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Protective Role of Granulocyte Colony Stimulating Factor and Umbelliferone on Testes

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Abstract:

Granulocyte-colony stimulating factor (G-CSF) is a glycoprotein with hormone-like properties. G-CSF organizes proliferation, differentiation, and survival of haemopoietic progenitor cells. Clinically, G-CSF is an approved drug for treatment of leukocytopenia and for mobilization of haemopoietic stem cells from bone marrow towards peripheral circulation. Recently, growing evidence support that G-CSF has many important non haemopoietic functions in other tissues Umbelliferone (UMB) (7-hydroxycoumarin) is a natural product found in plants of the Rutaceae and Umbelliferae families. UMB possesses several pharmacological activities, including antioxidant, anti-inflammatory, antidiabetic, and antiapoptotic effects**.** The antioxidant and radical scavenging activity of UMB has been reported in several studies by reducing cellular oxidative stress by decrease ROS generation and increase expression of antioxidant enzymes. Also, UMB ability to work locally within the testes to improve the spermatogenesis or centrally by regulating the pituitary-gonadal axis.

Keywords: Granulocyte Colony Stimulating Factor, Umbelliferone, Testes.

In 1960, several studies were done to detect how white blood cells (WBCs) are regulated within the blood circulation. An in-vitro studies demonstrated that the growth of WBCs colonies was based on the presence of unknown proteins that were given the name of colony-stimulating factors (CSFs). In 1980, different laboratories performed work to purify and classify CSF proteins. Resulting from these efforts, four CSF proteins with different activities were discovered. They were classified and named based on the type of cell colonies they stimulated. They were granulocyte-macrophage colony stimulating factor (GM-CSF) stimulated both granulocyte and macrophage colonies, macrophage colony stimulating factor (M-CSF) stimulated macrophage colonies, Granulocyte-colony stimulating factor (G-CSF) stimulated granulocyte colony formation and multi-CSF (known as interleukin 3, IL-3) stimulated multiple hematopoietic cell colonies **(1)**.

Granulocyte-colony stimulating factor (G-CSF) is a polypeptide that belongs to the colony stimulating factor glycoprotein group. It is located on chromosome 17 and encoded by CSF3 gene. This gene encodes two different messenger ribonucleic acid (mRNA) products: G-CSFa contains 177amino acids (18.8kD) and G-CSFb contains 174 amino acids (19.6kD). The difference between the two types is that G-CSFa contains additional amino acids; valine, serine and glycine. The principal sources of G-CSF are monocytes (the most abundant source). It is also synthesized by macrophages, lymphocytes, fibroblasts, endothelial cells, bone marrow stromal cells and natural killer (NK) cells **(2, 3)**. The G-CSF is also expressed in E. coli and yeast system **(4)**. **Michailov et al. (5)** added that GCSF is also produced by interstitial cell of Leydig, Sertoli and interstitial macrophages.

The major target cell of G-CSF is polymorphonuclear leukocytes (PMNL). G-CSF not only promotes neutrophils proliferation but also modulates the function and activity of developing and mature neutrophils **(6)**. G-

CSF also has trophic effects on different cell types including the neurons **(7)**. Inflammatory factors such as bacterial lipopolysaccharide, interleukin-1β, tumor necrosis factor α (TNF- α) , IL-1 and IL-17 from T helper cells induce G-CSF expression via intracellular signaling. The increase in the number of circulating neutrophils reduces the production of G-CSF in the bone marrow **(8).**

Granulocyte-colony stimulating factor (G-CSF) usage as hematopoietic stem cells (HSCs) mobilizing agent has multiple advantages relative to natural bone marrow collection. Its administration decreases the need for post-transplantation platelet transfusions. G-CSF remains as the most commonly used agent for hematopoietic stem cells (HSCs) mobilization. G-CSF induces the mobilization of hematopoietic stem cells (HSCs) from bone marrow (BM) towards the peripheral circulation after splitting the links between them and niche where they are stored. Peripherally, G-CSF influences the survival and chemotaxis of neutrophils because of release of arachidonic acid, alkaline phosphatase, myeloperoxidase and superoxide anion. G-CSF can be used not only in hematological malignancies for hematopoietic stem cell transplantation but also as an effective drug for the treatment of chemotherapy-induced neutropenia **(9-11).**

Clinically, G-CSF is commonly used. Filgrastim is a synthetic form of G-CSF that is manufactured in Escherichia coli (E. coli). It is clinically used to treat neutropenia that is usually caused by chemotherapeutic drugs. In addition, it is used to help bone marrow recovery after bone marrow transplantation **(12, 13)**.

Dale et al. (14) mentioned that filgrastim was the first drug that obtained Food and Drug Administration (FDA) approval in 1991 and has been used to induce mobilization of allogeneic or autologous hematopoietic stem cells (HSCs) and collect peripheral blood progenitor cells. Granulocyte-colony stimulating factor has been purified, cloned, and produced through recombinant DNA techniques. The recombinant factors have been shown to have biologic properties and actions that are similar to the naturally occurring factors. The availability of quantities of the recombinant factors has resulted in their introduction into clinical trials and into the market **(15)**.

Granulocyte-colony stimulating factor acts by binding to a G-CSF specific transmembrane receptor (belonging to the class I cytokine receptor family). This receptor (G-CSF-R) is present on myeloid progenitor cells, myeloid leukemia cells, mature neutrophils, platelets, monocytes, lymphoid cells and some T cells and B cells. In addition to these cells of hematopoietic lineage, receptors for G-CSF are found in several non- hematopoietic cell types, including endothelial cells, placenta cells, trophoblastic cells and granulosa lutein cells **(16)**. **Michailov et al (5)** demonstrated G-CSF receptors in sperms for the first time. G-CSF binds to its transmembrane receptor (G-CSFR), and initiates a signaling cascade by phosphorylating/activating Janus kinase 2 (JAK-2). The activated JAK-2 can triggers multiple signaling mechanisms resulting in transcription of genes important for cell proliferation, differentiation or in the inhibition of apoptosis. Patients with hypomorphic mutation in G-CSF receptors usually exhibit marked neutropenia **(1, 17)**.

Self-replacing hematopoietic cells give rise to multi-potent stem cells, which in turn give rise to lymphoid progenitors, erythroid progenitors, megakaryocytes, basophil progenitors, eosinophil progenitors or granulocyte–monocyte progenitors. Granulocyte–monocyte progenitors give rise to neutrophils and monocytes by the stimulation of G-CSF with additional cytokines and growth factors such as IL-3, GM-CSF, and M-CSF **(18).**

The release of G-CSF into the blood stream by tissues also stimulates the mobilization of neutrophils from the bone marrow. Furthermore, the locally produced G-CSF within tissues also influences the function of neutrophils at the site of infection. G-CSF can inhibit neutrophil apoptosis **(19)**. In addition to its proliferation and survival-promoting activity, G-CSF treatment induces a rapid and sustained elevation in absolute peripheral neutrophil numbers. G-CSF shortens the transit time of developing granulocytes through the bone marrow compartment and accelerates the release of neutrophils that undergo recent cell division. Moreover, G-CSF stimulates an increase in the absolute number of hematopoietic progenitor cells (HPC) in peripheral blood in a process that is known as ‘‘stem cell mobilization” **(20)**.

Traditionally, hematopoietic cells for both autologous and allogeneic transplantation were obtained by collecting large volumes of bone marrow, aspirated from the pelvic crests under general anesthesia. However, it has been shown that GCSF could mobilize hematopoietic cells in large numbers from the marrow into the circulation. The use of G-CSF mobilization has the advantage of increasing the number of hematopoietic cells collected, with consequent reductions in the time taken post-transplant to restore neutrophil and platelet numbers to clinically safe levels, and improvements in transplant safety **(19)**.

Therefore, it is hypothesized that G-CSF mobilize the MSCs that play a pivotal role in preventing and alleviating stem cell transplantation complications. Several other studies showed that administration of Granulocyte colony-stimulating factor (G-CSF) might be effective in the treatment of ischemic diseases, such as stroke, cardiovascular diseases as well as limb ischemia **(21-23)**.

Benavides-Garcia et al. (24) demonstrated G-CSF receptor (CSF3R) upon undifferentiated spermatogonia in mouse testis. They suggested that G-CSF acts as a growth factor that maintains the process of spermatogenesis after cancer treatments.

Kotzur et al (25) documented that G-CSF has also a protective effect on spermatogenesis after chemotherapy. The mode of G-CSF action is by promotion of spermatogonial proliferation, leading to enhanced spermatogenic regeneration from surviving spermatogonial stem cells (SSCs). Since the receptor for G-CSF, colony-stimulating factor 3 receptor (CSF3R), has been previously detected on the cell surface of cultured undifferentiated spermatogonia, it was possible that G-CSF may act directly on undifferentiated spermatogonia and have a role in promoting normal steady state spermatogenesis. In addition, the protective role of G-CSF has been suggested to be mediated by its anti-apoptotic activity in various organs **(26)**.

Umbelliferone (UMB)

Umbelliferone (UMB) also known as 7-hydroxycoumarin, hydrangine, or skimmetine is one of the most common plant-based coumarins in the flowers, fruits and roots of almost all higher plants mainly from Umbelliferae/Apiaceae family that possesses a wide variety of pharmacological properties. Umbelliferone's name is derived from the umbelliferae family that have umbrella-shaped inflorescences, each called umbel. The umbelliferae family also includes many economically important herbs as alexanders, angelica, asafoetica, celery, cumin, fennel and parsley. Umbelliferone is a benzopyrone compound and was isolated in 1820 **(27, 28)**.

Umbelliferone is yellowish-white needle like crystals that are slightly soluble in hot water, but have high solubility in ethanol and dioxane. Its molecular formula is C9H6O3 as shown in **Diagram (1)** and has a molecular weight of 162.144g/mol, melting point: 230-233^{0}C. The dimensions of a single crystal grown by the cryostat process are 5.4 mm X 4.2 mm X1.85 mm. It absorbs ultraviolet light strongly at several wavelengths **(29)**.

UMB largely present in fruits and roots plants, such as, apple, the bitter orange, and carrot possessing several pharmacological activities, including antioxidant with free radical scavenging properties, anti-inflammatory, antidiabetic, and antiapoptotic effects **(27, 30)**. Its molecular formula is C9H6O3 as shown in **Diagram (1)**.
It is a yellowish-white, crystalline bioactive molecule has a slight solubility in hot water, but high solubility in ethanol, dioxane **(29)**.

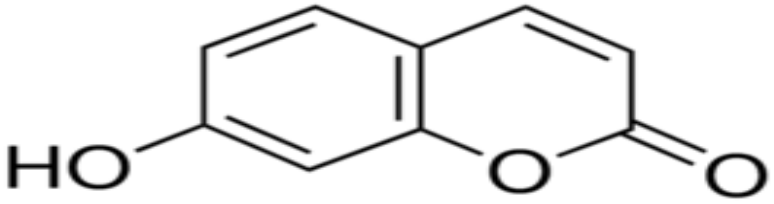

Diagram (1): Chemical Structure of UMB **(29)**.

Oxidative stress occurs when there is an imbalance between the production of free radicals and antioxidant enzymes. This imbalance caused by an excessive production of reactive oxygen species or by a decrease in antioxidant

capacities **(31)**. Among the reactive oxygen species (ROS), the peroxyl radicals (ROO•) are considered to be a highly toxic species due to their ability to initiate a whole cascade of radical reactions from other organic structures **(32)**. Inhibition of these radical species breaks the oxidation chain, or at least delays it, and therefore reduces oxidative stress. However, peroxyl radicals are not very reactive species and few antioxidants can effectively inhibit them **(33)**.

At the molecular Level, the free radical scavenging activity of antioxidants is complex and may be mediated by one or more of the following mechanisms: formal hydrogen transfer (FHT), radical adduct formation (RAF), sequential proton loss electron transfer (SPLET), and sequential electron transfer proton transfer (SETPT) **(34, 35)**. In all of these mechanisms, the antioxidant turns into a radical form that is much more stable than ROS and, in principle, harmless to biological systems. **(36, 37)**.

The antioxidant and radical scavenging activity of UMB have been proved in several studies by its ability to reduce reactive oxygen species (ROS) generation and increase the expression of antioxidant enzymes. The antioxidant activity of UMB is related to its direct scavenging of free radicals, up-regulation of superoxide dismutase (SOD) and enhancement nuclear factor erythroid 2-related factor2 (Nrf-2) activity **(38)**. Moreover, UMB inhibits lipid peroxidation and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway and the ability of to suppress the increment of pro-inflammatory cytokines such as IL-6 and TNF-α **(39, 40).**

Previous studies reported the beneficial effects of UMB in different experimental models such as protective effects against cerebral ischemia-reperfusion induced damage **(30)**, myocardial infarction induced isoproterenol **(41)**, hepatotoxicity induced by N-nitrosodiethylamine **(42)**,cyclophosphamide-induced liver damage **(38)**, and renal injury in type I diabetes **(43)**.

Alotaibi et al (44) and Hassanein et al (45) claimed that UMB may act locally within the testes to improve the spermatogenesis or centrally to regulate the pituitary-gonadal axis. Moreover, it is proved the effects of pre-treatment with UMB in a rat model of testicular torsion. We found for the first time that UMB pre-treatment protected testicular tissue against ischemia/reperfusion injury through attenuation of oxidative stress and potentiating the antioxidant defenses **(46)**. However, the effect of UMB upon the testis in psychotic patients treated by antipsychotic drugs has not been investigated.

Proliferating cell nuclear antigen (PCNA) is an evolutionarily well-conserved protein found in all eukaryotic species. PCNA is a nuclear protein, which related to cell proliferation, and can be measured by a variety of antibodies **(47)**.

PCNA is not cell cycle-specific and it functions as a cofactor for DNA polymerase-δ, which reaches its maximal synthesis during the S phase and during DNA synthesis associated with DNA damage-repair mechanisms. However, besides DNA replication, PCNA functions are associated with other vital cellular processes such as chromatin remodeling, DNA repair, sister-chromatid cohesion and cell cycle control **(48)**.

CD34 has been used as a marker for identification and purification of primitive hematopoietic cells **(49)**. In this sense, CD34 antibodies are regularly used to identify and isolate hematopoietic stem cells (HSCs) for bone marrow transplant. Expressed in mice, humans, rats and other species, CD34 has been used for more than 40 years as a hematopoietic stem and progenitor cell marker. It was later found that muscle satellite cells and epidermal precursors can also be identified with the aid of CD34. It was first discovered on human hematopoietic progenitor and stem cells **(50, 51)**.

These cells are found in low frequencies in the peripheral blood and their frequency can be increased by chemotherapy as well as cytokines **(52)**. Other than the blood, CD34 is found on vascular endothelial cells, on the luminal surfaces, on muscle satellite cells, keratocytes, interstitial cells, fibrocytes **(53-56).**

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