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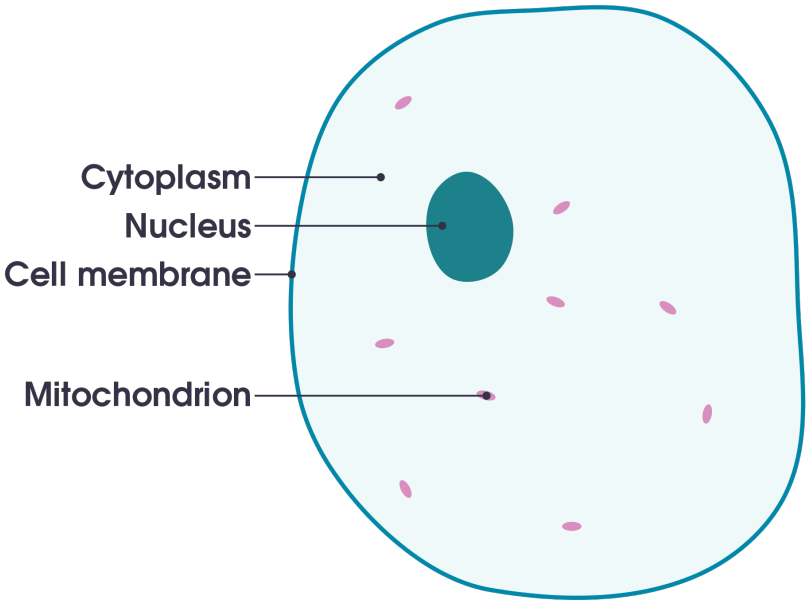

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Review

Stem cell drugs: the next generation of pharmaceutical products

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Abstract

Stem cells represent a new treatment option in medicine and pharmacy. Stem cells have been increasingly used for the treatment of many diseases. In fact, they have spurred a new age of medicine called regenerative medicine. In recent years, regenerative medicine has become a new revolution in disease treatment, especially with the use of stem cell drugs. Stem cell drugs refer to live stem cell based products that used as drugs for particular diseases. Unlike autologous stem cell transplantation, stem cell drugs are "off-the-shelf" products that are ready to be used without requirement of any further manipulation. This review aims to summarize some of the approved stem cell drugs, and discuss the revolution of regenerative medicine and personalized medicine. As well, the review will discuss how stem cell drugs have led to a new direction in stem cell therapy, providing a new platform for patient needs.

Keywords

Stem cells, Stem cell drug, Pharmaceutical, Stem cell therapy, Stem Cell transplantation, hematopoietic stem cell, mesenchymal stem cell, HLA matching

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Introduction

Stem cells are considered as the origin of the growth and development of human beings. The capability and relative of stem cell isolation, proliferation and modification gave rise to the field of stem cell application for disease treatment. Today, there is a huge potential benefit for the use of stem cells in disease treatment (stem cell therapy). To date, there are more than 100 different diseases that have been treated with stem cell transplantation. The first application of hematopoietic stem cells (HSCs) in 1950s by Donald et al. showed that stem cells could be used as drugs to treat diseases (Appelbaum 2007).

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Clinical applications of HSCs rapidly increased with more and more diseases treated with HSCs. From 1970s to 2000s, all HSC transplantations (HSCTs) were indicated for hematologic malignancies. In recent years, HSCTs have also been used in solid tumor treatment (Kotloff et al., 2004; Rodenhuis et al., 2003; Tallman et al., 2003).

Human leukocyte antigen (HLA) matching is an important component for successful HSCT (Lee et al., 2007; Petersdorf et al., 1998; Sasazuki et al., 1998). Therefore, in the initial years of HSCT, almost all cases were performed as autologous transplantation of HSCs. The stem cells were collected from bone marrow, umbilical cord blood or peripheral blood. However, autologous HSCT had some limitations, especially with regard to the quality and quantity of HSCs. More importantly, autologous HSCs have some mutations in their genome which limit their use in treating genetic diseases. To overcome these limitations, allogenic HSCT was suggested as a better platform; the use of selected HSC samples with greater quality and quantity can be controlled. In light of the demand for allogeneic HSCT, stem cell banks have been developed in several countries, such US, Japan, Korea, England and Germany (Armson et al., 2015). With greater characterization of HSCs (including HLA typing), stem cells were able to be commercialized as pharmaceutical products for transplantation in the US, beginning in the 2000s. Ducord and HemaCord are two HSC drugs introduced around 2010 in the US. However, since the HLA matching ratio is very low in the human population (about 1/100,000), the business of HSC drugs has only slowly developed.

From 2012 till now, stem cell drugs have advanced with mesenchymal stem cell (MSC) based products. Similar to HSCs, in the first clinical applications of MSCs, the MSCs were administered as autologous transplantation. Indeed, HLA matching as well as immune rejection are taken into consideration before transplantation. Some allogenic transplantations of MSCs have used immunosuppressive drugs to prevent or reduce the immune rejection of grafted cells in the host. However, increasing studies have confirmed that MSCs exhibit strong immune modulation capacity that can regulate the host's immune system; as well, they exhibit low immunogenicity compared to HSCs and other cells (Ankrum et al., 2014; Hoogduijn et al., 2010; P De Miguel et al., 2012; Rasmusson, 2006).

MSCs have been transplanted into patients without immune suppression and without HLA matching with safety (Hare et al., 2009; Karussis et al., 2010; Lalu et al., 2012; Wang et al., 2012). Based on such results, MSC based products have now been developed as drugs for patients with clear indications. The first MSC based stem cell drug was officially approved by the Food and Drug Agency of Canada in 2012. This drug was indicated to treat graft versus host disease (GVHD) related to HSCT.

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This review aims to summarize and discuss the development and growth of stem cell drugs in medicine and pharmacy. Stem cell drug has now become a new and potentially important member of regenerative medicine (**Fig. 1**).

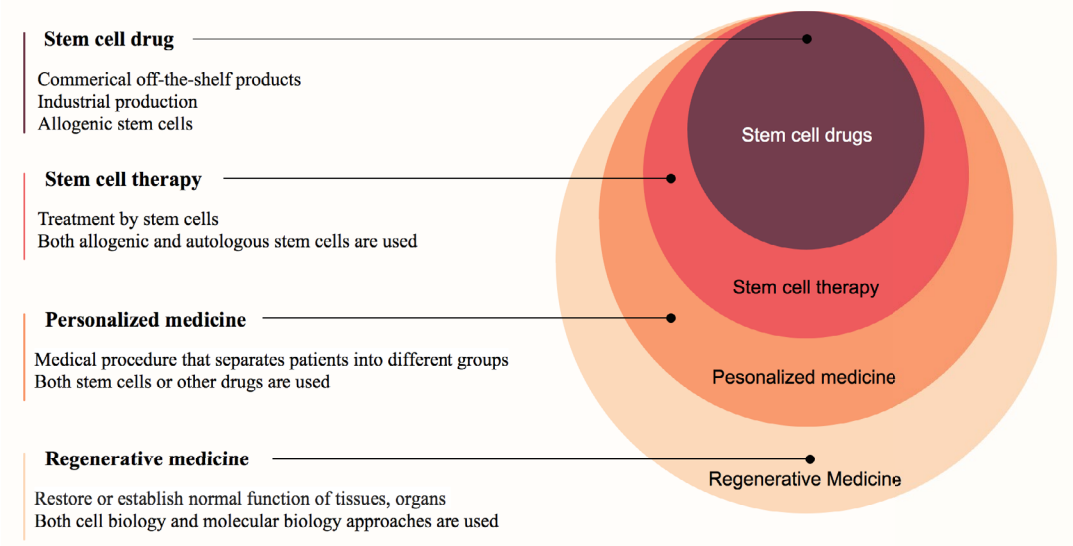

Figure 1. Stem cell drugs in regenerative medicine. Stem cell drugs are new members of stem cell therapy, personalized medicine and regenerative medicine. The development of stem cell drugs have impacted and advanced the stem cell industry as well as the pharmaceutical industry.

What are stem cell drugs?

A drug is defined as any substance other than food that when inhaled, injected, smoked, consumed, absorbed via a patch on the skin or dissolved under the tongue causes a physiological change in the body. In pharmacology, a drug (or pharmaceutical drug) is a substance used to treat, cure, prevent or diagnose a disease or to promote well-being. According to this definition, a drug must satisfy some criteria, such as having indication to treat any disease and is an off-the-shelf product. Therefore, by definition stem cell drugs are off-the-shelf products based on stem cells that are indicated to treat, cure, prevent or diagnose a disease or to promote well-being.

As off-the-shelf products, stem cell drugs are used in the allogeneic setting in stem cell transplantation. There are key differences between allogenic stem cell transplantation and stem cell drugs. The biggest difference between them is that the stem cell drug is a product, while allogenic stem cell transplantation is a procedure using the stem cell drug. Moreover, the former is approved as a drug and the latter is approved as a medical device. The details of the main differences are listed in **Table 1**.

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Table 1. Main differences between stem cell drugs and allogenic stem cell transplantation

Stem cell drugs	Allogenic stem cell therapy
Products of stem cells	Procedures using stem cells
Off-the-shelf products	Using off-the-shelf products or directly use from donors
Produced with large quantity with consistent quality	Quality differs from batch to batch
Quality of products are controlled from batch to batch according to GMP guidelines	Quality differs from batch to batch depending on donors and production procedures
Approved as drugs	Approved as medical devices

Stem cell drugs: from personalized to universalized

Personalized medicine is a medical procedure that separates patients into different groups—with medical decisions, practices, interventions and/or products being tailored to the individual patient based on their predicted response or risk of disease. Stem cells offers a new approach in personalized medicine. Indeed, the definition of "personalized medicine" using stem cells has become a popular term in the last 10 years. To date, there are at least 2 approaches using stem cells in personalized medicine, as presented in **Fig. 2**.

In this review, we mainly discuss the application of stem cells in stem cell therapeutic transplantation. In stem cell transplantation, autologous stem cells from a patient are used to treat his or her disease. However, the reduction in quality and quantity of stem cells due to aging is a major limitation of this approach. However, after Yamanaka's success with induced pluripotent stem cell production from skin fibroblasts (Takahashi and Yamanaka, 2006), scientists hoped that personalized medicine would lead to greater clinical applications using autologous reprogrammed stem cells (**Fig. 3**). To date, this approach has faced many limitations and proven to be quite difficult. Even if all the technical difficulties and limitations related to this approach could be solved in the near future, the high price of this procedure would remain a challenge. Indeed, the process of fibroblast isolation, culture and reprogramming for clinical applications is very complex, time-consuming and expensive.

Stem cell drug for universalized medicine has become a new option in stem cell therapy. Stem cell drugs can overcome all the major limitations of autologous stem cells. Particularly, the quality, quantity and price can be controlled. While personalized medicine requires more time for development and is, in some

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ways, a form of medicine for the "future", universalized medicine is more "real" in that the "off-the-shelf", allogeneic stem cell drugs can be used for many patients.

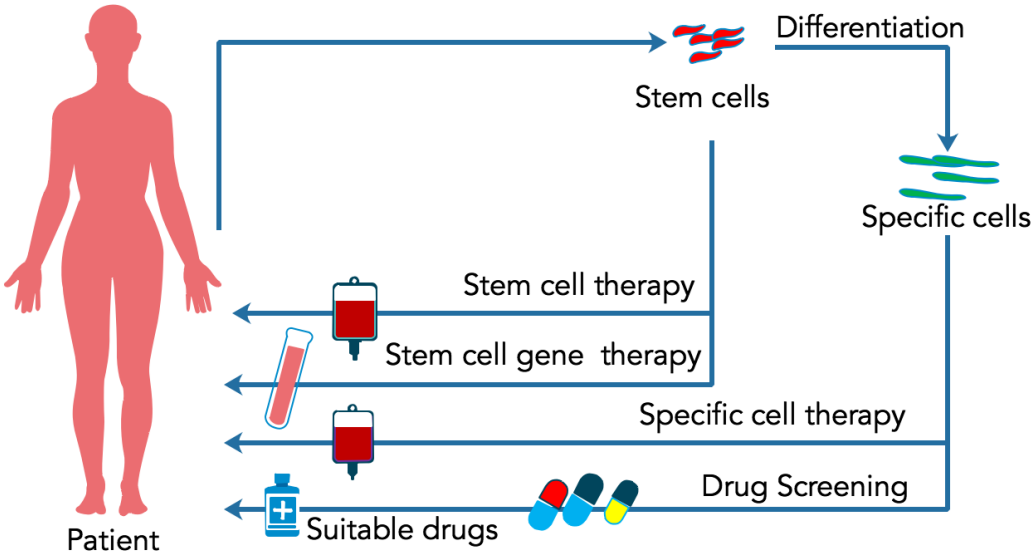

Figure 2. Applications of stem cells in personalized medicine. Stem cells can be used in personalized medicine in various settings, including disease modeling for drug screening or evaluation, stem cell gene therapy for gene correction, and stem cell therapeutic transplantation.

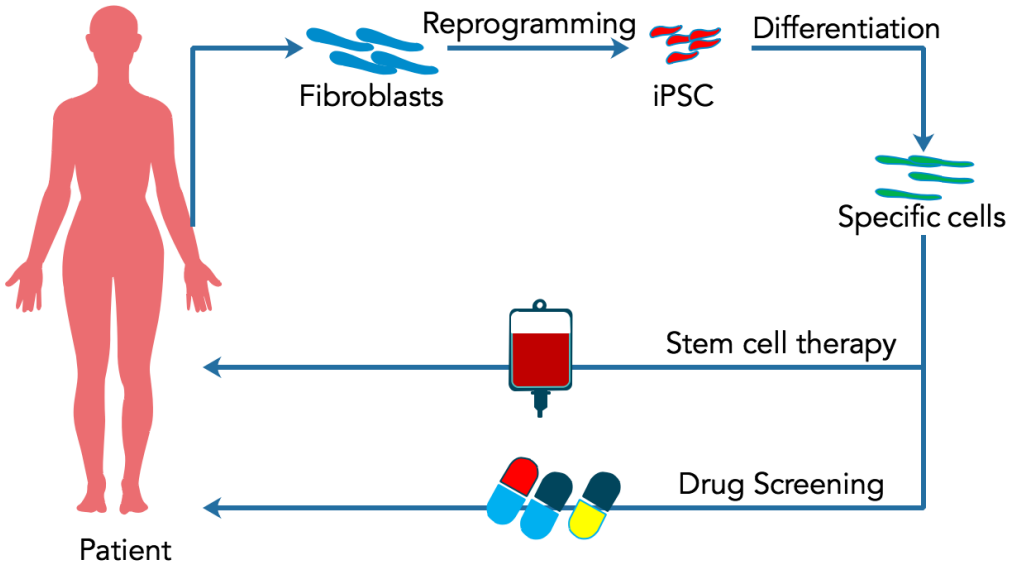

Figure 3. Induced pluripotent stem cells for personalized medicine. Fibroblasts initially isolated from skin are then reprogrammed into induced pluripotent stem cells (iPSCs). The iPSCs are further differentiated into specific cell types before transplantation into patients.

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Stem cell drugs: Drug mechanisms and properties

Stem cell drugs are mainly produced from HSCs and MSCs (**Table 3**). However, another kind of stem cell (limbal stem cells) can also be used to produce some products for corneal regeneration. The mechanisms of action of these stem cell drugs are different. While HSC based drugs can regenerate the hematopoietic system in treated patients (via homing and differentiation to functional cells), MSC based drugs typically target the immune system and facilitate healing at injured sites by paracrine or endocrine factors.

Hematopoietic stem cell based drugs

HSCs are stem cells that can produce blood cells, including white blood cells, red blood cells and platelets, through the process of hematopoiesis. In adults, HSCs are located in the bone marrow and maintain the blood system in the body. The definition of HSC has evolved since the time HSCs were first discovered in 1961 (Till and Mc, 1961). The hematopoietic tissue contains cells with long-term and short-term regeneration capacities as well as committed multipotent, oligopotent and unipotent progenitors. Nowadays, HSCs are found in and mostly isolated from bone marrow, peripheral blood and umbilical cord blood. The first successful bone marrow derived HSC transplantation was performed in 1950s by E. Donnall Thomas at Fred Hutchinson Cancer Research Center (Washington, USA); his work was later recognized with a Nobel Prize in Physiology or Medicine. Thomas infused bone marrow cells to repopulate the bone marrow and produce new blood cells. Furthermore, the first physician to perform a successful human bone marrow transplant for a disease other than cancer was Robert A. Good at the University of Minnesota in 1968. To date, HSCTs have been evaluated in a variety of malignant and non-malignant diseases (**Table 2**).

The roles of HSCs in both malignant and non-malignant diseases were determined by homing and differentiation of HSCs at bone marrow to form a new hematogenesis system. However, HSCs exert strong immunogenicity on the host immune system. Therefore, HLA matching is critical prior to HSCT. The requirement of HLA matching, however, restricts the development and advancement of HSC based stem cell drugs since there is extremely low HLA matching in the human population. Moreover, it is difficult to induce stem cell proliferation *in vitro* to increase cell quantity. Although umbilical cord blood seems like a good candidate for an unlimited source of HSCs, it too has challenges which limit its application, including the high cost and time for umbilical cord blood cell collection, enrichment and characterization (e.g. HLA typing).

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Table 2. Indications of HSC transplantation

Malignant	Non-malignant
Acute myeloid leukemia (AML)	Thalassemia
Chronic myeloid leukemia (CML)	Sickle cell anemia
Acute lymphoblastic leukemia (ALL)	Aplastic anemia
Hodgkin's lymphoma	Fanconi anemia
Non-Hodgkin's lymphoma	Immune deficiency syndromes
Neuroblastoma	Inborn errors of metabolism
Ewing's sarcoma	Autoimmune diseases
Multiple myeloma	
Myelodysplastic syndromes	
Gliomas, other solid tumors	

Mesenchymal stem cell based drugs

Mesenchymal stem cells (MSCs) are the most popular stem cells in the human body. They are present in almost all tissues but the most common sources are bone marrow, adipose tissue, umbilical cord tissue, umbilical cord blood, and placenta. Although the MSCs from the various tissues share common properties, they also exhibit different properties, as suggested by Dominici et al. (2006) (Dominici et al., 2006). Unlike other kinds of stem cells, MSCs are multifunctional; they not only differentiate into multiple cell lineages but they also produce a pool of cytokines and growth factor to execute immune modulation and promote injury healing and tissue regeneration (Caplan and Dennis, 2006; Chen et al., 2008; Hocking and Gibran, 2010; Majumdar et al., 2000; Ren et al., 2008).

MSCs are favorable for clinical applications of stem cell therapy due to their multiple lineage differentiation potential. Stem cells can be differentiated *in vitro* or *in vivo* into functional cells which can replace aged or damaged cells. Indeed, some applications of stem cell therapy have entailed differentiating cells from stem cells *in vitro* and then transplanting them into the recipient as cellular therapy or in combination with biomaterials as tissue engineering therapy. In stem cell transplantation, scientists are also evaluating and trying to promote *in vivo* differentiation in the microenvironment. Ideally, stem cells can home to injured tissue sites in the body and persist for a long time. Persistence of stem

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cells has been observed in HSC transplantation and, in some cases, in autologous MSC transplantation. Indeed, in MSC transplantation more than 50% of grafted cells typically die in the recipient from rejection by the immune system and selection in the microenvironment. Autologous transplantation or HLA matching, therefore, are necessary to overcome the kinds of challenges.

Autologous stem cells, however, cannot be produced to mass (industrial) scale and thus stem cell based drugs are highly advantageous. As aforementioned, one important component of stem cell based drugs is the capacity for industrial scale production with similar quality from batch to batch. In most cases, MSCs were used as the source for stem cell based drugs. The two main mechanisms of therapy mediated by stem cell drugs are immune modulation and paracrine/endocrine effects. Immune modulation is the most common mechanism of commercialized stem cell drugs generated nowadays; about 80% of stem cell drug products act via immune modulation. This means that the host immune system can be regulated by either indirect or direct interactions between stem cells and host immune cells.

Immune modulation has been observed and documented for mesenchymal stem cells from bone marrow (Bai et al., 2009), adipose tissue (Mello et al., 2015), umbilical cord (Barcia et al., 2015; Cutler et al., 2010), and Wharton's jelly (Prasanna et al., 2010; Weiss et al., 2008). Unlike immune suppression (whereby all immune cells are inhibited in their function), immune modulation is a dynamic process whereby only some cells are affected, i.e. only some cells stimulated. By immune modulation, MSCs can effectively suppress both acute and chronic inflammation. Some stem cell drugs based on MSCs have been approved for use in certain countries and have shown their capability to exert immune modulation; one example is the first stem cell drug (Prochymal) that was evaluated for treatment of GVHD and approved by the Canadian Food and Drug Agency.

The second main mechanism of stem cell drugs relates to the growth factors produced by stem cells. MSCs can produce a pool of growth factors and cytokines which exert biological effects on other cells. MSCs can produce factors that stimulate host stem cells, inhibit apoptosis, and stimulate angiogenesis (Caplan and Dennis, 2006; Chen et al., 2008; Hocking and Gibran, 2010; Majumdar et al., 2000; Ren et al., 2008). Some cytokines exert paracrine effects while others may have endocrine effects.

The future of stem cell drugs

In recent years, stem cell therapeutics studies has progressed from use of whole stem cells to components derived from stem cells. These components have included stem cell extracts, microvesicles and exosomes, all of which exhibit various biological activities. For example, exosomes from MSCs have functions

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similar to whole MSCs, including repair of tissue damage, suppression of inflammatory responses, and modulation of the immune system. Hu et al. (2016) demonstrated that exosomes from human adipose derived stem cells can accelerate cutaneous wound healing via optimizing the characteristics of fibroblasts (Hu et al., 2016). Similar to MSCs, extra-cellular vesicles (EVs) from MSCs can modulate the immune system (Burrello et al., 2016).

Table 3. Updated stem cell products approved for clinical use

Trade name	Stem cell	Company	Approved by	Indications
ALLOCORD	HPC, Cord Blood	SSM Cardinal Glennon Children's Medical Center	2013 US, FDA	For use in unrelated donor hematopoietic progenitor cell transplantation procedures in conjunction with an appropriate preparative regimen for hematopoietic and immunologic reconstitution in patients with disorders affecting the hematopoietic system that are inherited, acquired, or result from myeloablative treatment.
Hemacord	HPC, Cord Blood	New York Blood Center, Inc.	2013 US, FDA	For use in unrelated donor hematopoietic progenitor cell transplantation procedures in conjunction with an appropriate preparative regimen for hematopoietic and immunologic reconstitution in patients with disorders affecting the hematopoietic system that are inherited, acquired, or result from myeloablative treatment.
Ducord	HPC, Cord Blood	Duke University School of Medicine	2012 US, FDA	For use in unrelated donor hematopoietic progenitor cell transplantation procedures in conjunction with an appropriate preparative regimen for hematopoietic and immunologic reconstitution in patients with disorders affecting the hematopoietic system that are inherited, acquired, or result from myeloablative treatment.
None	HPC, Cord Blood	Clinimmune Labs, University of Colorado Cord Blood Bank	2012 US, FDA	For use in unrelated donor hematopoietic progenitor cell transplantation procedures in conjunction with an appropriate preparative regimen for hematopoietic and immunologic reconstitution in patients with disorders affecting the hematopoietic system that are inherited, acquired, or result from myeloablative treatment.

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None	HPC, Cord Blood	LifeSouth Community Blood Centers, Inc.	2013 US, FDA	For use in unrelated donor hematopoietic progenitor cell transplantation procedures in conjunction with an appropriate preparative regimen for hematopoietic and immunologic reconstitution in patients with disorders affecting the hematopoietic system that are inherited, acquired, or result from myeloablative treatment.
None	HPC, Cord Blood	Bloodworks	2016 US, FDA	For use in unrelated donor hematopoietic progenitor cell transplantation procedures in conjunction with an appropriate preparative regimen for hematopoietic and immunologic reconstitution in patients with disorders affecting the hematopoietic system that are inherited, acquired, or result from myeloablative treatment.
Prochymal	MSCs	Osiris Therapeutics	2012 Canada	Treatment of graft versus host disease (GVHD)
Cartistem	MSCs	MEDIPOST Co., Ltd.	2012 Korean FDA	Treatment of knee cartilage defects caused by degenerative osteoarthritis or repeated trauma
Temcell HS Inj.	MSCs	JCR Pharmaceuticals Co. Ltd. (Licensee of Mesoblast Limited)	2015 Japan FDA	Treatment of acute graft versus host disease (aGVHD) in children and adults
Stempeucel	MSCs	Stempeutics Research PVT Ltd.	2016 Indian FDA	CLI patients due to Buerger's disease

Exosomes from stem cells can also affect other systems and organs, such as the cardiovascular system, kidney, liver, nervous system and musculoskeletal system. In the cardiovascular system, exosomes have been suggested as cardioprotective agents (Lai et al., 2010); they have been shown to have proangiogenic effects (Bian et al., 2014) and involvement in reduction of apoptosis and cardiac fibrosis (Feng et al., 2014). Arslan et al. (2013) showed that a single intravenous administration of exosomes was effective in enhancing cardiac function and geometry after myocardial infarction due to bioenergetics re-establishment (increased ATP production), oxidative stress reduction and pro-survival signaling activation (enhanced PI3K/Akt signaling) (Arslan et al., 2013). Recently, Zhang et al. (2016) showed that MSC derived exosomes promoted cardiac stem cell proliferation *in vitro* (Zhang et al., 2016). In kidney, studies have

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shown that acute kidney injury can be effectively treated with MSC based exosomes (Jiang et al., 2016; Zhou et al., 2013). Moreover, MSC derived exosomes can also be used to treat fibrotic liver disease (Hyun et al., 2015; Li et al., 2013). In some diseases of the musculoskeletal system, exosomes can trigger differentiation of MSCs into osteoblasts (Narayanan et al., 2016) and stimulate skeletal muscle regeneration by enhancing myogenesis and angiogenesis (Nakamura et al., 2015). In the nervous system, MSC derived exosomes have been evaluated in the treatment of neurological and neurodegenerative diseases; they have been shown to enhance angiogenesis and neurogenesis, reduce inflammation and improve spatial learning and sensorimotor function (Kim et al., 2016; Zhang et al., 2015).

Taken together, the aforementioned discoveries suggest the dawn of a new era of stem cell therapy, i.e. stem cell drugs. Here, components (mRNA, protein and peptides) from stem cells, in microvesicles or exosomes, can be effective over whole stem cells. Certainly, the advantages of stem cell drugs include reduction of immunogenicity and easy processing, storage and delivery. Stem cells free drugs may play a potentially important and emerging role in regenerative medicine.

Conclusion

Stem cell drugs are new members of pharmaceutical medicines that are produced from stem cells. From 2012 to now, more than ten stem cell drugs have been approved in various countries for clinical applications. These products may contain live hematopoietic stem cells or mesenchymal stem cells. With their advantages such as decreased immunogenicity and ease of processing, stem cell drugs have emerged as a promising new platform in the field stem cell therapy around the world. As a new product of pharmaceutical medicine, it is anticipated that stem cell drugs will significantly contribute to both the pharmaceutical and medical industries in the near future. In clinical applications, besides the stem cell drugs which contain live and whole stem cells, new stem cell drugs containing components from stem cells (such as extracts, exosomes and vesicles) are in development and expected to be launched soon.

Lists of abbreviations

EVs: xtra-cellular vesicles; FDA: Food and Drug Angency; GVHD: graft versus host disease; HLA: Human leukocyte antigen; HPC: Hematopoietic progenitor cells; HSC: Hematopoietic stem cells; HSCT: HSC transplantations.

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Original Research

Epidemiology, incidence and mortality of oral cavity and lips cancer and their relationship with the human development index in the world

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Abstract

Introduction: According to the importance of knowledge about incidence and mortality of oral cavity and lip cancer in health planning, this study was performed with the aim of investigating the incidence and mortality rate of oral cavity and lip cancer and its relation with the Human Development Index in the world in 2012. **Methods:** The study was conducted based on data from the world data of cancer and the World Bank (including the HDI and its components). Data about the age-specific incidence and mortality rate (ASR) for every country in 2012 were getting from the global cancer project.

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To analyze data, correlation tests between incidence and death rates, and HDI and its components were employed with a significance level of 0.05 using SPSS software. **Results:** In 2012, 300373 cases of oral cavity and lip cancer and 145353 cases of death from it have occurred in the world. A positive correlation of 0.221 was seen between the standardized incidence rate of oral cavity and lip cancer and HDI but this correlation was not statistically significant (p=0.114). On the other side, a correlation of 0.295 was seen between the standardized mortality rate of oral cavity and lip cancer with HDI that this correlation was statistically significant (p<0.001). **Conclusion:** The incidence and mortality of oral cavity cancer is high in the Asian countries especially south eastern of Asia. Performing preventive plans in high incidence and mortality rate regions and also obtaining etiological studies in these regions is recommended for diagnosing the causes of high incidence and mortality rates.

Keywords

Incidence, mortality, development, world, oral cavity, lips cancer

Introduction

Today, after heart disease, the cancer is the most common cause of death in many countries Unfortunately, the number of cancer patients is increasing (Ghoncheh et al., 2016; Rafiemanesh et al., 2016; Sciubba jj, 1999) as more than 10 million new cases and more than 6 million deaths occur each year worldwide (Petersen, 2009).

Among cancers, oral cancer is one of the most common cancers. According to the International Agency for Research on Cancer, the amount of the sufferers from the disease is increasing in coming years (Pereira et al., 2007). In most cases, predominant form of this cancer type is squamous cell carcinoma (Mukherjee et al.) that because of side effects and high mortality rates, is considered as one of the important threats in public health (Stîngă et al., 2011). Squamous cell carcinoma is a malignant neoplasm that arises from squamous epithelium which can be found on both oral cavity (oral mucosa, gums, hard palate, tongue, and mouth) and on lip (Batista et al., 2010).

In most countries, oral cavity cancer in men is more than women. Its risk increases by aging and mortality occurs in 50 years and older. The incidence of oral cancer (except lip) is more in South and Southeast Asia (Sri Lanka, India, Pakistan and Taiwan), parts of the West (France) and East Europe (Hungary, Slovakia and Slovenia), parts of Latin America and the Caribbean (Brazil, Uruguay and Puerto Rico) and in the Pacific (Papua New Guinea and Melanesia). The highest incidence of lip cancer has been reported between white populations in Canada and Australia that is rare among non-whites (Warnakulasuriya, 2009a).

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The main cause of the incidence of this cancer is the high consumption of tobacco, especially among consumers of smokeless tobacco, excessive alcohol consumption, and exposure to the sun's ultraviolet rays (Ariyawardana and Johnson, 2013; Listl et al., 2013). Also infection of human papillomavirus (Dodd et al., 2016) has been reported a as risk factor for this cancer.

WHO (World Health Organization) has considered necessary actions for controlling the oral cavity cancer as a health priority (Priya and Lando, 2014). So that can be detected through routine examination, but the 5 year survival rate is low (Ahluwalia et al., 1998). Oral cancer is preventable by controlling tobacco, alcohol and sun exposure (Elter et al., 2005).

To avoid overload of non-communicable diseases especially in low and middle-income countries that already include 80% of burden of disease worldwide, a global action is needed. Cancer is the main cause of morbidity and mortality in many regions of the world.So the HDI (Human Development Index) and considered as a marker of socioeconomic development and incidence and mortality of cancer (Bray et al., 2012; Mahdavifar et al., 2016; Pakzad et al., 2015; Razi et al., 2016). HDI is a composite index which has three dimensions: education, life expectancy and national income. That according to the United Nations Development, the countries are categorized on 4 levels: low, medium, high and very high (Soltani et al., 2015). Since the knowledge about the incidence and mortality of oral cavity cancer can be useful for health programs and research activities and with regard to the possible role of the Human Development Index, this study has taken place with the aim of investigating the incidence and mortality of lip and oral cavity cancer and its relationship with development index and its components in the world in 2012.

Methods

This study was an ecologic study in the World for assessing the correlation between age-specific incidence and mortality rate (ASR) with HDI and its details, including life expectancy at birth, mean years of schooling, and Gross national income (GNI) per capita. Data about the age-specific incidence and mortality rate (ASR) for every country in 2012 were get from the global cancer project that available in http://globocan.iarc.fr/Default.aspx (Ferlay J et al., 2016) and HDI from Human Development Report 2013 (Malik, 2013), that includes information about HDI and its details for every country in the word in 2012.

Method for estimating the age-specific incidence and mortality rates in global cancer project by international agency for research on cancer:

Age-specific incidence rate estimate

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The methods of estimation are country specific, and the quality of the estimation depends upon the quality and on the amount of the information available for each country. In theory, there are as many methods as countries, and because of the variety and the complexity of these methods, an overall quality score for the incidence and mortality estimates combined is almost impossible to establish. However, an alphanumeric scoring system which independently describes the availability of incidence and mortality data has been established at the country level. The combined score is presented together with the estimates for each country with an aim of providing a broad indication of the robustness of the estimation.

The methods to estimate the sex- and age-specific incidence rates of cancer for a specific country fall into one of the following broad categories, in priority order:

1- Rates projected to 2012 (38 countries)

2- Most recent rates applied to 2012 population (20 countries)

3- Estimated from national mortality by modelling, using incidence mortality ratios derived from recorded data in country-specific cancer registries (13 countries)

4- Estimated from national mortality estimates by modelling, using incidence mortality ratios derived from recorded data in local cancer registries in neighboring countries (9 European countries)

5- Estimated from national mortality estimates using modelled survival (32 countries)

6- Estimated as the weighted average of the local rates (16 countries)

7- One cancer registry covering a part of a country is used as representative of the country profile (11 countries)

8- Age/sex specific rates for "all cancers" were partitioned using data on relative frequency of different cancers (by age and sex) (12 countries)

9- The rates are those of neighboring countries or registries in the same area (33 countries) (Ferlay J et al., 2016).

Age-specific mortality rate estimate

Depending on the degree of detail and accuracy of the national mortality data, six methods have been utilized in the following order of priority:

1- Rates projected to 2012 (69 countries)

2- Most recent rates applied to 2012 population (26 countries)

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3- Estimated as the weighted average of regional rates (1 country)

4- Estimated from national incidence estimates by modelling, using country-specific survival (2 countries)

5- Estimated from national incidence estimates using modelled survival (83 countries)

6- The rates are those of neighboring countries or registries in the same area (3 countries) (Ferlay et al., 2015).

Human Development Index (HDI)

HDI is a composite measure of indicators along three components, including life expectancy, educational attainment, and command over the resources needed for a decent living. All groups and regions have seen notable improvement in all HDI components, with faster progress in low and medium HDI countries. On this basis, the world is becoming less unequal. Nevertheless, national averages hide large variations in human experience. Wide disparities remain within countries of both the North and the South, and income inequality within and between many countries has been rising. According to HDI, countries in the world are divided into four categories as follows: countries with very high HDI (HDI≥ = 0.80), countries with a high HDI (0.80> HDI> 0.710), medium HDI countries (0.710≥ HD≥0.535), and countries with a low HDI (HDI < 0.535) (Malik, 2013).

Statistical analysis

In this study, we used correlation bivariate method for assessment of the correlation between age-specific incidence and mortality rate (ASR) with HDI and its details, which include life expectancy at birth, mean years of schooling, and GNI per capita. Statistical significance was assumed if $P< 0.05$. All reported P-values are two-sided. Statistical analyses were performed using SPSS (Version 15.0, SPSS Inc.).

Results

The incidence number of lip and oral cavity cancer

In 2012, 300373 cases of lip and oral cavity cancer had occurred in the world that 198975 cases of them were men and 101398 cases were women (Sex ratio=1.96). Among all cases, 92338 cases were in countries with very high HDI, 45734 cases in countries with a high HDI, 121240 cases in average HDI countries and 40954 cases in countries with a low HDI. Five countries that the highest number of Lip and oral cavity cancers have had occurred in them included India with 77003 cases, America with 26064 cases, 21413 cases in China, Bangladesh with 10550, and Pakistan with 12761 cases. Five countries with the highest

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number of lip and oral cavity cancer in men were India with 53842 cases, America with about 17325 cases, China with about 13656 cases, Russia with 7451 cases, and Bangladesh with 7120 cases , respectively. 5 countries which have the most cases of lip and oral cavity cancer include India with 23161 cases, America with 8739 cases, china with 7757 cases, Pakistan with 5693 cases, and Brazil with 3509 cases.

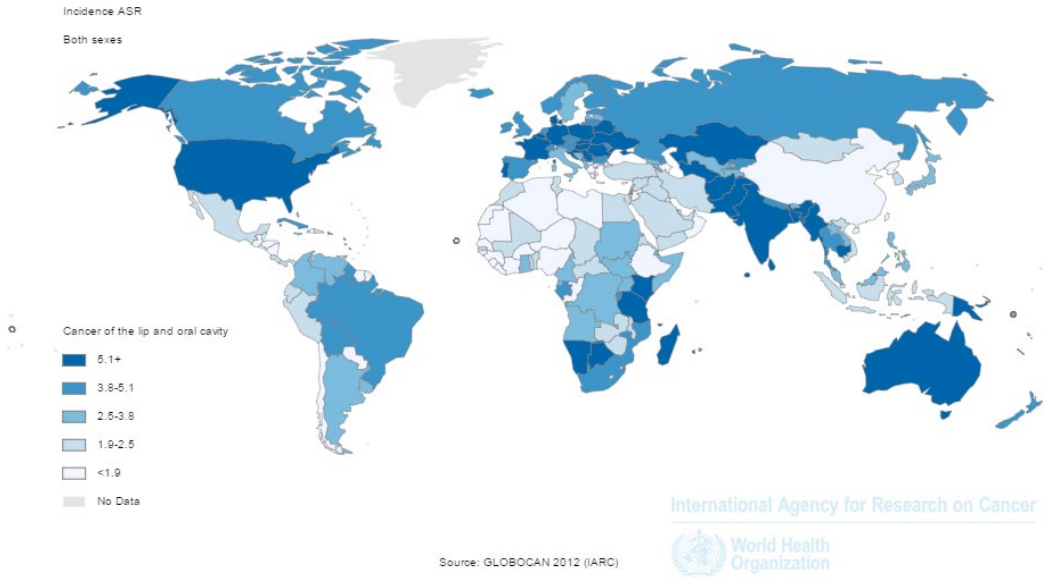

Figure 1. Distribution of the standardized incidence rate of oral cavity and lips cancer in the world (extracted from GLOBOCAN 2012)**.**

Age-standardized incidence rate of Lip and oral cavity cancer

The standardized incidence rate of lip and oral cavity cancer was4 in every hundred thousand in the world that it was 5.5 in men and 2.5 in women per hundred thousand. Standardized incidence rate of lip and oral cavity cancer in countries with very high HDI was 2.7 per 100 thousand people, in countries with high HDI was 2.1 per hundred thousand people, in countries with average HDI was 2.2 per hundred thousand, and in countries with low HDI, it was 4 per hundred thousand people. Five countries with the highest age-standardized incidence rate of lip and oral cavity cancer were Papua New Guinea with 25 cases per hundred thousand people, Maldives with 11 cases per hundred thousand people, Sri Lanka with 10.3 cases per hundred thousand people, Pakistan with 9.8 cases per hundred thousand people, and Hungary with 9.7 cases per hundred thousand people, respectively. 5 countries with the highest age-standardized incidence rate of lip and oral cavity cancer for men include: Papua New Guinea with 30.3 cases per hundred thousand people, Hungary with 15.7 cases per hundred thousand people, Sri Lanka with 15.4 cases per hundred thousand people, Maldives with 15.4 cases per hundred thousand people, and France, La Reunion with 13.7 cases per hundred thousand people. Also 5

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countries with the highest age-standardized incidence rate of lip and oral cavity cancer for women include Papua New Guinea, with 21.1 cases per hundred thousand people, Pakistan with 9.1 cases per hundred thousand people, Brunei with 9 cases per hundred thousand people, Maldives with 6.4 cases per hundred thousand people, and Bangladesh with 5.9 cases per hundred thousand people **(Fig. 1)**.

The mortality number of lip and oral cavity cancer

In 2012, 145353 deaths occurred from lip and oral cavity worldwide from which about 97940 cases related to men and 47413 cases related to women (Sex Ratio = 2.06).The number of deaths from cancer in very high HDI countries was about 26970 cases; about 19615 cases in high HDI countries, 73503 cases in average HDI countries and 25235 cases in low HDI countries. The five countries with the highest number of deaths from lip and oral cavity cancer included India with 52067 deaths, China with 11,333 deaths, 7266 deaths in Pakistan, Bangladesh with 6071 deaths, and Russia with 5658 deaths. Five countries with the most cases of death from lip and oral cavity cancer in men included India with 36,436 deaths, China, with 7370 deaths, Russia with 4472 deaths, Bangladesh with 4094 deaths, and Pakistan with 4046 deaths. Also, five countries with the most cases of death from lip and oral cavity cancer in men included India with15631 deaths, China with 3963 deaths, Pakistan with 3220 deaths, Bangladesh with 1977 deaths, and 1806 cases of death in Japan.

Age-standardized mortality rate of lip and oral cavity cancer

In 2012, the standardized mortality rate of lip and oral cavity cancer was 1.9 per hundred thousand people in the world that this rate was2.7 in men and 1.2 in women per hundred thousand people. The standardized mortality rate of lip and oral cavity cancer in countries with very high HDI was 1.2 per hundred thousand people, in countries with high HDI 1.6 per hundred thousand people, in countries with average HDI 2 per hundred thousand, and in countries with low HDI was 3.3 per hundred thousand people.5 countries which had the highest standardized death rate of lip and oral cavity cancer were Papua New Guinea with 16 per hundred thousand people, Pakistan with 5.9 per hundred thousand, Bangladesh with 5.6 per hundred thousand, Afghanistan with 5.1 per hundred thousand people, and India with 4.9 per hundred thousand people, respectively. 5 countries which had the highest standardized mortality rate of lip and oral cavity cancer for men were Papua New Guinea with 19.4 per hundred thousand, Hungary with 7.9 per hundred thousand, Bangladesh with 7.7 per hundred thousand, Belarus with 7.1 per hundred thousand people, and India with 6.7 per hundred thousand people, respectively. Also,5 countries which had the highest standardized mortality rate of lip and oral cavity cancer for women were Papua New Guinea with 13.6 per hundred thousand people, Pakistan with 5.4 per hundred thousand, Afghanistan with 4.3 per hundred thousand, Comoros with 4.3 per hundred thousand people, and Bangladesh with 3.5 per hundred thousand people, respectively **(Fig. 2)**.

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Figure 3 shows standardized incidence and mortality rate of lip and cavity cancer in different parts of UN. As it is clear, the standardized incidence rate of lip and oral cavity cancer in undeveloped countries and South Asia is more than developed countries. But mortality of lip and oral cavity cancer is higher in less developed countries than in developed and developing ones **(Fig. 3)**.

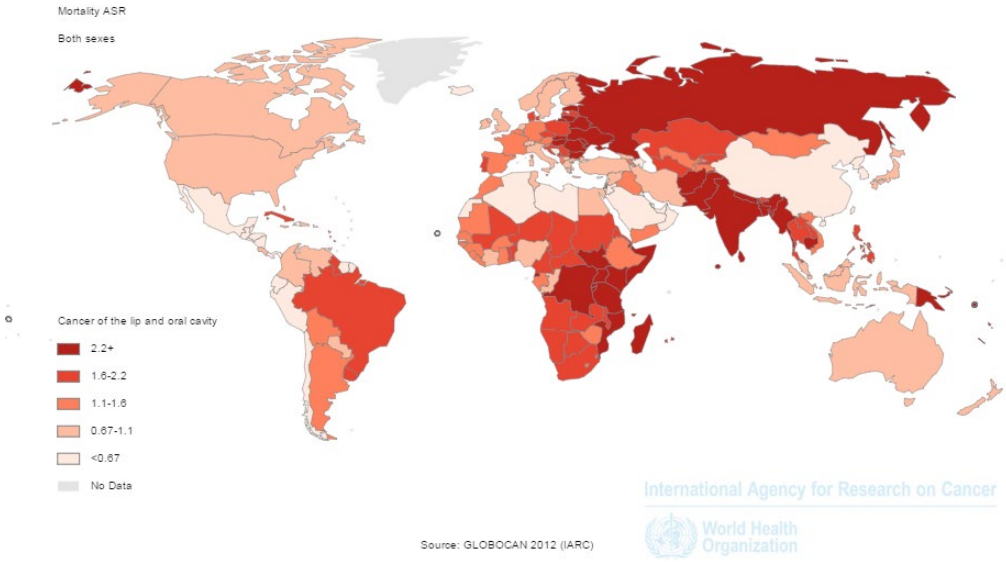

Figure 2. Distribution of the standardized mortality rate of oral cavity and lips cancer in the world (extracted from GLOBOCAN 2012)**.**

The relationship between the standardized incidence rate of lip and oral cavity cancer and the human development index

A positive correlation of 0.122 was seen between the standardized incidence rate of lip and oral cavity cancer and HDI but it was not statistically significant (p=0.114).

Also, a positive correlation was seen between components of the human development index and standardized incidence rate of lip and oral cavity cancer. So that positive correlation between standardized incidence rate with life expectancy at birth was 0.116 (p = 0.134), with mean age of education equaled to 0.123 (p = 0.111), and with the level of income per person of the population was 0.072 (p = 0.352) that none were statistically significant **(Fig. 4 and Table 1)**.

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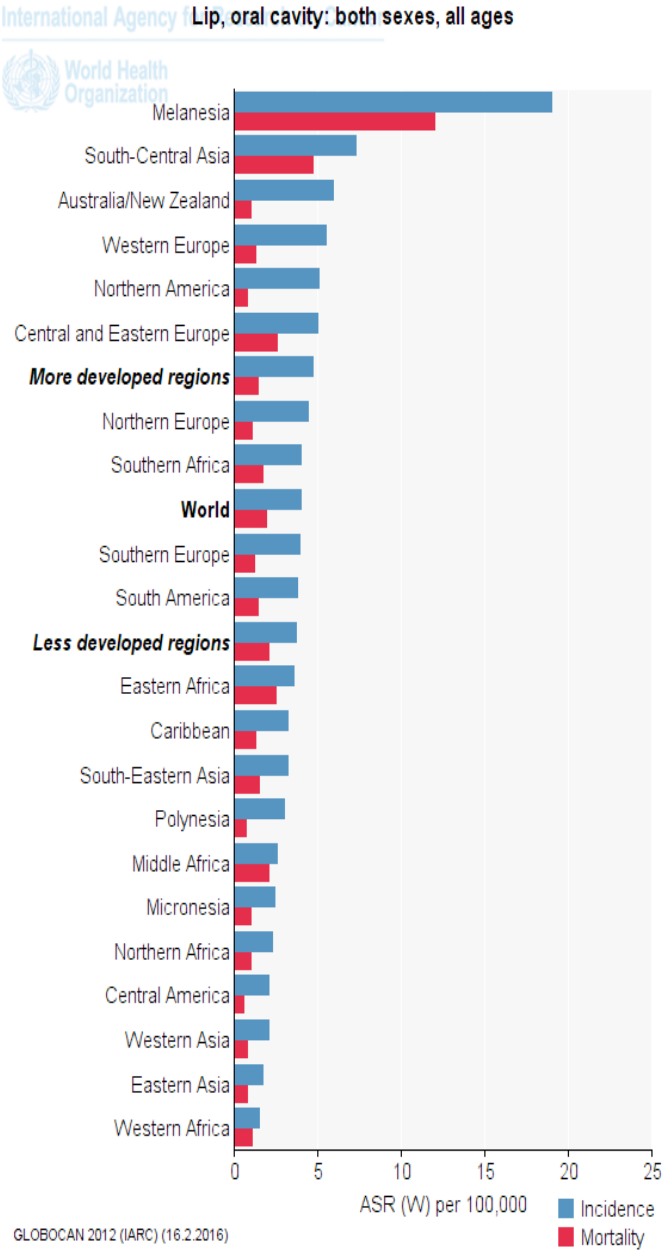

Figure 3. Standardized incidence and mortality rate in different regions of UN (extracted from GLOBOCAN 2012)**.**

The relationship between the age-standardized mortality rate of Lip and oral cavity cancer and the Human Development Index

A negative correlation of -0.295 was seen between the standardized mortality rate of lip and oral cavity cancer and the Human Development Index, that this association was statistically significant ($p < 0.001$). Also, a negative correlation was seen between components of the Human Development Index and standardized mortality rate of Lip and oral cavity cancer. So that a negative correlation of -0.264 ($p = 0.001$) was seen between standardized mortality rate and life expectancy at birth, a correlation of -0.26 with the average years of education ($p = 0.001$), and a

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negative correlation of -0.248 with income level per each person of population (p = 0.001) **(Fig. 5 and Table 1)**.

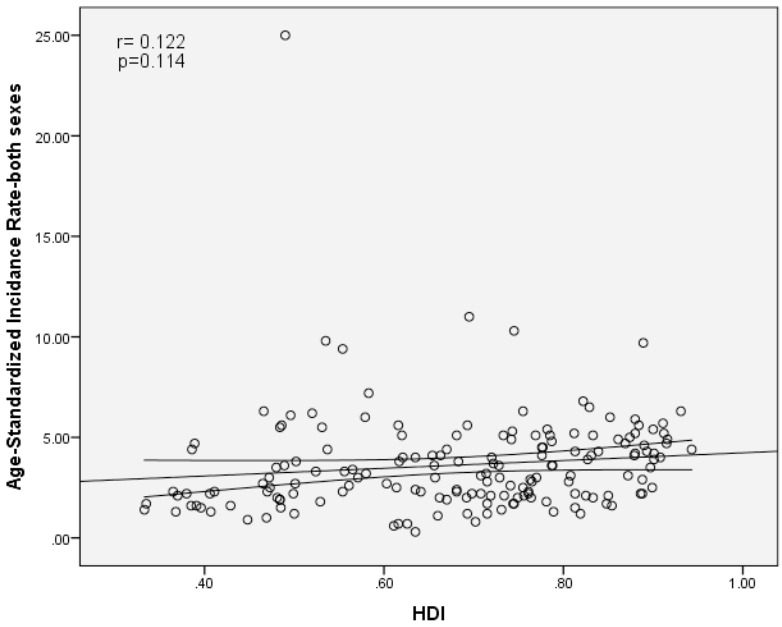

Figure 4. The relation between the standardized incidence rate and the human development index.

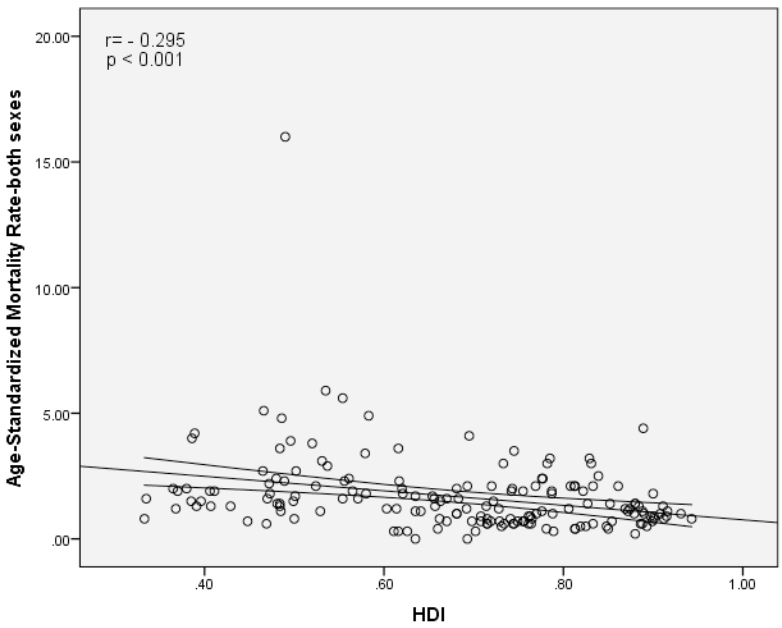

Figure 5. The relation between the standardized mortality rate and the human development index.

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Table 1. Correlation between ASIR, ASMR with HDI and its components

Variables	HDI	LEY	Literacy	GNP
ASIR	r = 0.122 p = 0.114	r=0.116 p=0.134	r=0.123 p=0.111	r=0.072 p=0.483
ASMR	r=-0.295 p<0.001	r=-0.264 p=0.001	r=-0.26 p=0.001	r=-0.248 p=0.001

ASIR: Age-standardized Incidence Rate, ASMR: Age-Standardized Rate

Discussion

Cancer is a major cause of death worldwide which was the cause of 6.7 million deaths in 2008 (Soerjomataram et al., 2012). Oral squamous cell carcinoma is one of the most common tumors in head and neck that can affect both mouth and lip squamous area (Oliveira-Neto et al., 2012). About 264,000 new cases and 128,000 deaths from it have happened in 2008 that about 172,000 cases and 97,000 deaths were in developed countries (Sankaranarayanan et al., 2013).

The concern about lip and oral cavity area is related to high incidence of malignant tumors in this areas compared to other areas of head and neck. complications and mortality which occur from squamous cell carcinoma diagnosis which is a the diagnosed tissue, that can be found 40% in lip and oral cavity, 25% in the larynx and 15%in the throat with a low incidence in oral area (Ribeiro et al., 2015). In addition, oral cancer disease burden is high due to the high cost of treatment, permanent impairment and mortality (Rao et al., 2013).

The results of this study showed that the highest standardized incidence rate of lip and oral cavity cancer is related to Papua New Guinea, Maldives, Sri Lanka, Pakistan and Hungary. The first four countries are located in medium level and Hungary is located in high in terms of Human Development Index .On the other hand, Papua New Guinea, Pakistan, Bangladesh, Afghanistan and India have the highest standardized death rates that those mentioned countries were in average and low levels in the terms of the HDI (Soltani et al., 2015).

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The Mortality and incidence rates of oral cancer varies widely throughout the world as the highest amount has been recorded in developing countries including India, Pakistan, Bangladesh, Hong Kong, Singapore and the Philippines that oral cancer is the most common form of cancer in it (La Vecchia et al., 1997). Standardized incidence rate of oral cancer in West Europe are rising unevenly in two decades (Warnakulasuriya, 2009a). The incidence of oral cancer is high in Asian countries, especially South East Asia (Rao et al., 2013). Asians have the highest risk of oral cancer compared to other populations and other races/ethnicities which is more associated with their lifestyles (Warnakulasuriya, 2010). In South Asia, India is mentioned as the country with the highest incidence of oral cancer (Warnakulasuriya, 2009a).

Smoking, alcohol consumption, sun exposure and viral infections, previous events of head and neck cancer and socioeconomic status can be related to the occurrence of lip and oral cavity cancer (Ribeiro et al., 2015).In most countries of the world, oral cancer occurs more in men than in women. Differences in gender is due to differences in indulgence in risk full behavior habits (tobacco and alcohol) by men and prolonged exposure to sunlight (lip cancer) (Warnakulasuriya, 2010).Lip cancer in white people is more than black people (Wurman et al., 1975). In white men of United States of America, lip cancer occurs more among all mouth cancers that its incidence is about 9/3% in 100000 (Douglass and Gammon, 1984).The highest incidence of oral cavity cancer occurs in Melanesia, South and Central Asia and Central and Eastern Europe and the lowest in Africa, Central America and East Asia for both sexes. Oral cavity cancer mortality rate among men has dropped significantly in many countries including European and Asian countries over the past decade. But the continued rise has been seen in Hungary and Slovakia in several Eastern European countries. Increase in oral cavity cancer in women in European countries, mainly reflects the tobacco epidemic (Jemal et al., 2011).

Human Development Index is combination of three dimensions: a long and healthy life (based on life expectancy at birth), access to knowledge (based on a combination of the adult literacy rate) and a decent standard of life (based on gross national income) (Bray et al., 2013). Factors such as life expectancy, gross national income, literacy, health expenditures, physician density and efficiency of care systems play a constructive role in control of oral cancer mortality (Rao et al., 2013). As almost 47% of cancer cases and 55% of cancer deaths occur in less developed areas of the world meaning countries with low or medium human development index (Soltani et al., 2015).

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In this study, a positive correlation was seen between life expectancy and standardized incidence rate which was not statistically significant. While, a negative correlation was seen between life expectancy and standardized mortality rate which was statistically significant. Increase in life expectancy helps increase in global cancer burden (Soerjomataram et al., 2012). Life expectancy of oral cancer varies considerably depending on the location of lesion, so that lip cancer rates are highest in middle ages among all sufferers (Welch and Nathanson, 1937) and it is also 63.38% for oral cavity cancer (Soerjomataram et al., 2012).

In this study, a positive correlation was seen between level of education and standardized incidence rate which was not significant. However, a negative correlation was observed between standardized incidence rate and standardized mortality rates which was statistically significant that could be due to the delay of referral and unthreatening in patients with low literacy levels. Studies from India, Pakistan and Turkey showed that a correlation exists between education and cancer. Those with lower education levels are at greater risk. A study in India found that even more illiteracy is more associated with oral cancer in comparison with lower literacy levels, (Rao et al., 2013). Another study showed that less education in population, increases death rates from cancer 2.6 folds compared to those who had higher education (Patel et al., 2012). Education Level may be related to behavior, health conditions or access to knowledge and resources that directly or indirectly affect the cancer and getting rid of it (Mahdavifar et al., 2015).

In this study, a positive correlation was seen between income level and the standardized incidence rate which was not statistically significant. While, a negative significant correlation was seen with the standardized mortality rate. Low socioeconomic status was significantly associated with increased risk of oral cancer in low and high-income countries across the universe (Allam and Windsor, 2014; Hobdell et al., 2003; Pawar et al., 2012). A meta-analysis of 41 case-control study around the world proved that, low socioeconomic status is an independent risk factor for oral cancer. People with manual jobs such as agriculture, trade and industry are at risk for oral cancer. In Sri Lanka, for example, tea garden workers are at greater risk (Rao et al., 2013). Another meta-analysis study showed that low socioeconomic and deprivation are significantly related to the risk of oral cancer compared to high-income socio-economic level people (Warnakulasuriya, 2010). One study showed that the elimination of socio-economic inequalities in black Americans may omit deaths from early cancers occurrence as much as 2 folds which is a kind of racial inequality elimination (Patel et al., 2012). Low socioeconomic status may be related to low awareness about health, lack of access to health care, poor

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nutrition, poor work environment factors and poor living conditions associated with an increased risk of oral cancer (Warnakulasuriya, 2009b).Socio-economic status is associated with nearly all health outcomes in most countries. People with more education or income, have a long life and will experience less adverse events related to health (Crimmins and Saito, 2001).

Conclusion

Mortality rates and the incidence of oral cancer varies widely throughout the world as the highest amounts have been recorded in developing countries including India, Pakistan, Bangladesh, Hong Kong, Singapore and Philippines which oral cancer is recorded as the most common form of cancer in it. The prevalence of oral cancer is high in Asian countries, especially Southeast Asia. A positive and statistically non-significant correlation was observed between the lip and oral cavity cancer and human development index and its components including life expectancy at birth, average education level and income level per person. But also a negative significant correlation was observed with the standardized mortality rate and HDI and its components. This research is essential for better treatment in the world to reduce the incidence and mortality of cancer and to create a suitable platform for performing studies with the aim of determining the causes of increased incidence and mortality in the world.

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Original Research

Potential evaluation of central nervous system anti-depressant activity of *Cleome rutidosperma* in mice

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Abstract

Introduction: This investigation was carried out to analyze the central nervous system (CNS) depressant effect of the plant *Cleome rutidosperma* extract, after it was found to have been used by the local people in the Philippine for that purpose. **Methods:** In this study presented below, the CNS depressant effects of the extract was evaluated in *in vivo* mice models; using the standard procedures of Open field, Hole cross and Thiopental sodium induced sleeping time tests. **Results:** Using two test extracts at a concentration of 100 and 200 mg/kg, it was seen that the extracts showed significant ($p< 0.01$) dose dependent suppression of motor activity in both open field and hole cross test, 4.67 ± 0.68** and 3.00 ± 0.45**, respectively at 200 mg/kg. It also showed significant ($p< 0.01$) decrease in the time for the onset of sleep (5.00 ± 0.45 at 200 mg/kg); and an increase in sleeping duration (70.20 ± 0.66 at 200 mg/kg), when compared with the positive control Thiopental sodium. **Conclusion:** Overall, the study demonstrates that the extracts used, showed promising CNS depressant effect. Further study needs to be carried out on the extract to isolate the active constituent, so that it can be assessed for therapeutic use.

Keywords

CNS, anxiolytic, hole cross test, thiopental, motor neuron activity

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Introduction

Anxiety and depression are two most prevalent psychiatric diseases that have been reported in these days. More than 20% of the mature population suffers from these diseases at some part of their lives (Buller and Legrand, 2001; Titov et al., 2010; Yadav et al., 2008). Sedative and hypnotics drug classes are being used to cure anxiety and these drugs produces relaxation by reducing onset of sleep time as well as increasing duration of sleep (Katzung et al., 2011). Thus, present demand has increased use of these drugs largely, to treat different psychiatric disorders like, anxiety and insomnia. However, continuous use of this sedative-hypnotic therapeutics may cause some serious side effects ranging from respiratory and immune system disorders to damaging the cognitive nerve function, and can also cause physical dependency (Dhawan et al., 2003). The development of a new sedative-hypnotic drug is therefore needed with fewer side effects and hence, a promising approach to prevent different psychiatric disorders.

Cleome rutidosperma, is a flowering plant species, which belongs to the genus Cleome of the family Cleomaceae, and is commonly known as Fringed Spider Flower or Purple Cleome. This species is a kind of invasive weed that are found everywhere in the low, wet tropical regions of Asia and Oceania continent. It is a very common weed of lawns. Crude methanol, chloroform and petroleum ether extracts of *Cleome rutidosperma* shows significant analgesic effect and depressed locomotor activity substantially compared to control treatment with chlorpromazine (Bose et al., 2004).

Based on this finding, the experiment was carried out to further assess the extracts potentiality on the Central Nervous System (CNS). We found that the extract of *Cleome rutidosperma*, significantly reduced the locomotor activity and motor coordination in mice. Furthermore, pretreatment with this extract potentiated thiopental sodium-induced hypnosis in mice by decreasing the onset of sleep and prolonging sleeping duration. Therefore, our findings strongly support the sedative and hypnotic activities of *Cleome rutidosperma* extract and suggest that, upon successful isolation of the active molecule from the extract, it can be used in future as treatment of different psychiatric disorders including insomnia.

Materials-Methods

Extract preparation

C. rutidosperma DC was collected from Mirpur area, Dhaka, Bangladesh in October, 2013. The specimen of the plant was identified by the taxonomists of

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National Herbarium, Dhaka, Bangladesh (Accession No. 38625). The leaves of the plant was washed, dried, and mashed to powder. About 500 g of the dried plant powder was dissolved in 1500 ml of methanol and stirred rigorously for the following three days. Then the filtrate was collected using a sterilized cotton filter and dried in a rotary evaporator. Finally, 37.67 g (yield 7.53%) of plant extract was obtained and stored at freezing condition for future use.

Animal model sampling

20-25 g (male) of Swiss Albino mice were obtained from the International Center for Diarrheal Disease Research, Bangladesh (ICDDR, b). Then, under the standard environmental conditions, (temperature: 24.0 ± 1.0^{0}C; relative humidity: 55-65%; 12 h light and dark cycle), these animals were housed. Mice were provided with the food and fresh water, *ad libitum*. According to the Ethical Principles and Guidelines for Scientific Experiments on Animals (1995) formulated by The Swiss Academy of Medical Sciences and the Swiss Academy of Sciences, treatment for all the experimental animals were designed. The protocol was examined, and then accepted by the Ethics Committee of School of Health and Life Sciences, North South University, Dhaka-1229, Bangladesh.

Acute oral toxicity testing

Mice were grouped into the control and three test groups (n = 5). The test groups received MECR (methanolic extract of *C. rutidosperma*) orally at the doses of 1500, 2000, and 3000 mg/kg body weight. Then they caged separately and provided free excess to food and water. They were observed for the next three days (Imam and Sumi, 2014; Walker et al., 2008) for possible changes in the behavior, allergic reactions (like skin rash, itching) and mortality.

Open field test

This test was carried out in an apparatus having a floor of about half square meter in area and surrounded by a wall of 50 cm in height (Gupta et al., 1971). The floor consisted of small squares alternately, colored in black and white. Four test groups containing five mice in each, was classified as control, positive control and two test groups. The control group was treated with vehicle (1% Tween 80 in water) and the positive control was treated with diazepam (1 mg/kg). The test groups were treated with 100 mg/kg and 200 mg/kg of the extract, respectively.

The number of squares visited by the mice was counted and noted for an interval of 3 minutes; before and after 30, 60, 90 and 120 minutes of the oral administration of the vehicle, diazepam and the test extracts.

Hole cross test

This test was carried out in a closed chamber, surrounded by wooden walls measuring 30 cm × 20 cm × 14 cm, with no roof top (Takagi et al., 1971). A fixed

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wooden partition was placed in the middle of the chamber, which divided the chamber into two parts. The partition had a hole cut in it, measuring 3.5 cm and a hole height of 7.5cm.

Four test groups, with each group containing five mice each were selected for this test. The groups were classified as control, positive control and two test groups; with the test groups receiving 100 mg/kg and 200 mg/kg of the extract. The control and the positive control group received vehicle (1% Tween 80 in water) and diazepam (1 mg/kg), respectively.

The number of times it took for each mice to cross in between the chambers via the hole, was measured for an interval of 3 minutes; before treatment and 30, 60, 90 and 120 minutes after the oral administration of the vehicle, diazepam and the doses of 100 and 200 mg/kg of the extract .

Thiopental sodium induced sleeping time

For this experiment, four groups with five mice in each group were taken, similar to the previous experiments. The groups were classified as control, positive control and two test groups; with the test groups receiving 100 mg/kg and 200 mg/kg of the test extract. The control and the positive control group received vehicle (1% Tween 80 in water) and diazepam (1 mg/kg), respectively (Ferrini et al., 1974).

Thirty minutes after the treatment, thiopental sodium was administered intraperitoneally at a dose of 20 mg/kg to the mice in each group and they were placed in separate chambers. The latent period (time between thiopental sodium administration and loss of righting reflex) and the duration of sleep (time between the loss and recovery of righting reflex) was observed for each mouse. The onset and duration of sleep were recorded for the four groups.

Statistical Analysis

The results have been portrayed as Mean ± SEM. The one-way ANOVA test along with Dunnett's post hoc test had been used for the inspection of data using GraphPad Prism 6 software. $p < 0.05$-0.001 were considered as statistically significant.

Results

Open field test

In the Open field test, it was observed that, the squares visited by the different groups of mice; before the treatment were 101.00, 88.33, 99.33 and 98.00 for the control, positive control and the test groups treated with 100 and 200 mg/kg **(Table 1, Fig. 1)** respectively.

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As time passed on, it was seen that, the number of squares visited by the mice of the different groups, decreased over time for an interval of 2 hours. For the control group, the numbers of squares visited were nearly constant, from values ranging from the highest value of 88.67 to the lowest value of 77.00 **(Table 1, Fig. 1)**.

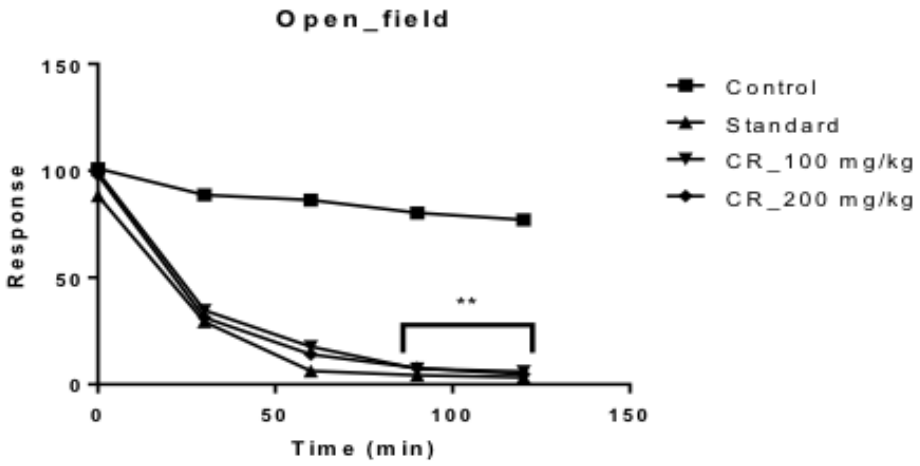

Figure 1. Effect of methanolic crude extract of *C. rutidosperma* in the open field study neuropharmacological activity evaluation. Values are means and standard error mean (mean ± SEM) represented by vertical bars (n=5). Mice were given methanol extract of *Cleome rutidosperma* (100 mg/kg and 200 mg/kg body weight) or Diazepam (standard; 1 mg/Kg) or only water (control) by oral administration for a period of experiments. Mean values marked with an asterisk (*) were significantly different from those of respective control rats (**P <0.001) (derived from repeated-measures ANOVA and adjusted using Bonferroni correction or Dennett's test).

Table 1. Effect of *C. rutidosperma* extract on open field test

Groups	Time (min)				
	0	30	60	90	120
Control	101±3.38	88.67±2.91	86.33±1.57	80.33±1.13	77±1.18
Standard	88.33±3.39	29.33±1.37	6.33±0.68*	4.33±0.68**	3.33±0.68**
CR-100mg/kg	99.33±4.16	34.67±0.93	17.67±1.37	7.33±1.44	6.00±1.18
CR-200mg/kg	98.00±5.16	31.33±1.13	14.00±1.34	7.67±2.11	4.67±0.68

*(Value is presented as the mean ± SEM (n=5); * p<0.05 and **p<0.001; compared with control group (Dunnett's test followed by ANOVA))*

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For the 100 and 200 mg/kg test groups, it was seen that the test group treated with 200 mg/kg of the extract showed the lower number of squares visited by the mice over time, compared to the test group treated with 100 mg/kg .The highest and lowest value for the 200 mg/kg treated group were 31.33 and 4.67 during the two hour period, respectively. For the 100 mg/kg treated group, it was 34.67 and 6.00 **(Table 1, Fig. 1)** respectively. At all times, the values for the positive control group was the lowest among the four groups, with the highest being 29.33 at 30 min and the lowest being 3.33 at 120 min **(Table 1, Fig. 1)** for an interval of 2 hours.

Hole cross test

Like the Open field test, it was observed in the hole test that, the number of times taken by the mice to cross between the chambers, decreased gradually as time passed on for a period of 2 hour **(Table 2, Fig. 2)**. For all the groups, the numbers of crossing between the chambers were higher in the pretreatment period, where no extract or positive control was given to the mice.

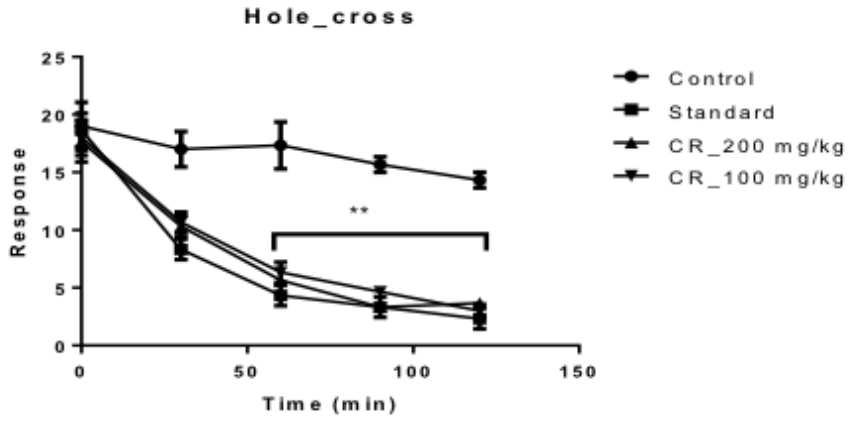

Figure 2. Potentiality of *C. rutidosperma* in the hole cross test to justify neuropharmacological effect. Values are means and standard error mean (mean ± SEM) represented by vertical bars (n=5). Mice were given methanol extract of *Cleome rutidosperma* (100 mg/kg and 200 mg/kg body weight) or Morphine Sulphate (5mg/Kg) or only water (control) by oral administration for a period of experiments. Mean values marked with an asterisk (*) were significantly different from those of respective control rats (⁎*P <0.001) (derived from repeated-measures ANOVA and adjusted using Bonferroni correction or Dennett's test).

As time passed on from 30 min to 120 min, it was seen that, the mice belonging to the positive control group showed the highest change in the number of crossing between the chambers with the lowest value of 2.33 at 120 min whereas, the test group treated with 200 mg/kg showed a considerable decrease in the number of crossing with a value of 3.00 at 120 min **(Table 2, Fig.**

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2), when compared with the Control group and the test group treated with 100 mg/kg.

Table 2. Neuropharmacological effect of *C. rutidosperma* extract was evaluated through hole cross test

Groups	Time (min)				
	0	30	60	90	120
Control	19.00±1.61	17.00±1.18	17.33±1.57	15.67±0.52	14.33±0.52
Standard	18.67±1.13	8.33±0.68	4.33±0.68*	4.33±0.68**	2.33±0.68**
CR-100mg/kg	18.00±1.18	10.67±0.68	6.33±0.68	4.67±0.26*	3.67±0.26*
CR-200mg/kg	16.67±1.37	10.33±0.68	5.67±0.26*	3.33±0.26**	3.00±0.45**

*(Each value is presented as the mean ± SEM (n=5); * p<0.05 and **p<0.001; compared with control group (Dunnett's test followed by ANOVA))*

Thiopental sodium induced sleeping time

When measuring the Onset of sleep, it was seen that the onset was most rapid in the positive control group with only 3.60 mins **(Table 3)**. The test Group 2 had the lowest onset of sleep time of 5 mins when compared with the Control group and the test Group 1 **(Table 3 and Fig. 3)**. When measuring the duration of sleeping time, it was seen that, the duration of sleeping time was the highest in the positive control group with 80 mins; the next being the mice in the group 2 and group 1 with a duration of 70.20 and 59.80 **(Table 3, Fig. 4)** respectively.

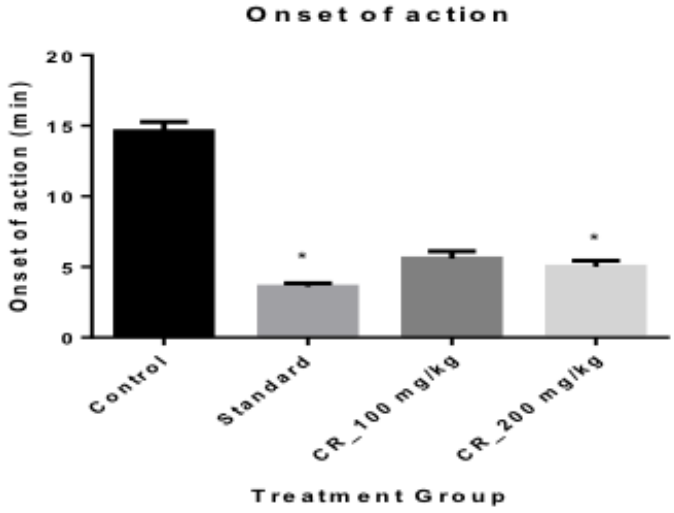

Figure 3. Determination of the onset of sleep induction capability of methanolic crude extract of *C. rutidosperma* after its induction to animal model. Values are means and standard error mean (mean ± SEM) represented by vertical bars (n=5). Mice were given methanol extract of *Cleome rutidosperma* (100 mg/kg and 200 mg/kg body

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weight) or Diazepam (1mg/Kg) or only water (control) by oral administration for a period of experiments. Mean values were significantly different from those of respective control rats (derived from repeated-measures ANOVA and adjusted using Bonferroni correction or Dennett's test).

Table 3. The potency of methanolic crude extract of *C. rutidosperma* in the elongation of sleeping time and onset of sleeping induction

Onset of action		Duration of sleep	
Groups	**Time (min)**	**Groups**	**Time (min)**
Control	14.60±0.68	Control	40.60±0.87
Standard	3.60±0.25	Standard	80.00±1.92
CR-100 mg/kg	5.60±0.51	CR-100 mg/kg	59.80±1.43
CR-200 mg/kg	5.00±0.45	CR-200 mg/kg	70.20±0.66

Each value is presented as the mean ± SEM (n=5)

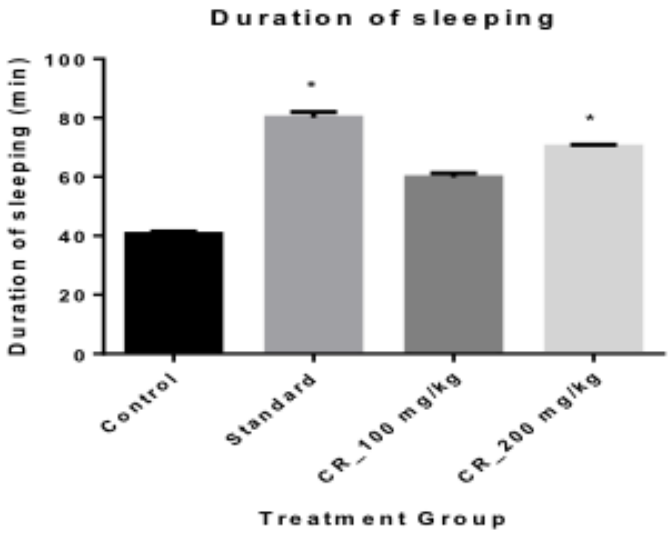

Figure 4. Determination of time duration of sleeping induced by methanolic crude extract of *C. rutidosperma*. Values are means and standard deviations (mean ± SEM) represented by vertical bars (n=5). Mice were given methanol extract of *Cleome rutidosperma* (100 mg/kg and 200 mg/kg body weight) or Diazepam (1mg/Kg) or only water (control) by oral administration for a period of experiments. Mean values were significantly different from those of respective control rats (derived from repeated-measures ANOVA and adjusted using Bonferroni correction or Dennett's test).

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Discussion

This research has demonstrated that the administration of different doses (100 and 200 mg/kg body weight) of methanol extract of *C. rutidosperma* leaves, showed strong sedative and anxiolytic properties. Both doses potentiated sleep during the thiopental sodium induced sleeping time tests. "Thiopental" that is a hypnotic agent, and basically cause hypnosis by potentiating GABA mediated postsynaptic inhibition through allosteric modification of GABA receptors at a proper dose. Substances that consist of CNS depressant activity either decrease the onset or prolong the duration of sleep or dose both (Hasan et al., 2009; Nyeem et al., 2007). GABA-benzodiazepine receptors are the most abundant inhibitory receptor (Braestrup and Squires, 1977) system in the CNS and binding of a benzodiazepine agonist to its binding site results in an increase in chloride ion flux (Trofimiuk et al., 2005) that in turns hyperpolarizes the postsynaptic membrane at a lower threshold of spike generation. This mechanism of GABA agonists made them available for generation of hypnosis in the treatment of anxiety. In addition, the hole cross and open field methods carried out to test the locomotor activity, which shows both doses of methanol extract from the leaves of *C. rutidosperma* decreased the frequency and the amplitude of movements. Since, locomotor activity is a measurement of the level of excitability of the CNS (Mansur et al., 1980), this decrease in spontaneous motor activity could be attributed to the sedative effect of the plant extracts (Rakotonirina et al., 2001). Both doses significantly, decreased locomotion in mice. The locomotor activity lowering effect was evident from the 2nd observation of (30 min) and continued up to the 5th observation period at (120 min) of the hole cross and the open field test. The results were also dose dependent and statistically significant (p<0.001).

The research has examined some neuropharmacological activities of methanolic crude extracts of *C. rutidosperma* leaves. The plant extracts possessed CNS depressant activity that has been shown by the decrease in exploratory behavior in mice. It also showed a marked sedative effect by the reduction in gross behavior and potentiation of thiopental induced sleeping time. Substances which possess CNS depressant activity either reduce the onset of sleep time or prolong the duration of sleep or dose both (Nyeem et al., 2007). Moreover, the research on locomotor activity, as measured by hole cross and open field tests, showed that both extracts of the dried leaves of *C. rutidosperma* (100 and 200 mg/kg) reduced the incidence and the amplitude of movements. Since, locomotor activity is a measure of excitability of the CNS (Mansur et al., 1980), this inhibition of continuous motor activity could be attributed to the sedative effect of the plant extracts (Öztürk et al., 1996; Rakotonirina et al., 2001).

The medicinal effect of a plant usually results from the combination of a secondary metabolites present within it, through additive or synergistic action of several chemical compounds acts on single or multiple target sites associated with a physiological process (Briskin, 2000). According to Kaufman *et al.*, (1999)

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(Kaufman et al., 1999), preliminary phytochemical analysis with this plant revealed the presence of alkaloids, tannins, glycosides, steroids, flavonoids and tannins. These secondary metabolites especially flavonoids individually or in combination with other phytochemicals, might account for the observed pharmacological effects exerted by this plant. However, many flavonoids were found to be ligands for the gamma aminobutyric acid type A (GABAa) receptors in the CNS, which led to the hypothesis that they acts like benzodiazepine molecules. Thus, the sedative and anxiolytic effects exhibited by the *C. rutidosperma* leaves extracts might be due to the interaction of flavonoids with the GABA/benzodiazepine receptor complex in brain (Trofimiuk et al., 2005). This is findings supported by their behavioral effects in animal models of anxiety, sedation and convulsion (Marder and Paladini, 2002). Electrophysiological experiments with flavone and flavanone derivatives have shown that some of them can modulate GABA-generated chloride currents, either positively or negatively. Due to the increased knowledge of the diversity of GABAa receptor sub-types, the number of studies with cloned receptors of defined subunit composition has risen recently and experiments with some natural and synthetic flavones and flavonones have shown that they can modulate gamma aminobutyric acid (GABA)- generated chloride currents, either positively or negatively (Campbell et al., 2004; Goutman et al., 2003; Johnston, 2005; Kavvadias et al., 2004). Thus the decreased spontaneous motor activity could be attributed to the CNS depressant activity of the leaves of *C. rutidosperma*.

Conclusion

It can be concluded from the above experiment that, the extracts of *Cleome rutidosperma* possess significant sedative and hypnotic activities. With all the doses, used in the above experiment, it was clearly visible that, the effects were statistically significant. However, further studies must be carried out to isolate the active constituent from the extract; which is responsible for the CNS depressant activity, and hence investigate its potentiality for therapeutic use in future and to understand its molecular mechanisms responsible for that pharmacological activity.

Abbreviations

ANOVA: Analysis Of Variance; CNS: Central Nervous System; icddr'b: International Centre for Diarrheal Disease Research, Bangladesh

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Ethical Approval

We declare that this study was done for the human beneficiary and under proper supervision of ethical committee of North South University. Nothing has been done that violate animal rights as this study involves use of a number of animals. We took our ethical consent from icddr'b (International Centre for Diarrheal Disease Research, Bangladesh) during collection of models for this experiment and also our institutional (Department of Pharmaceutical Sciences, North South University, Dhaka-1229, Bangladesh) authority approved us on this protocol to use animal model. And Prof. JMA Hannan led the ethical committee along with other intellectual body. They have inspected the protocol and estimated the animal use. After inspection, it is decided that the animal to be sacrificed in this protocol is justified. Then the committee issued an ethical approval stating that this protocol is eligible to use animal model for its purpose.

Competing Interests

We declare that we have no competing of interest.

Authors Contribution

SA and AC carried out the study design, participated in experiment. AC, FFH and SI contributed in the manuscript preparation. SA, SG and AU have done all data calculation and statistical analysis. MAHKB, MSK and MR have collected the plant and was involved in the herbarium of the plant. All the authors have scrutinises whole manuscript for gramatical and typographical mistake.

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Case Report

Umbilical cord derived stem cell (Modulatist™) transplantation for severe chronic obstructive pulmonary disease: a report of two cases

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Abstract

Introduction: Chronic obstructive pulmonary disease (COPD) is a chronic disease affecting the airway of the respiratory system. COPD cases have rapidly increased in recent years, with the disease becoming the fourth leading cause of death worldwide. Stem cell transplantation is a new approach to treat COPD. In this study we report in two cases the use of transplanted stem cells to treat COPD. **Methods:** Umbilical cord derived stem cells (Modulatist™) were used in the study. Modulatist™ was prepared according to previous published studies. Two patients with late stage COPD (stage IV) were transfused with Modulatist at a dose of 10^6 cells/kg. Patients were evaluated by the COPD assessment test (CAT) score as well as the Modified Medical Research Council Dyspnea Scale (mMRC) score, before and after transplantation (1, 3 and 5 months post transplantation). **Results:** Results showed that Modulatist™ transplantation significantly improved sever COPD, especially after 3 months. At that time point, the two patients receiving Modulatist™ showed a significantly improvement, from late-stage of COPD (stage IV) to stage I. **Conclusion:** Although these initial results suggest that Modulatist™ transplantation is a promising therapy, more clinical studies in COPD patients are warranted to evaluate efficacy.

Keywords

COPD, chronic obstructive pulmonary disease, Modulatist™, umbilical cord derived stem cells, CAT, mMRC

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Introduction

COPD is the third-leading cause of death in the United States (Kochanek et al., 2011; Minino, 2011) and fourth leading cause of death in the world. Therefore, many studies have been carried out to develop effective treatment methods. To date, development of treatments, such as pharmaceutical drugs, have led to reduction of symptoms. However, these treatments cannot attenuate disease progression or reverse COPD and emphysematous changes.

Increased interest in stem cells and their unique properties have led to investigations of stem cell transplantation as an alternative treatment for COPD. The most popular kind of stem cells for disease treatment has been mesenchymal stem cells (MSCs). MSCs can be isolated and cultured easily from various tissues in the human body, such as bone marrow and adipose tissue. However, procedures to collect MSCs from these tissues are invasive and laborious. Therefore, MSCs from umbilical cord blood, placenta or umbilical cord have emerged as alternative sources. Unlike other kinds of stem cells, MSCs exhibit unique properties; they secrete paracrine factors, play a role in immune modulation, and undergo multiple lineage differentiation. Given their immune modulatory properties, MSCs have been used effectively to treat various immune-related diseases.

MSCs have been investigated in mice for treatment of various lung diseases. These conditions include ventilator-induced lung injury (Curley et al., 2012), bleomycin-induced fibrosis (Moodley et al., 2009; Ortiz et al., 2003), cigarette smoke-induced or elastase-induced COPD/emphysema (Antunes et al., 2014; Chen et al., 2015; Zhao et al., 2014), bronchopulmonary dysplasia (Aslam et al., 2009; Tropea et al., 2012), and bacterial pneumonia (Gupta et al., 2012; Krasnodembskaya et al., 2012). A systemic and meta-analysis of 20 eligible preclinical studies using MSC transplantation for COPD treatment has shown that MSC administration significantly attenuates acute lung injury, stimulate lung tissue repair and improves lung function (Liu et al., 2016). The mechanism of action mediated by MSC transplantation in COPD was also investigated and reviewed in that study; the most common mechanism was amelioration of airway inflammation (Liu et al., 2016).

The promising preclinical studies have prompted clinical investigations to evaluate the application and efficacy of stem cells (particularly MSCs) in patients (Shroff, 2015; Stessuk et al., 2013; Weiss et al., 2013). In the first study reported by Stessuk et al., the authors infused autologous bone marrow mononuclear cells into 4 patients with advanced pulmonary emphysema (Stessuk et al., 2013). These patients were followed up for 3 years. The results showed that this procedure was safe for patients with chronic obstructive pulmonary disease, with no adverse effects recorded. Moreover, patients were showed improvement in

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clinical condition and quality of life (Stessuk et al., 2013). In a subsequent study by Weiss et al., the authors performed a placebo-controlled, randomized trial using MSCs from bone marrow (Prochymal; provided by Osiris Therapeutics Inc.). Contrary to the hypothesis, the clinical trial showed that Prochymal cell transplantation showed low efficacy in COPD, despite significantly reduced serum C-reactive protein (C-RP) levels in the patients who received MSC administration (Weiss et al., 2013). Recently, a case report using human embryonic stem cells to treat emphysematous COPD was reported (Shroff, 2015). In this case, human embryonic stem cell transplantation resulted in improved symptoms of the emphysema patient (Shroff, 2015). Our report herein represents 2 cases of patients with severe COPD who received treatment with umbilical cord derived stem cells (Modulatist™; provided by RegenMed Lab.). The safety and efficacy of transplantation were followed for 5 months.

Methods

Umbilical cord derived stem cells (Modulatist™) were isolated according to our published protocol (Pham et al., 2016). All cryopreserved Modulatist™ cells were thawed and re-plated overnight to select for viable (adherent) cells. The next day, the adherent cells were detached and collected. Cell viability and cell number were analyzed by flow cytometer (Accuri C6; BD Biosciences, San Jose, CA). Only cell samples with greater than 95% cell viability were used for transplantation.

The patients were diagnosed with severe COPD (stage IV) based on forced expiratory volume in one second (FEV1). After transplantation with stem cells, patients were monitored and re-evaluated for their CAT score (i.e. COPD assessment test score), which is based on a validated test for evaluation of COPD impact on health status. Moreover, patients were evaluated on the Modified Medical Research Council Dyspnea Scale (mMRC), which uses a simple grading system to assess a patient's level of dyspnea, i.e. shortness of breath).

Case presentation

For the first patient (male; born in 1959) with stage IV COPD, evaluations included CAT, mMRC and FEV1. The results showed that FEV1 was low (at 20.8%), while CAT and mMRC scores were high (28 and 2, for CAT and mMRC, respectively). After diagnosis, the patient was transplanted with Modulatist™ at 10^6 cells/kg by transfusion into arm vein. All cells were prepared in 250 mL of sodium chloride (0.9%). The cell suspension was transfused in 30-45 minutes. Patients was monitored in the hospital for 1 week after transplantation for evaluation and recording of any side effects related to contamination. Following hospital discharge, the patient was monitored as an out-patient for 5 months after transplantation. The CAT and mMRC scores were evaluated after 1, 3 and 5

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months. All traditional treatments were maintained and applied for the patient. The results showed the CAT score significantly decreased from 28 (before transplantation) to 9, 7 and 8 (at 1 month, 3 months and 5 months, respectively) (**Table 1**). However, the mMRC score stayed consistently at 2, before or after transplantation (1, 3 and 5 months). While the FEV1 slightly increased after Modulatist™ transplantation to 22.8% after 3 months and to 23.1% after 5 months, compared to 20.8% (before transplantation). The patient felt better and much healthier, and showed a significant reduction in acute exacerbation; the patient has been monitored for 12 months to date.

Table 1. Scores of Patients before and after transplantation of Modulatist™

Patients	Scores	Before transplantation	After transplantation		
			1 Month	3 Months	5 Months
LVT (yr 1959, Stage IV)	CAT	28	9	7	8
	mMRC	2	2	2	2
	FEV1	20.8%		22.8%	23.1%
NBH (yr 1938, stage IV)	CAT	18	11	5	5
	mMRC	4	2	2	2
	FEV1	59.6%		72.4%	

For the second patient (male; born in 1938) with stage IV of COPD, evaluations also included CAT, mMRC and FEV1 **(Table 1)**. The results showed that FEV1 was low (at 59.6%), while CAT and mMRC scores were high (18 and 4, for CAT and mMRC, respectively). Similar to the first case, after diagnosis the patient was transplanted with Modulatist™ at 10^6 cells/kg by transfusion into arm vein. Likewise, all cells were prepared in 250 mL of sodium chloride (0.9%). The cell suspension was transfused in 30-45 minutes. Patient 2 was monitored in the hospital and out of the hospital, according to the same regimen and schedule as patient 1. The results showed that compared to the scores before transplantation, the CAT score significantly decreased 5 months post transplantation (from 18 to 5) and FEV1 increased 3 months post transplantation (from 59.6% to 72.4%). No complaints or side effects were noted for Modulatist™ during the 5-month monitoring post transplantation. Importantly, the number of hospital admissions related to exacerbations significantly reduced, from 13 admission per year to 0 admission during the 5 month follow-up.

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Discussion

COPD is a prevalent and global disease, ranking worldwide as the fourth leading cause of mortality. Although there have been many medicines developed to treat this disease, the present-day medicines only reduce symptoms and pain. This study investigated the application of umbilical cord derived stem cells (called Modulatist™; produced by RegenMed Lab Ltd.) to treat 2 patients with severe COPD.

Although there were only 2 patients, and the monitoring was relatively short term, these results from these 2 patients are promising. The umbilical cord derived stem cells (Modulatist™ cells), produced by Modulatist™ technology, showed positive effects in the COPD patients. Firstly, during the 5 months after transplantation of Modulatist™, there were no recorded complications or side effects related to Modulatist™ in any of the patients. Secondly, with regard to treatment efficacy, administration of Modulatist reduced COPD symptoms, improved all scores (CAT, mMRC, and FEV1), and improved the patient's quality of life. Particularly, the rate of acute exacerbation was significantly reduced in both patients.

These effects arise from properties and characteristics of Modulatist™ cells which have been published in our previous study (Pham et al., 2016). For instance, Modulatist™ cells exhibit strong immune modulation, more so than adipose derived stem cells or bone marrow derived stem cells. As MSCs, Modulatist™ cells isolated from umbilical cord can inhibit T cells, B cells, and NK cells through various different mechanisms. Moreover, Modulatist™ cells can control inflammation as well as immune reactions inside the transplanted patients (Pham et al., 2016). Given that inflammation is the main process contributing to COPD, the ability of the umbilical cord derived stem cells (Modulatist™) to modulate inflammation is highly beneficial.

COPD is a result of chronic inflammation at the airway of the respiratory system. Acute exacerbations of chronic obstructive pulmonary disease is characterized by increased pulmonary and systemic inflammation (Tan et al., 2016). This process is triggered and increased by smoking or by air pollution. The long-term effects of inflammation leads to the obstructive condition, i.e. COPD. Moreover, the COPD attack considered to lead to the greatest risk of death is related to intense inflammation. With these reasons, Modulatist™ cells should be effective against COPD via immune modulation.

MSCs have shown some potential and success as treatment of diseases related to immune system, such as graft versus host disease (GVHD), autoimmune disease and liver cirrhosis. However, MSCs probably mediate their effects in different diseases via different mechanisms. Some mechanisms may include paracrine factors and in vivo differentiation of grafted cells. In animal models of COPD, MSCs have been shown to play a key role in stimulating lung tissue repair (Liu et al., 2016). In a mouse model of COPD, it has been demonstrated

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that MSC transplantation can promote proliferation of endogenous lung stem cells (Liu et al., 2015).

Conclusion

This study represents the first two cases of CODP patients treated with umbilical cord derived stem cells (Modulatist™). The transfusion of Modulatist™ into arm vein of late-stage COPD patients can significantly improve CAT and mMRC scores of the patients. Patient quality of life also improved with a significant reduction of acute exacerbation. Importantly, there were no adverse side effects recorded during the 5 month follow-up. Our results suggest that a clinical trial with more COPD patients needs to be evaluated to further confirm the safety and efficacy of umbilical cord derived stem cells (Modulatist™) transplantation in late-stage COPD treatment.

Lists of abbreviations

COPD: Chronic obstructive pulmonary disease; CAT: COPD assessment test; GVHD: graft versus host disease; mMRC: Modified Medical Research Council Dyspnea Scale; FEV1: forced expiratory volume in one second; MSC: Mesenchymal stem cell.

Ethics approval

This study was approved by Ministry of Health, Viet Nam. ClinicalTrials.gov Identifier: NCT02645305

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Authors' contributions

PTBL and TMD: did clinical treatment, transfused stem cells into patients, acquired the data. NBV and PVP: prepared the stem cell products, did analysis and wrote the manuscript. All authors approved this manuscript.

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