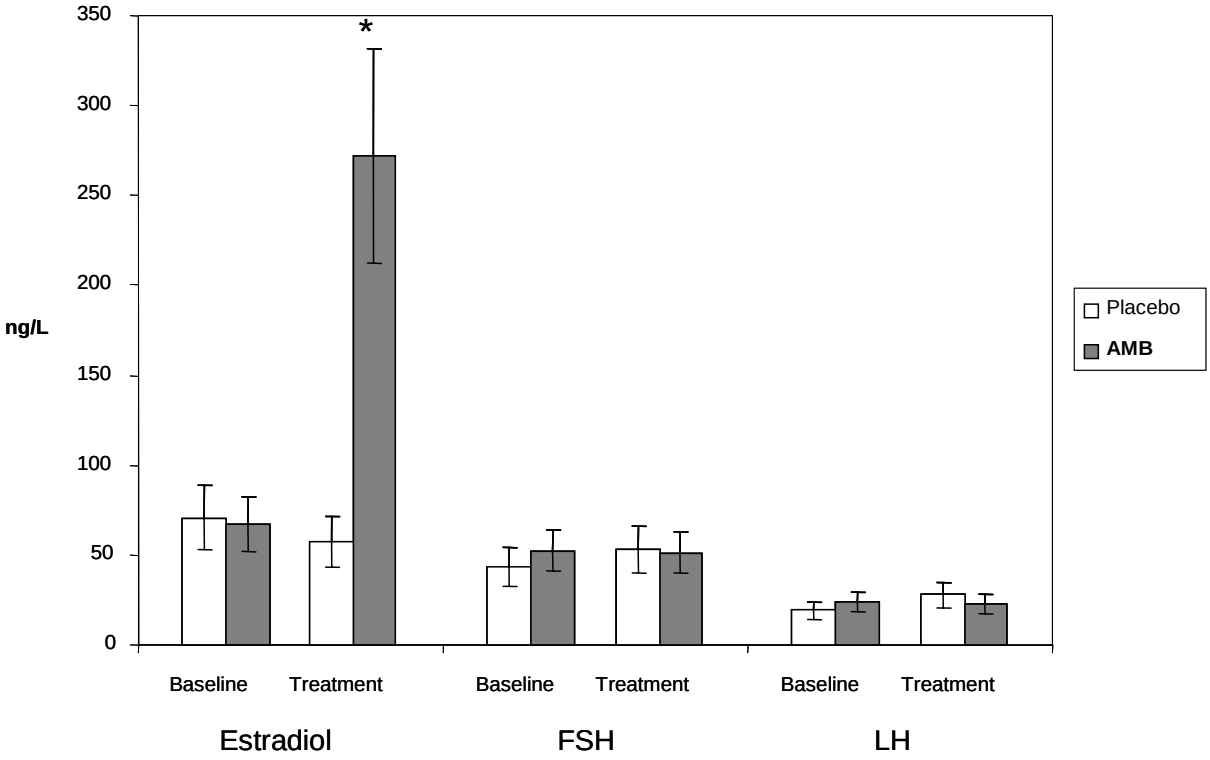

Figure 3. Blood levels of FSH and LH in menopausal women treated with SBC or placebo. *P<.001 compared with both pretreatment levels and placebo.

Out of 20 neurovegetative presentations of menopausal syndrome evaluated by physicians according to the Kupperman Index, the following disturbances were largely alleviated by Amberen: headache, vestibulopathies, increased excitability, somnolence, increased excitability, somnolence, insomnia, and frequency of hot flushes (Table 2). Placebo, as a rule, improved some parameters, albeit without any statistical significance. After Amberen treatment, the difference between the impact of Amberen and placebo was not statistically significant. The anxiety levels

measured by the Spielberger's State Anxiety Inventory test declined significantly from 54.8 ± 1.8 to 47.3 ± 1.7 ($p < 0.002$) in the SBC-treated group, and from 53.1 ± 2.4 to 50 ± 2.0 (n.s.) in the placebo group).

Table 2.
Neurovegetative presentations of menopausal syndrome in SBC or placebo-treated women (numerical score). Physician's evaluation according to the Kupperman scale with Uvarova`s modification.

Parameters	Amberen		Placebo	
	before treatment (n = 50)	after treatment (n = 50)	before treatment (n = 20)	after treatment (n = 20)
Arterial blood pressure increase	0.98 ± 0.12	0.70 ± 0.11	1.40 ± 0.26	1.20 ± 0.25
Arterial blood pressure decrease	0.40 ± 0.12	0.24 ± 0.10	0.25 ± 0.17	0.25 ± 0.17
Headache	1.54 ± 0.10	0.92 ± 0.10**	1.35 ± 0.16	1.30 ± 0.18
Vestibulopathies	0.72 ± 0.12	0.38 ± 0.08*	0.55 ± 0.15	0.55 ± 0.15
Paroxysms of tachycardia	1.24 ± 0.15	0.98 ± 0.14	1.25 ± 0.26	0.90 ± 0.23
High temperature intolerance	0.84 ± 0.14	0.66 ± 0.13	0.50 ± 0.18	0.40 ± 0.17
Chill /shiver	0.96 ± 0.14	0.68 ± 0.12	1.00 ± 0.24	0.90 ± 0.21
Numbness	1.16 ± 0.14	1.00 ± 0.13	1.35 ± 0.27	1.35 ± 0.50
Dermographism	1.04 ± 0.13	1.02 ± 0.14	1.25 ± 0.21	1.20 ± 0.21
Xerodermia	0.56 ± 0.13	0.56 ± 0.13	0.70 ± 0.19	0.45 ± 0.16
Hyperhidrosis	1.26 ± 0.14	1.00 ± 0.13	1.00 ± 0.23	0.80 ± 0.22
Inclination to edemata	0.98 ± 0.13	0.84 ± 0.12	0.95 ± 0.38	1.00 ± 0.16
Allergic reactions	0.38 ± 0.11	0.28 ± 0.09	0.15 ± 0.08	0.25 ± 0.09
Exophthalmus, eye luster	0.12 ± 0.05	0.12 ± 0.05	0	0
Increased excitability	1.12 ± 0.13	0.46 ± 0.10**	1.05 ± 0.17	0.60 ± 0.16*
Somnolence	1.34 ± 0.15	0.68 ± 0.13**	1.15 ± 0.24	1.10 ± 0.23
Insomnia	1.32 ± 0.14	0.60 ± 0.13*	1.35 ± 0.24	0.95 ± 0.24
Hot flushes per day	1.12 ± 0.10	0.68 ± 0.08**	0.95 ± 0.12	0.70 ± 0.12
Asthmatic fits	0.30 ± 0.10	0.06 ± 0.04*	0.05 ± 0.04	0.05 ± 0.04
Sympathoadrenal crises	0.08 ± 0.06	0	0	0

Comparison with the state before treatment: *$p < 0.05$, ** $p < 0.01$.

Compared with the psychological state before treatment, Amberen-administered women showed an evident alleviation of practically all psychological presentations of menopausal syndrome (Table 3), but a statistically significant difference between the impact of Amberen and placebo was observed only for neurotic syndrome.

Table 3.
Psychological presentations of menopausal syndrome in SBC or placebo-treated women (numerical score). Physician's evaluation according to the Kupperman scale in Uvarova`s modification.

Syndromes	Amberen (n = 50)		Placebo (n = 20)	
	before treatment	after treatment	before treatment	after treatment
Asthenic	0.92 ± 0.10	0.50 ± 0.08**	0.95 ± 0.09	0.70 ± 0.10
Depressive	0.42 ± 0.10	0.14 ±0.05*	0.30 ± 0.12	0.15 ± 0.08
Anxious-phobic	0.66 ± 0.11	0.32 ± 0.07*	0.55 ± 0.19	0.30 ±0.14
Neurotic	1.04 ± 0.08	0.48 ± 0.07** #	0.95 ± 0.11	0.90 ±0.12
Hypersexual	0	0	0	0
Hyposexual	1.18 ± 0.12	0.58 ± 0.12**	1.10 ± 0.20	0.90 ± 0.20

Comparison with syndrome score before Amberen treatment: *p < 0.05, ** p < 0.01.
Comparison between the impact of Amberen and placebo: # p< 0.02.

In addition, data gathered from the personal questionnaire journals demonstrated that SBC-treated patients noted a significant alleviation of 11 symptoms: cardiac pain, dizziness, hot flashes, attention impairment, headache, irritability, depression, anxiety, insomnia, and muscle and joint pains; the difference between Amberen and placebo was statistically significant for a number of registered symptoms (Figure 4).

The biochemical blood and urine tests indicated a slight decrease of glucose levels in the Amberen-treated group, with the other 9 parameters comparable between pre-treatment and post-treatment measurements (Table 4).

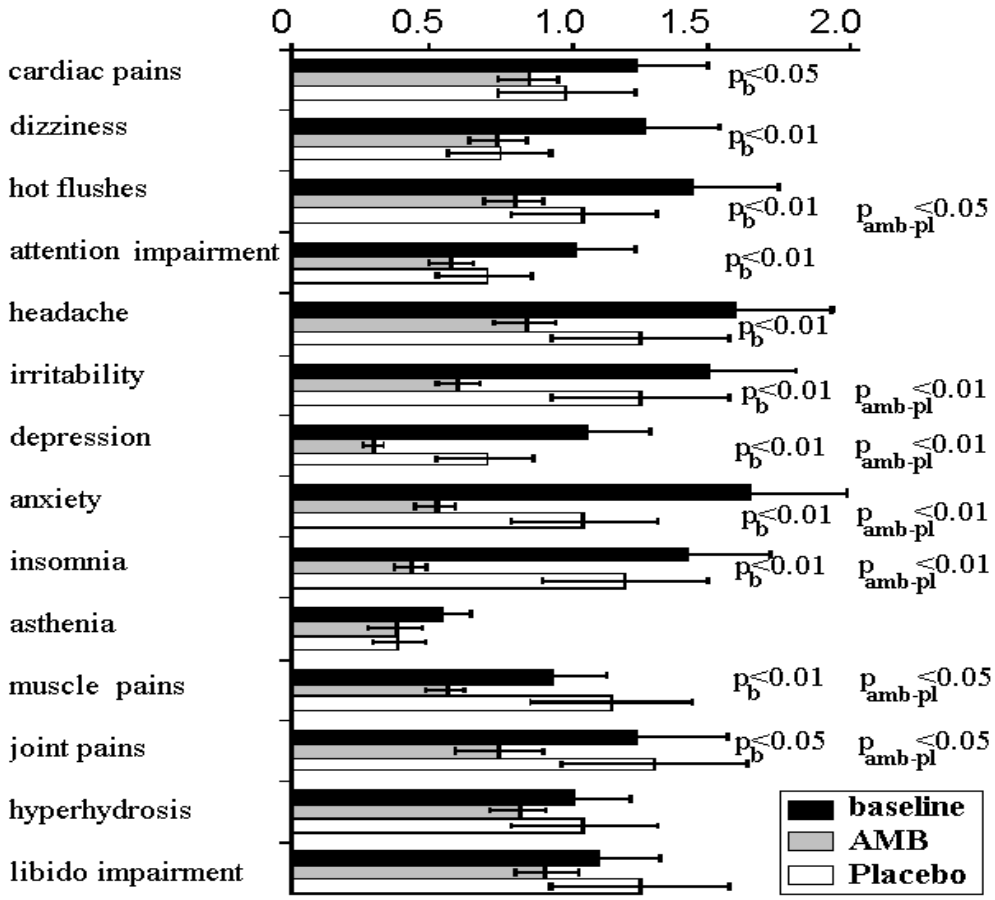

Figure 4. Subjectively evaluated state of menopausal women (numerical score) treated with SBC or placebo (p_b is the significance level in the t-test comparison of SBC effect to the level observed before SBC treatment; p_{sbc-pl} is the comparison between SBC and placebo).

The ultrasonography scans exhibited no difference in the size of the uterus or ovaries (data not shown). In the Amberen-treated group, endometrial thickness decreased by 0.61 ± 0.28 mm on average (versus 0.24 ± 0.18 mm in the control group), compared with an initial thickness of 4.58 ± 0.95 mm. Also, the endometrium appeared more homogeneous in the SBC-treated group.

Placebo did not influence biochemical blood test parameters to any statistically significant extent.

Table 4.

Biochemical blood test parameters in women with menopausal syndrome, measured before and after SBC or placebo treatment.

Parameter, units	Amberen		Placebo	
	before treatment	after treatment	before treatment	after treatment
Glucose, mmol/L	4.73±0.14	4.19±0.16 p<0.02	4.85±0.26	4.68±0.24
Total cholesterol, mmol/L	4.62±0.31	4.83±0.44	4.92±0.54	5.55±0.64
α-cholesterol, mmol/L	1.27±0.04	1.34±0.05	1.20±0.07	1.44±0.10
Atherogenic coefficient	2.65±0.19	2.71±0.30	3.12±0.48	2.98±0.53
Triglycerides, mol/L	1.27±0.11	1.36±0.16	1.50±0.26	7.14±10.59
β-lipoprotein, mmol/L	3.82±0.24	4.44±0.33	4.34±0.82	4.77±0.81
LDL, mmol/L	2.69±0.22	3.38±0.30	2.95±0.44	3.60±0.49
VLDL, mmol/L	0.57±0.05	0.65±0.07	0.69±0.12	0.78±0.18
Total protein, g/L	71.08±1.59	75.11±1.23	68.92±2.23	71.89±2.78
Prothrombin index, %	90.3±1.27	90.8±1.11	90.85±1.91	91.1±2.08

Discussion

Our experimental results demonstrate that SBC administration has a favorable effect on the appearance, behavior, EC structure, and bone mass in aging mice, but these parameters in adult and aging (old) animals were only influenced slightly. SBC treatment provided the restoration of duration of the fertile estrus phase in old mice to the level of young mice, protected against age-related bone mass loss, and promoted an increase in water, organic compound, and total calcium content in femoral bone tissue. Because bone loss and calcium resorption both occur as a result of estrogen deficiency, it has been suggested that SBC treatment increases the level of estrogen in aging mice.

In our double-blind, placebo-controlled clinical study, 4-week Amberen treatment sharply increased estradiol concentrations in the blood of menopausal women without any shifts in FSH and LH levels. Simultaneously, Amberen therapy improved the subjective state in menopausal women and relieved many neurovegetative symptoms, such as headache, vestibulopathies, increased irritability, somnolence, insomnia, and frequency of hot flashes. Furthermore, Amberen treatment reduced the manifestations of asthenic, depressive, anxious-phobic, neurotic, and hyposexual syndromes in menopausal women.

We attribute the observed effects of SBC treatment to the recently discovered substrate-signal function of succinate and its influence on the metabolism and regulatory relationship of the HPO axis. It is necessary to emphasize that in our study, animals and women received very low doses of SBC (Amberen), the main components of that are mitochondrial intermediates. These doses are significantly lower than the "substrate" doses required by mitochondria. Our experiments in animal and clinical studies showed that significant physiological effects are induced by essentially smaller succinate doses, concentrations of which are comparable to those of signal mediators and hormones. Correspondingly, we designate this the "signal effect".

We have demonstrated adrenomimetic effects of succinate in animals - which can be eliminated with propranol, a beta-adrenoblocator - and in healthy humans as long as ammonium succinate doses do not exceed 2 mg per kg of body weight.[17] This signal effect implies the existence of succinate receptors, which have been identified independently of our study.[11] Thus, when administered in signal-level doses, succinate is a strong physiological regulator. In particular, it influences the renin-to-angiotensin conversion[11,12] connected with adrenalin/noradrenalin synthesis. Succinate can stimulate activity of the suppressed hypothalamic-pituitary system and correspondingly restore ovary function.[5]

According to Dilman's theory, hypothalamic sensitivity decreases so much with age that the center ceases to react to signals from ovaries and equally halts the cyclic stimulation of ovaries by the hypothalamus.[3] However, although female reproduction function ceases, the potential of ovaries is not yet exhausted. Despite its recommendation for alleviation of menopause-related pathological symptoms, HRT is accompanied by adverse reactions and might increase the risk of developing cancers.

In our studies, an HRT-like effect was achieved by administering a natural, non-hormonal compound based on succinate and other natural mitochondrial metabolites that essentially mimic

the effects of hormones. Our approach, which focuses on correcting metabolism and neuro-endocrine control by using a signal energy intermediate, SBC, instead of exogenous hormones, is the first of its kind and represents a step forward in metabolic mitochondrial medicine. It offers hope in the development of safer treatments for a wide range of diseases, particularly those associated with aging.[2] Our results on the effects of SBC in senile mice and Amberen on the neurovegetative status of menopausal women suggest that the same approach may be also effective in the therapy of other menopause-related symptoms in women.

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