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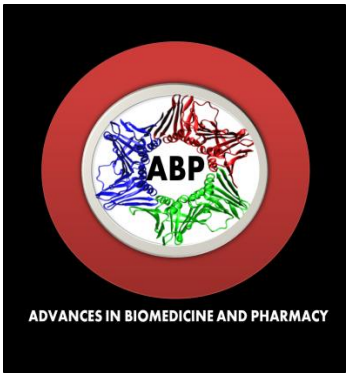

Original Article

Synergistic activity of Herbal Extracts on Dog Blood Progenitor cells

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Abstract

Peripheral blood is an accessible adult stem cell source for both basic and applied research including clinical applications. Peripheral blood mononuclear cells (PBMCs) were reported to possess multipotent progenitor cell populations and has the potential to differentiate into blood cells, endothelial cells, hepatocytes, cardiomyogenic cells, smooth muscle cells, osteoblasts and so on. They can be used to predict *in vivo* toxicity only if independent exposure and pharmacokinetic disposition and metabolism studies are available. In the present study adaptogenic herbs with beneficial properties such as *Withania somnifera, Ocimum sanctum, Phyllanthus amarus* were tested for inducing proliferative activity on blood derived stem cell population. It was observed that the blood progenitor cells treated with tested herbs were not toxic and there were no significant adverse effects on the livability of cultured cells during the observation period. The extracts of *Withania somnifera, Ocimum sanctum, Phyllanthus amarus* showed synergistic activity on stimulation of cell proliferation.

Keywords: Synergistic, Herbal Extracts, Dog Blood CD 34+ve Progenitor cell, proliferation

Introduction

Hematopoietic stem cells are endowed with different characteristic features. Among the core properties are the ability to choose between self-renewal and differentiation. They actively home and migrate to the bone marrow and sometimes to the circulation at the hour of need. In addition, HSCs are subjected to regulation by apoptosis. The balance between these activities determines the number of stem cells that are present in the body. Hematopoietic stem cells (HSCs) and hematopoietic progenitor cells (HPCs) play key roles in the production of mature blood cells and in the biology and clinical outcomes of hematopoietic transplants. The more accessible, blood-derived adult stem cells are now being evaluated as a potential source for different cell lineages. Several methods have been used to provide the evidence of multi-potential cells in circulating blood [1]. Studies conducted by Porat and Colleagues found that isolation of human cell population from the peripheral blood was found to be rich in CD31Bright, CD34+, CD45 Dim and CD34 Bright, which composed of multipotent progenitor cells. The progenitor cells differentiated into a variety of cell lineages on defined culture conditions. The resulting cells exhibited morphological, immunocytochemical and

functional characteristics of angiogenic, neural or myocardial lineages [2]. The isolation of pluripotent precursor cells from equine peripheral blood was tested for their differentiation potential and it was found that equine peripheral blood-derived cells had osteogenic and adipogenic differentiation capacities comparable to cells derived from bone marrow [3].

Certain plants and herbs have been used since ancient days for the treatment of several diseases. Many herbs are active against neoplastic diseases by inhibiting cell growth and proliferation. Herbal stem cell therapy promotes endogenous stem cell proliferation and differentiation and sued in the treatment of various human diseases [4]. The active components of *Plastrum testudinis* (PT) involved in promoting proliferation of MSCs [5]. Extracts from Buzhong Yiqi Decoction (BYD), a well-known ancient tonic prescription which had a promoting effect on BMSCs. It was found that BYD extracts had positive effects on the proliferation of rat BMSCs [6]. Many natural compounds promote healing effects on stem cells which have not investigated in detail. The effects of natural compounds such as blueberry, green tea, catechin, carnosine and vitamin D_3 were found to increase cell proliferation of human bone marrow cells in a dose dependent manner. The combination of nutrients produces a synergistic effect to promote proliferation of human hematopoietic progenitors [7]. National research council (NRC) recommends the species specific authentication on using natural compounds in foods and as drugs. Hence the validation of activities of natural compounds in dog blood progenitors would be appropriate to analyze the activity of herbal compounds in food and drug use.

In the present study, isolated peripheral blood derived progenitor cells from dogs were used for the analysis of synergistic activity of the herbs with adaptogenic property which was found to be on par with resveratrol, the pure bioactive of grape skin extract.

Materials and Methods

Herbs such as *Phyllanthus amarus, Ocimum sanctum* and *Withania somnifera* were obtained from National Institute of Siddha referred pharmacy, Chennai, India as per the authentication done by Dr. S. Thamilalagan. The herbal samples were extracted using methanol and sterile water as reported earlier [8].

Culture of canine peripheral blood derived stem cells

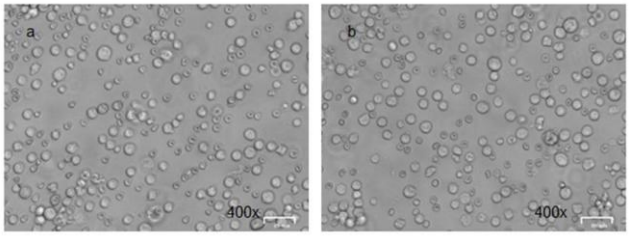
Peripheral blood (12 mL) was obtained from domesticated dogs with due consent from the pet owners. The blood was collected in 5 mL tubes with sodium heparin. Immediately after collection the blood was diluted in one volume of PBS (Invitrogen) and mixed with the buffer by inverting the tube several times or by drawing the mixture in and out of a pipette. 3 mL of Ficoll-Paque media (Sigma-Aldrich) was added to the centrifuge tube. Diluted blood sample (4 mL) was layered onto the Ficoll-Paque solution carefully without mixing. The monolayer fraction was harvested after a density gradient centrifugation step of 20 min at 1600 g and rinsed twice. The cell pellet was re-suspended by gently drawing them in and out of a pipette and centrifuged at 500 × g for 10 min at 20°C. The cell pellet was re-suspended in culture medium (2 mL) and subjected for CD34+ phenotypes by Magnetic activated cell sorter (MACS) separation using anti CD34+ antibodies (Origene). Briefly, Primary dog anti CD 34 antibodies in the ratio of 1:1000 were incubated for 20 minutes, followed by FITC conjugated secondary antibodies in the ratio of 1: 2000 was incubated for 20 minutes. Then incubated with MACS anti FITC antibodies and washed. Magnetically labeled antibody bound cells were retained and unbound fraction were collected aseptically and subjected for suspension culture under 5% CO_2 maintained at 37 ^{o}C (Thermo Scientific).

Population Doubling Time

Cells were sub cultured and third passage cells were used for PDT analysis. (Td=ln, 2/x, x is the cell growth rate). Cells were trypsinized and viability of the cells was analyzed using trypan blue dye staining in Neubauer chamber [10]. The results were tabulated and plotted in a graph and the PDT was estimated. The assay was done by seeding $2x10^4$ cells/well in DMEM-high glucose (Gibco) supplemented with FBS (Gibco) (4%) and antibiotic (1%). Resveratrol (Sigma-Aldrich) was used as the positive control. The selected herbs were compared to the efficiency of Resveratrol.

Results

Canine peripheral blood derived cells with herbal incorporation showed a mixed population of cells during initial culture days (Fig 1) Initial Mononuclear cell population recovered was 2.1± 0.01 $x10^9$ mononuclear cells. The CD 34 positive population recovered was 0.018±0.02 x 10^4 cells.



Canine blood progenitors

Figure 1: Morphology of cultured canine peripheral blood-derived progenitors

The population doubling time of two culture system with and without herbal supplementation using CD34 phenotypes showed that the herbal extract has a significant activity on CD34 phenotypes (Fig 2). The synergistic activity of the herbal extracts was found to be comparable to the activity of resveratrol, the pure bioactive of grape skin extract.

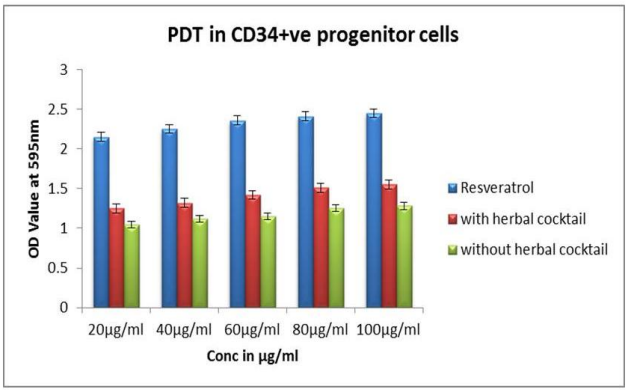


Figure 2: Population doubling time of dog blood derived CD34+ve cells

Discussion

In vitro studies provide a basis for comparing cytotoxicity data across species. They can be used to predict *in vivo* toxicity only if independent exposure and pharmacokinetic disposition and metabolism studies are available [9, 10]. A recent National Research Council (NRC) study explored how the safety of dietary substances should be assessed in horses, dogs, and cats. A common misconception is that products widely found in human food (e.g., garlic and chocolate) are assumed to be safe for pets. In contrast, the major conclusion of the NRC report was that safety in humans or any one species does not guarantee safety in animals. Such studies serve as a bridge to similar *in vitro* cytotoxicity evaluations conducted in other laboratory species or humans [11]. Scientific research shows that *Ocimum sanctum* has strong antiviral, antimicrobial and antioxidant properties. It also contains anti-fungal and insect-repelling characteristics which can be use against dogs. Basil also helps to treat against canine arthritis. Holy basil is a well-known calming supplement for decreasing stress hormone levels, specifically corticosterone, which causes anxiety in dogs. Ashwagandha has anti-inflammatory properties which helps with all kinds of aches and pains and is especially useful in degenerative arthritis conditions in pets. It helps to increase oxygen carrying red blood cells in cats suffering from anemia (www.dogsnaturallymagazine.com).

In the present study, it has been shown that the blood progenitor cells treated with tested herbs were not toxic and there were no significant adverse effects on the liveability of cultured cells during the observation period. Major activity of *Withania somnifera, Ocimum sanctum, Phyllanthus amarus* showed synergistic activity on stimulation of cell proliferation [13, 14]. It has been shown that the blood progenitor cells treated with these herbs were not toxic and there were no significant adverse effects on the livability of cultured cells during the observation period. Supporting our findings, herbs were checked for the cytotoxicity assessment in 4 canine cell types to 20 different food components provided a baseline that begin to illustrate how such an *in vitro* panel could be used for hazard assessment [9].

Conclusion

These adaptogens increase the body's resistance to physical, biological, emotional and environmental stressors and promote normal physiological function. The present research indicates that the crude extracts of the selected herbs has got intense *in-vitro* proliferation effect and may have potential use in traditional medicine.

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