From cellular to chemical approach for acute neural and alternative options for age-induced functional diseases

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Abstract

Endogenous "stem cell niche" (SCN) accompanying vessels contains immune system components which *in vivo* determine differentiation of multi potent stem cells toward proper cell types in given tissue. Combinations of sex steroids may represent novel chemical approach for neuronal areas of regenerative medicine, since they cause transformation of vascular smooth muscle stem cells into differentiating neuronal cells. Circulating sex steroids are present during pregnancy and can be utilized where needed, when various embryonic/fetal tissues develop from their stem cells. Utilization of induced regeneration of tissues (regenerative medicine) is expected being more effective in sudden failures of younger individuals carrying intact SCN, as compared to established chronic disorders caused by SCN alteration. An essential component of SCN are monocyte-derived cells exhibiting tissue-specific "stop effect" (SE) preventing, for instance, an aging of neuronal cells. Its alteration causes that implantation of neuronal stem cells will also result in their differentiation toward aging cells. When we repair the SE by supply of circulating mononuclear cells from young healthy individuals, we may be able to provide novel regenerative treatments of age-induced neural diseases by sex steroid combinations. Questions regarding some age-induced body alterations are also addressed.

Key words: Cellular differentiation; Endogenous stem cells; Neurological disorders; Regenerative medicine; Sex steroid combinations; Stem cell niche; Treatment of aging diseases

Core tip: Sex steroid combinations have been found to transdifferentiate *in vitro* the vascular smooth muscle cells into neurons. Therefore, utilization of sex steroid combinations may enable regeneration of neural tissues affected by acute/traumatic disorders. In aging individuals, however, an altered immune system components in the stem cell niche may be unable to preserve regenerating cells in the functional stage due to the immune system regression with age advancement. This could be improved by transfer of blood mononuclear cells from young healthy individuals. Beside that, the local and systemic utilization of honey

bee propolis alone has been found to ameliorate some age-induced disorders.

INTRODUCTION

To deal with regeneration of tissues, we need to better understand their preservation in the appropriate functional state. Under normal embryonic and fetal development, the normal tissue function is established and programmed to last for certain period of subsequent life. In other words, even in normally developed individuals, functional longevity in distinct tissues is different. The most extended functional tissue life is established for such tissues that evolved functionally as earliest, and the opposite applies for those which functionally developed as the latest, with variations between these states. For instance early developing functional human heart can work for one hundred years, while the proper function of late developing fetal ovary will last for a much shorter period. In reality, the ovarian dysfunction is initiated between 35 and 40 years of age, due to the age-induced alteration of the ovarian stem cell niche (SCN). Due to the absence of corpora lutea during fetal immune adaptation toward self tissues, they are considered to be a "graft", which results in their cyclical rejection during menstrual cycles in adulthood (reviewed in[1]). The corpus luteum rescue during pregnancy accompanies immune tolerance of fetal allograft, and both effects required for successful pregnancy are considered to be caused by trophoblast-derived chorionic gonadotropin and other endocrine factors[2,3].

The perivascular tissue-specific SCN enables regeneration of tissues from endogenous stem cells. We still do not have a detailed information regarding role of SCN in distinct tissues. The intent of the current regenerative medicine is to deal with a replacement of dysfunctional cells with various types of exogenous stem cells, utilizing for instance induced pluripotent stem cells (iPSCs), embryonic stem cells (ESC), or somatic stem/progenitor cells, which are able to differentiate into functional cells of particular tissue[4]. Such an approach, however, carries a number of complications, including ethical concerns, immune responses toward implanted cells, possibility of teratoma development, lack of ability of implanted cells to regenerate, failure of their cell cycle, lack of their preservation in functional stage, and apoptosis[5-7]. Moreover, most attempts to establish ESC from large mammals have failed[8] and human iPSCs develop into teratomas when tested in immunosuppressed animals[9]. Recent observation also indicates that iPSCs carry a genome methylation memory of their former differentiation history, which might alter their therapeutic utilization[10].

Another possibility is to stimulate autologous pluripotent stem cells to develop *in situ* into functional cells. Such approaches eliminate some of the hurdles mentioned above but don't deal with concerns about the success of the return of regular tissue function[5]. Chronic disorders are expected to be caused by an altered tissue-specific SCN which causes permanent tissue damage. Of particular interest are SCN alterations stimulating degeneration of functional tissue cells toward apoptosis in degenerative diseases.

An essential role in the maintenance of tissues by SCN plays so called "stop effect" (SE) encoded at monocyte derived cells (MDC) in tissue-specific manner. The SE enables tissue cells to differentiate into the proper stage for their function, but not beyond. Stage of cellular differentiation needed for proper tissue function differs among the tissues. For instance brain neural cells should be prevented to undergo advanced differentiation toward apoptosis, while superficial cells of epidermal stratified epithelium should differentiate into apoptotic squamous cells for the proper epidermal function and protection. The SE is established epigenetically, during the fetal immune adaptation. Lower or higher cellular differentiation during that period than required for proper tissue function will cause dysfunction of that tissue during subsequent life (reviewed in[1]). In aging individuals, there is a "shift up" of the SE, causing age-induced functional diseases, *e.g.*, Alzheimer's disease.

In other words, improper function of tissues is caused by altered tissue-specific SCN, and may result in either functional immaturity or aging of such tissues.

Utilization of stem cells alone will not improve altered tissue function unless we will be able to induce the SCN repair[1] either endogenously or by allogeneic body rejuvenation (reviewed in[11]).

Utilization of animal models for the treatment of degenerative disorders was slightly encouraging as compared to the human experimental trials[6], possibly due to the persistence of an unaltered tissue-specific SCN after initiation of a disorder in otherwise normal healthy animals.

WHAT IS THE SCN?

Stem cells in distinct tissues are present in a perivascular location of microvessels as pericytes that function as mural vascular cells[12]. They secrete growth factors factors which stimulate constitutional tissue progenitor cells[12]. Some pericytes exhibit properties of mesenchymal stem cell and exhibit nuclear reprogramming into such cells as testicular Leydig cells[13], cardiomyocytes[14], cartilage and bone[15], and neural and neuronal type cells[16]. Pericytes and neural cells originate from the neural crest[17], but from different germ layers. The functional behavior of SCN is modulated by autonomic innervation[18], which influences numbers of

pericytes and inhibits or enables their activity[19,20].

Another essential component of the perivascular SCN are MDC accompanying microvasculature. Depletion of MDC in one testis alters differentiation of Leydig cells from their precursors[21] represented by vascular pericytes[13]. Circulating CD14+ primitive MDC (pMDC) play important roles in tissue morphostasis, in addition to their roles in the immune responses[22]. The pMDC enable division of progenitor cells and influence by SE the persistence of tissue-specific cells in the epigenetically encoded particular differentiation state[1]. Hence, the MDC exhibit several roles in the perivascular SCN.

The SCNs accompanying body microvasculatures represent basic "tissue control units" incorporated into a more complex tissue control system (TCS)[1], which shows distinct diversity between the tissue types. The tissues differ in their proper stage of cellular differentiation, ranging between stem cells and cellular apoptosis. Extent of cellular differentiation of certain tissues ceases relatively early, e.g., skeletal muscle or brain. A moderate stage of proper cellular differentiation is present in gut epithelium, where intraepithelial lymphocytes are involved, and cellular apoptosis with binding of immunoglobulin is required for the proper function of stratified epithelium of epidermis and uterine ectocervix (reviewed in[1]).

Developmental tissue alteration prior reaching its functional stage will cause its permanent immaturity, e.g., muscular dystrophy. On the other hand, when differentiation of tissue cells is enhanced toward higher stage then required for their proper function, the autoimmune and degenerative diseases, e.g., rheumatic diseases, atherosclerosis, or Alzheimer's disease, will occur[23,24].

17 BETA ESTRADIOL IN VASCULAR SMOOTH MUSCLE CELL CULTURES AFTER TESTOSTERONE, PROGESTERONE AND TESTOSTERONE + PROGESTERONE TREATMENT

Our former research[25] has shown that initial utilization of estradiol (E2) is necessary to induce transdifferentiation of ovarian stem cells (OSCs) and granulosa cells to the neural stem cells (NSC) and neurons following subsequent progesterone (PG) + testosterone (TS) application. However the transdifferentiation was also sometimes observed after PG + TS alone, because of the PG and TS transformation into E2 by aromatase. This possibility was investigated in smooth muscle cell (SMC) cultures. Fresh and spent control medium exhibited E2 1050 ± 150 pg/mL and 1477 ± 742 pg/mL SEM only. However TS pre-treatment caused significant E2 increase (22086 ± 4650 pg/mL) and PG pre-treatment was even three times more efficient (71920 ± 9090 pg/mL). An addition marked E2 increase was

observed after pretreatment with PG + TS (198202 ± 40088 pg/mL)[16]. This observation indicates that cultures with PG and TS also contain significant E2 levels. It is apparent that vascular SMC contain aromatase capable to cause conversion of PG and TS into E2.

Estradiol is also produced in extragonadal localities, such as vascular SMC and brain[26]. The SMC associated with vessels exhibit aromatase and 17 beta-hydroxysteroid dehydrogenase type Ⅰ, which transform estrone produced by aromatase into the final E2[27]. This indicates that, similarly to in vitro experiments, in vivo treatment with PG and TS may cause a transdifferentiation of vascular SMC into pluripotent stem cells and induce a differentiation of neural/neuronal cells in those locations, which require that.

THE PG AND TS COMBINATION CAN BE SUFFICIENT

The high PG + TS combination is virtually absent in adult human females or males. The combinations of E2 + PG or E2 + TS we not found to transdifferentiate OSCs or vascular SMC into vascular stem cells and neurons. Suitable sex steroid combinations may, however, be present in the brain as neurosteroids[28]. Our observations[16,25] indicate that E2 + PG + TS or PG + TS combinations (assuming E2 is created by aromatase of target cells) could be considered.

During human prenatal development the estrogens, progestogens, and androgens are physiologically present in the circulation of developing fetus[29]. This indicates that such sex steroid combination is not harmful for the fetal development and it may act where actually needed, e.g., for the proper brain development.

Some studies indicate that utilization of PG limits central nervous system damage after injury, inhibits a loss of neural cells (CNS), and stimulates brain function (reviewed in[30]). This may be caused by an effect of supplied PG and in situ aromatase-created E2. Yet, utilization of PG + TS combination can accelerate levels of E2 and cause vascular SMC differentiation into neural cells. This may work well for injured brain or spinal cord in younger individuals.

VASCULAR SMC

Since vascular SMC accompany as pericytes all vessels, including the CNS, their ability to differentiate into neural cells is of particular interest. Utilization of all three sex steroid combination can cause a fast conversion into pluripotent stem cells. Such cells can thereafter differentiate into mature vascular SMC. These cells, known as vascular pericytes, influence properties of endothelial cells and participate in the functional ability of blood vessels. Pericytes can serve for differentiation of other types of cells[31]. Collectively, the vascular SMC accompanying capillaries and post-capillary venules[32] can serve in various sites (brain, muscle, bone marrow, kidney, lung, vessels, liver, thymus and spleen) as

mesenchymal stem cells[33-36]. The mesenchymal stem cell populations in distinct tissues showed alpha smooth muscle actin expression, which suggests their origin from alpha smooth muscle actin positive pericytes[37].

Taken together, systemic or local (spinal cord injury) utilization of sex steroid combinations alone can represent a regenerative chemical approach with a perspective of improvement of neurological and vascular diseases, without a need to develop and implant particular stem cells *in vitro*.

A preservation of normal tissue functions continues to decline with age advancement, in association with a decline of the immune system function. For instance it has been shown that the immune system components play an essential role in the renewal of ovarian follicles during the prime reproductive period (till 38 ± 2 years of age), when the human female fertility is well preserved. After that period, however, women' fertility begins to decline along with age-induced alterations of immune system functions, beginning from the age of 35 years[38]. Due to the cessation of ovarian follicular renewal the ovaries begin to preserve aging oocytes unsuitable for regular or assisted reproduction (reviewed in[11]).

Simultaneously, additional alterations of certain tissue functions, like type 2 diabetes mellitus accompanied a pathology of pericytes[39] and degenerative neuronal diseases associated with apoptosis of neurons[40] may occur due to the age-induced shift up or lost SE of MDC. This indicates that regenerative medicine could be more successful in the treatment of acute tissue alterations in younger individuals with preserved appropriate SE of MDC than in chronic or age induced tissue disorders[41], unless there is an improvement of altered SE, for instance by a transfer of circulating mononuclear cells from young healthy individuals, as recently suggested[11].

CELLULAR REGENERATION WITHOUT CELLS

The regenerative medicine of neurologic disorders is currently expected to utilize *in vitro* developed NSC implanted into the CNS with expectation of renewal and repair of altered or degenerated mature neuronal cells. Such cells may be collected from CNS and expanded by growth factors with mitogenic effects[42,43]. Another origins of NSC are represented by ESC, blood from umbilical cord, amnion cells, stem cells from bone marrow, and blood-derived CD133+ cells[44-47]. After some passages, such cells can be differentiated into NSC by fibroblast growth factor-1, 12-O-tetradecanoylphorbol-13-acetate, which is an activator of protein kinase C, isobutylmethylxanthine, which is a non-specific inhibitor of phosphodiesterases, and either by forskolin, representing protein kinase A activator, or by all-trans-retinoic acid and 2-merkaptoethanol[48]. Such substances, however, can't be utilized for an *in vivo* treatment.

Another possibility is a "systemic regenerative therapy" or "*in situ* regenerative treatment" by substances capable to cross blood-tissue barrier, *e.g.*, the blood-brain barrier, due to their low molecular weight.

We have also realized that combinations of sex steroids are capable to transdifferentiate directly vascular SMC cultures, which have been shown to exhibit receptors for sex steroids[49-51], into the neural and neuronal cells[16], as pericytes accompany microvasculature of all tissues, including CNS.

It was also observed that appearance of vascular SMC stem cells can be induced by combinations of sex steroids, and such stem cells within few days develop back into mature SMC[16]. Sex steroids may stimulate renewal of vascular pericytes, which play by themselves an important role in the physiology of vessels. They influence endothelial cell properties and contributions to the proper vascular functions. Moreover, pericytes were considered to represent precursors for certain types of cells[31], *e.g.*, cardiomyocytes[14]. Therefore, systemic regenerative treatment may also improve vascular disorders, *e.g.*, type 2 diabetes, which is closely twisted together with microvascular pericytes throughout the body[52], improve functions of vessels affected by atherosclerosis, and induce regeneration of cardiomyocytes affected by myocardial infarction. Therefore, systemic and *in situ* treatment with sex steroid combinations may represent a novel regenerative approach for the stem cell therapy without the cells.

SEX STEROID ROLE IN THE NEURAL SCN

Depletion of androgens with age advancement in men is accompanied by depression and memory alteration. Decline of TS concentrations several years precede the diagnosis of Alzheimer's disease. The therapy with androgens, however, did not show any improvement of cognitive memory in altered aging individuals. Beside gonads and adrenals, some sex steroids are also produced within the brain. Such sex steroids are eventually called neurosteroids (reviewed in[28]).

Sex steroids could stimulate neuronal regeneration from persisting NSC, since they cross the blood-brain barrier and associate with their receptors important for the regulation of emotions, cognition, and behavior[53]. It appears, however, that utilization single sex steroid, such as androgen, does not prevent or treat neuronal degeneration (see above).

COMBINATIONS OF SEX STEROIDS AND REGENERATIVE STEM CELL THERAPY WITHOUT THE CELLS

The consideration of sex steroid treatment provided below is based on the sex steroid doses utilized for other clinical applications. It is recommended that, before any clinical trial, relevant doses of sex steroid combinations are tested in animal studies.
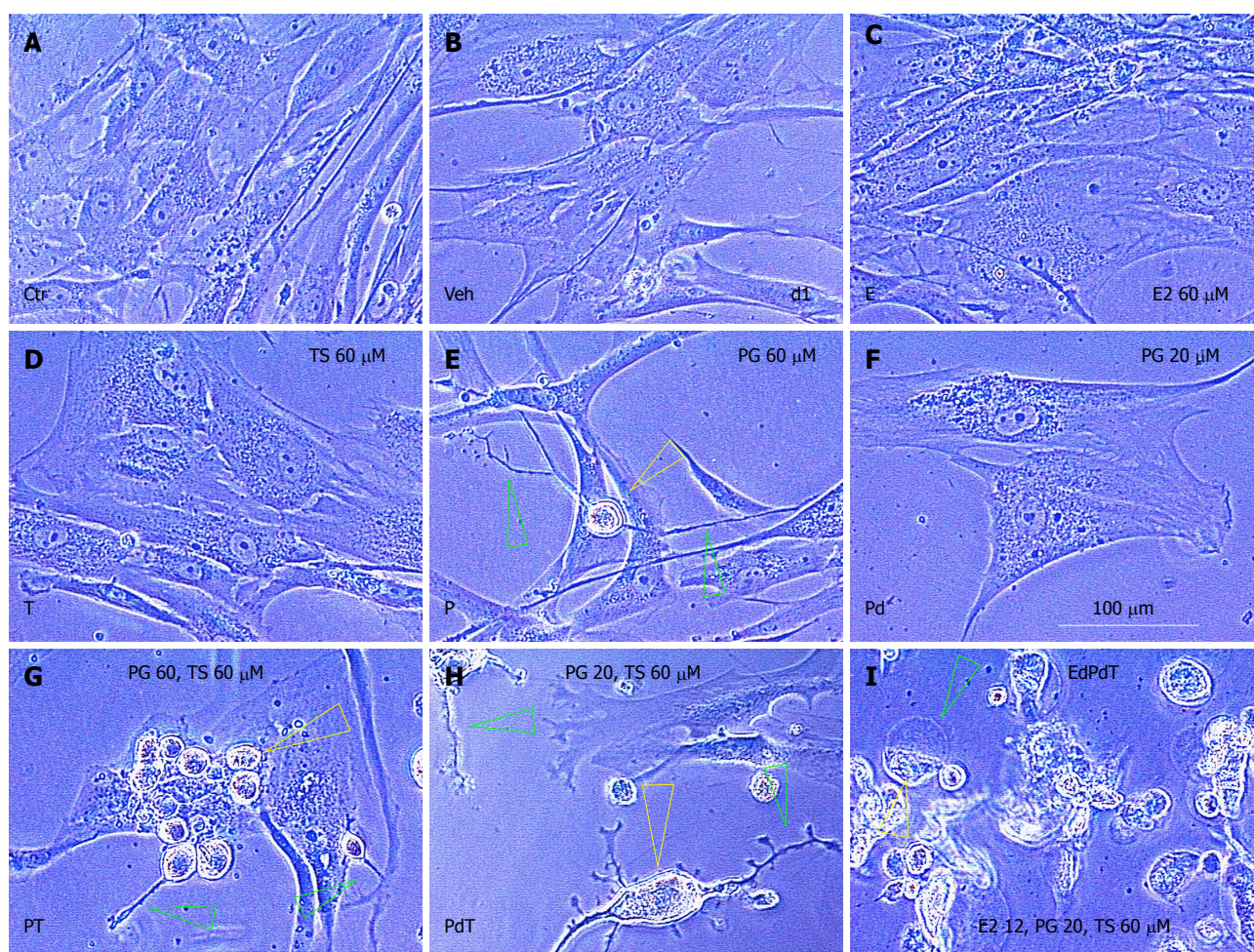
Figure 1 Sex steroid doses and combinations in vascular smooth muscle cell cultures. Reprinted from[68] with permission, ©Page Press Publications. A-D: Control (A), vehicle (B), high estrogen (E2 60 microM) (C), and high testosterone (TS 60 microM)-treated cultures of vascular SMC showed no morphological changes (D); E: High progesterone treatment (PG 60 microM) was, however, associated with occasional neuronal type cells; F: This was not observed in cultures with diluted progesterone (PG 20 microM); G: Cultures with high PG and high TS showed numerous neural type cells; H: Cultures with diluted PG and high TS showed direct nuclear transdifferentiation into neuronal type cells with branching extensions; I: Diluted estradiol and PG and high TS caused formation of undifferentiated stem type cells. Open yellow arrowheads indicate neural/neuronal type cell bodies, green arrowheads indicate extensions. Green arrowhead in panel I indicates bubble-like anchor. Ctr: Control; E: Estradiol; Ed: Estradiol diluted; P: Progesterone; Pd: Progesterone diluted; PT: Progesterone + testosterone; Veh: Vehicle; TS: Testosterone; PG: Progesterone.

The *in vitro* studies were performed in order to approach a question on which sex steroid combinations and their doses could be effective for the regenerative treatment. Figure 1A and B show that control cultures and media with a vehicle alone exhibited no transdifferentiation into neuronal type cells. When 60 microM E2 (Figure 1C) or 60 microM TS (Figure 1D) was applied, no changes were observed next day after the treatment. The 60 microM PG, however, showed sporadic emergence onset of neuronal cells as indicated by yellow arrowhead in Figure 1E. They exhibited typical neuronal extensions (green arrowheads). Such concentration of PG in tissue culture is relevant to the 16 mg/kg PG density, which was reported to diminish death, gliosis, and functional defects following trauma-induced rat brain alterations[54]. Therefore, the PG alone could exhibit certain protective effect *in vivo*. Decrease of PG to 20 microM, however, exhibited lack of the *in vitro* effect for an emergence of neural cells - see Figure 1F.

Combination of 60 microM PG with 60 microM TS, caused frequent appearance of small NSC shown by yellow arrowhead in Figure 1G, exhibiting some extensions (see green arrowheads). Therefore, many new NSC could develop using PG along with TS. In other words, the TS significantly potentiated a small PG neuronal effect.

When a reduced dose of PG (20 microM) was utilized with 60 microM TS, the cultured SMC exhibited a transdifferentiation to the well differentiated cells of neuronal type (see yellow arrowhead in Figure 1H) - note the branching extensions indicated by green arrowheads.

The dose of *in vitro* used PG is relevant to PG suppositories (200 mg), utilized 2 times a day to prevent a miscarriage or a preterm labor[55,56]. Relating the TS dose, it corresponds with weekly intramuscular androgen 600 mg injections, which were tested in human males[57,58]. Therefore, 400 mg PG daily with weekly 600

mg TS could be considered to treat dysfunctions of CNS.

In vitro utilization of a small concentration of E2 (12 microM) along with 20 microM PG and 60 microM TS, caused a development of many stem type cells in vascular SMC cultures (see yellow arrowhead in Figure 1I) exhibiting bubble-like anchors (green arrowhead). Within few days such stem type cells differentiated backward into typical mature SMC[16]. Therefore, it is possible to consider that vascular disorders might be treated with such therapy. The E2 12 microM dose used *in vitro* is relevant to 120 mg in humans. For hormone-replacement therapy a transdermal 17 beta-estradiol 50 mg was reported to be used twice a week[59].

As mentioned above, the pericytes were proposed to represent pluripotential stem cells for other cell types[31], incorporating cardiomyocytes[14]. Utilization of all three sex steroids, *i.e.,* PG daily + TS weekly + 50 mg of E2 twice a week during about four weeks could contribute to vascular regeneration after the stroke occurrence or for cardiomyocyte regeneration after the heart infarction.

It is considered that aging humans, and those having a possibility of inherited CNS or hart and vascular alterations, could be prevented by annual PG + TS or E2 + PG + TS combinations used about four weeks to prevent such disorders. Because the E2 could be derived from other two sex steroids, the PG and TS treatment might be sufficient. Such sex hormone supplementation might also be effective for the acute trauma of spinal cord, stroke, or myocardial infarction.

WHAT THE REGENERATIVE MEDICINE OPTIONS MIGHT BE

It is currently expected that the regenerative medicine should replace altered functional cells with newly developed mature cells formed from implanted stem cells or by *in vitro* reprogramming of already differ-entiated cells from one cell type into a needed distinct one.

In some instances, it might be possible to use the therapy resulting in the *in situ* transdifferentiation of *in vivo* existing autologous progenitor cells, *e.g.,* by substances crossing the blood barrier for the treatment of neurological diseases.

Utilization of exogenous sex steroids could be a preferred option since they are naturally occurring during the life *vs* other possible chemical approaches for regenerative medicine without the cells. Since regeneration of tissues is dependent on the homeostatic function of the immune system components[1,60], and the immune system exhibits progressive regression since 35 years of age[38], the effect of sex steroid combination is expected to be more effective in younger individuals, whose tissues exhibit normal ability to regenerate. Therefore, such treatment could be more effective in sudden traumatic and ischemic disorders of younger individuals exhibiting preserved SCN and TCS exhibiting regular function. In contrast, the degenerative

diseases caused be an altered function of the TCS will not be treatable, unless its normal components are restored, for instance by a blood transfusion from a young healthy individuals (see below).

IS THERE A POSSIBILITY TO AMELIORATE AGING?

Heterochronic parabiosis between aged and young mice was reported to improve the activity of progenitor tissue cells by the blood serum[61]. Subsequent studies supported this proposal (reviewed in[11]). The issue of rejuvenation by young blood is, however, more complex than the serum content alone. Blood transfusion, beside serum with immunoglobulins, polynuclear leucocytes and erythrocytes, contains immune system-related mononuclear cells, which are important for the morphostasis of various tissues as the components of the complex TCS[62]. Numerous studies have shown that the cells of the immune system, beside immunity, participate in regeneration of tissues and healing of the wounds. The TCS is involved in the regulation of tissue-specific cellular regeneration, preservation of developed cells in the proper functional state, and in the maintenance of each tissue in the proper quantity of the cells (reviewed in[1]).

There are several problems with heterochronic parabiosis. Firstly, this is an animal approach which has no therapeutic use in human beings *per se*. Secondly, long lasting heterochronic parabiosis has been shown to alter the young animal by the old animal blood components[63]. Thirdly, and most importantly, the utilization of a single dose of blood serum from young to older individual can not be expected to exhibit any long lasting rejuvenation of the recipient's tissues, and the same can be expected after the whole blood transfusion. Regarding the blood transfusion, it can only be utilized as a partial blood volume replacement for two reasons - to prevent an overdose of erythrocytes and to improve a proportionality between the young and aged blood.

Yet, the partial blood replacement with a temporary rejuvenation may have some therapeutic utilization, for instance to temporarily enable gonadal function in order to produce new gametes for the treatment of infertility. Longer lasting tissue rejuvenation after partial blood replacement will require an accompanying stimulation of the immune system morphostatic functions by the honey bee propolis or drugs with similar effects - reviewed in Ref.[11].

Regarding the role of the immune system related cells in tissue morphostasis, it has been shown that after a partial hepatectomy or unilateral nephrectomy the immune system is specifically activated to repair the liver or kidney tissue depletion. The T lymphocytes initiate and then stop the growth of the liver or kidney until the morphostasis reappears[64]. Our observations from the ovarian physiology indicate that the active

tissue growth is dependent on pericytes releasing Thy-1 differentiation glycoprotein, the most primitive member of the immunoglobulin gene superfamily[65]. Activity of pericytes for Thy-1 release is inhibited by vegetative innervation in the sites, where regeneration is not needed. In other words, the neural system regulates proper tissue quantity, and immune system cells stimulate the regeneration when asked to do so by the Thy-1 release from pericytes.

In addition, the incidence of malignant diseases progressively increases with age advancement. Albeit a single accidental consumption of an unprocessed shiitake mushroom caused a common severe skin erythema with pruritus[66] lasting for a couple of weeks. This immune system systemic hypersensitivity, however, successfully eradicated a rectal cancer. The subsequent weekly consumption of small amounts of unprocessed shiitake mushrooms in salads was without skin reaction and prevented cancer relapse and any occurrence of malignancy for many years thereafter[67].

An elaborated immunotherapy, consisting of human gammaglobulin to unblock antibodies against alloantigens, a single dose of cyclophosphamide to eliminate immune system support of cancer progression (see Ref.[1]), followed by two separate blood transfusions to enhance immune system reactivity against cancer alloantigens, and weekly intradermal applications of Bacterinum adnexitidicum to booster a reversion of immunosuppression, was capable to induce within few months a gradual regression of advanced human ovarian cancer, including complete regression of ascites and severe liver metastases, accompanied by a regeneration to the regular liver size (for details see Ref.[67]).

CONCLUSION

Available data indicate that sex steroid combinations will represent a novel approach of regenerative medicine enabling stem cell therapy of neuronal alterations without the cells. In other words, this approach can enable utilization of endogenous pluripotent cells to differentiate into new neurons where needed. Sex steroid levels are high during pregnancy in both the mother and the fetus, and do not alter their bodies in general, but contribute to the fetal development and its immune tolerance by the mother. Utilization of sex steroids in aging individuals exhibiting alteration of neuronal tissues may, however, not be effective, unless its morphostasis mediated by the immune system, is improved. A combined treatment with sex steroid combinations to enable a transdifferentiation of vascular pericytes into neuronal cells, and with a partial blood replacement (500 mL blood withdrawal with immediate replacement of the same fresh blood volume from the young healthy individual) can cause a temporary brain tissue rejuvenation, possibly including improvement of the Alzheimer's disease. To preserve the achieved regeneration will require a permanent utilization of drugs stimulating immune system role in normal tissue

morphostasis, as reported for the honeybee propolis.

The age advancement is also accompanied by malignancy development. Early cancer development in untreated individuals can be eradicated by a consumption of unprocessed (rough) shiitake mushrooms. The regression of advanced and metastasizing cancers in otherwise previously untreated patients could be achieved by an elaborated immunotherapy. The patients who had undergone a prolonged and/or high dose or long lasting cytostatic therapy, high dose irradiation, excessive surgery, and exhausted cases close to death, are unlikely to respond the elaborated immunotherapy of cancer.

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