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Review Article

Growth Factor as a Supportive Treatment in Chemotherapy

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Abstract

Even after a lot of advancements have been made in the treatment patterns of the various malignancies, there are still a number of dose-limiting toxicities associated with chemotherapy. Most of these toxicities are of hematological nature. Hence, there is always a need of supportive care treatment to assist these cytotoxic drugs. The main aim of supportive care is not only to relieve the adverse effects but also to improve the living quality of the sufferer. Hence, the pharmacologic agents that can readily improve the recovery of hematological functions can be the potential candidate for supportive care treatment. Growth factors are peptides having low molecular weight and are found to be active in the stimulation of cell proliferation, regulation of embryonic development and cellular differentiation. These small molecules are available as a supportive treatment to control and decrease the cytopenia, resultant from toxicity of chemotherapeutic treatment on bone marrow of cancer patients. These growth factors may include GM-CSF, G-CSF, and erythropoietin. All these growth factors can be utilized as a supportive care treatment in different diseases that may be induced after chemotherapy or after transplantations. The complete indications and the applications of these therapeutic agents as the supportive care treatment have been discussed in this article.

Keywords: Growth Factors; Hematopoiesis; G-CSF; Erythropoietin; Chemotherapy

Abbreviations:

ALL: Acute Lymphoblastic Leukemia;

AML: Acute Myeloid Leukemia;

ANC: Absolute Neutrophil Count;

BMP: Bone Morphogenetic Protein;

CFU-E: Colony Forming Unit-Erythroid;

CTGF: Connective Tissue Growth Factor;

EPO: Erythropoietin;

EPOR: Erythropoietin Receptor;

ERK: Extracellular Signal Related Kinases;

FGF: Fibroblast Growth Factor;

G-CSF: Granulocyte Colony Stimulating Factor;

GDF: Growth and Differentiation Protein;

GM-CSF: Granulocyte–Macrophage Colony-Stimulating Factor;

HB-EGF: Heparin Binding Epidermal Growth Factor;

Hb: Hemoglobin;

HGF: Hepatocyte Growth Factor;

IGF:Insulin-like Growth Factor;

IL-3: Interleukin-3;

JAK2: Janus-kinase-2;

MAPK: Mitogen-Activated Protein Kinases;

M-CSF: Macrophage Colony Stimulating Factor;

PDGF: Platelet-Derived Growth Factor;

PI3K: Phosphoinositide 3-Kinase;

RBC: Red Blood Cells;

rDNA: Recombinant DNA;

TGF-β: Transforming growth factor- β;

VD3: 1,25-dihydroxyvitamin D-3 Cholecalciferol;

VEGF: Vascular Endothelial Growth Factor

Introduction

The research on the growth factors and their mechanism of action has got much attention in the recent years. The reason for this attention is due to the involvement of the growth factors along with its downstream elements in the development of therapeutic modalities of various growth factors. Such factors are reported to be controlling almost all the phases of tumorigenesis. They have the potential to not only alter the process of proliferation, but also impact the motility of cell, differentiation and development of metstatic tumoral lesions [1]. With the advancements in the field of oncology, a number of cases of cancer are cured while others get relief from the painful symptoms to varying degrees. It becomes the responsibility of physician to comfort the patient to the maximum possible extent. Hence, the field of supportive oncology is growing, which provides not only the symptomatic relief, but also the palliative care to the patients.

The aim of supportive care is to improve the quality of living of the patient along with the survival, when given with the standard protocol based regimen. The availability of growth factors has an impact on patient care by making it possible to administer chemotherapy doses on schedule or at higher doses, [2] improving the recovery of cell counts after bone marrow or stem cell transplantation and [3] improving the ability to collect stem cells for transplantation and fight with infections [4].

Growth factors

Growth factors may be defined as a class of proteins that act as signaling agents inside the body. These proteins are generally secreted by the cells and act on a specific target cell or a group of cells. The action of these factors on the target cells can be of three types:

1) Autocrine: These factors that act on the cell, from which it has originated or phenotypically-similar cells,

2) Paracrine: The factors act on the cells in the vicinity that are phenotypically-different.

3) Endocrine: The factors that act on the phenotypically-different cell present out of the local region of these factors [5,6].

Hence, it can be said that growth factors exhibit their effects on various types of cells and initiate a cascade of cellular functions in different types of tissues. Growth factors form an important part of a broad communication network in the cells, which regulate some of the major functions like cell division as well as differentiation.

Mechanism of Action

On adhering to the receptor of the target cell, a signal is intracellularly transduced, that finally reaches the nucleus and initiates a biological response. The process of adhering of growth factor to a specific target receptor is widely defined as ligand-receptor interaction. The interactions may range from binding to a single ligand to that of the multiple ligands. Such interactions result in ligand-receptor effect. The most unique feature of these interactions is that different subtypes of a growth factor bind only to one receptor or various subtypes are activated with the help of a single ligand [7].

After this ligand-receptor interaction, there is a conformational change in the receptor, which activates it. The receptor consists of both extracellular and intracellular domains. The

extracellular domain interacts with the ligand, while the intracellular domain adheres to and results in the activation of signal transduction system. The transcription factor, which is an intracellularly located protein is activated by these signaling pathways and forms a component of transduction system. This transcription factor, on activation reaches the nucleus, adheres to the nuclear DNA, after which a new set of genes or a single gene is expressed [8,9]. These newly expressed genes are responsible for the alteration in the characteristics of the cell.

Types of Growth Factors

Growth factors are distinguished into two different families as shown in Figure 1:

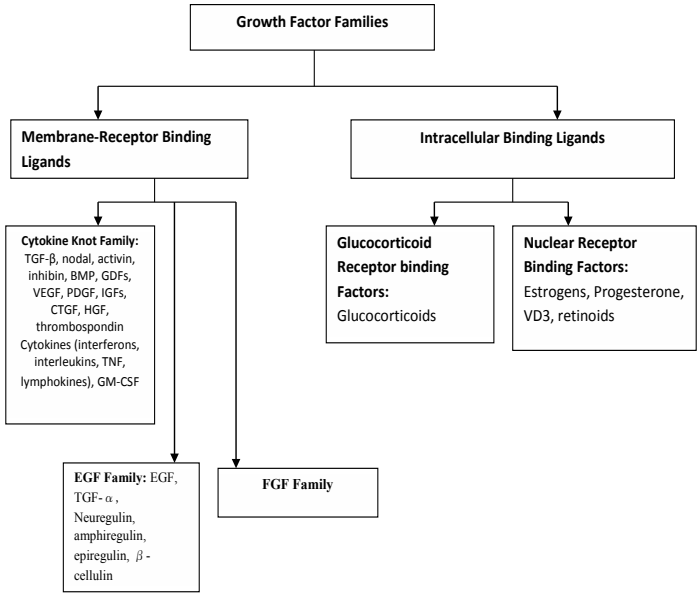

Figure 1. Different families of growth factors and their members [10].

Membrane Receptor Binding Growth Factors

Cystine-Knot Growth Factors

Cystine-knot proteins are the proteins of small size and comprise of around 30 amino-acid based residues, and has a particular tertiary fold [11,12]. Various growth factors like TGF-β, PDGF, G-CSF, and GM-CSF belong to this category [13].

The TGF-β group of family proteins acts by activating some particular membrane receptors, and then initiating a cascade of Smad signaling pathway. Another group of cystine knot proteins include cytokines and some hematopoietic growth factors. They are responsible for immune-cells' proliferation and differentiation along with hematopoiesis. The most notable examples in this group are interleukins, interferons, G-CSF and GM-CSF [10].

EGF Family

The growth factors in this family are responsible for the process of cell proliferation, apoptosis, tumorigenesis, and cell differentiation. The ligands belonging to this category include: EGF, TGF-α, epiregulin, amphiregulin, neuregulins and HB-EGF.

Fibroblast Growth Factor Family

This family consists of around 20 growth factors that are structurally related [14, 15]. Out of these 20 factors, FGFs 1-10 bind to their own receptors (FGFRs) and are paracrine in nature, while those from 11-14 have donot bind to their receptors and possess different functions [16,17]. FGFs 15, 19 and 21 have endocrine-based functions.

Intracellular Binding Ligands

The growth factors of this subfamily are characteristic of adhering to and functioning through intracellular receptors. The ligands of this class are distinguished as: (1) nuclear receptor binding growth factors, such as estrogen, progesterone, retinoids, and VD3, and (2) cytoplasmic receptor binding ligands such as glucocorticoids [10].

In the supportive care treatment, the hematopoietic growth factors are widely used and hence, discussed in detail with their applications in this article.

Hematopoietic Growth Factors

Hematopoietic growth factors belong to the family of cytokines and are associated with some particular receptors present on the hematopoietic cells [18,19]. These factors are responsible for controlling the activation of functionalities of some cells, in particular. These are the cells that come in contact with these factors and are vital for the cellular proliferation and cellular differentiation of progenitors of the process of hematopoiesis [18]. Various hematopoietic factors that have been identified till now include G-CSF, GM-CSF, M-CSF, EPO, IL-3, and thromobopoietin.

The Process of Hematopoiesis

Hematopoietic system acts in the form of hierarchy, where the multipotent stem cells arise from the pluripotent cells and gradually convert into mature hematopoietic cells that are non-replicative in nature. The cells present in the initial stages are known as stem cells, as they have the potential to differentiate and produce cells further, that progress and differentiate across all the lineages. The progenitors of the initial stages involve certain cytokine receptors and with the progress in the stages, they lose the potential to respond to some of the growth factors. Some of the important sources of these factors are fibroblasts, lymphocytes, monocytes, endothelial cells, and macrophages [20-22].

Growth factors like GM-CSF have a broader range of action on initial progenitors, which results in differentiation and generation of hematopoietic cells through various lineages. However, other factor like G-CSF usually acts on the later stage cell type like neutrophils, and presents fairly concessive alterations in them.

GM-CSF

This factor originates from endothelial cells, fibroblasts, and monocytes [22-24]. It functions as the regulator of neutrophil proliferation and differentiation [25-27. It enhances the neutrophil functioning and survival of these cells [26,28].

When the GM-CSF binds to its receptors of heterodimeric nature like α-subunits, that are specific to cytokines and IL-3, IL-5 associated βc-subunit, [29] the receptors are aggregated in the dodecamer quartenary structural form. This complex is vital for the receptor activation and initation of signaling [30].

The general side effects associated with GM-CSF includes: pain in the bone, increased levels of alkaline phosphatase. The levels of urate, and lactate dehydrogenase are also potentiated. The route of administration of GM-CSF is subcutaneous.

G-CSF

Granulocyte-stimulating factor is also an important growth factor, which is involved in myeloid progenitor differentiation, proliferation and survival of neutrophil and granulocyte based lineages. It originates from the similar sources to that of GM-CSF. It is a glycoprotein of 19kDa, and is found to be active intracellularly. The activated G-CSF is the monomeric form.

G-CSF exhibits a high affinity for G-CSFR, its own receptor. It is a transmembrane molecule having general structural features with an extracellular domain for ligand-binding, a transmebrane domain, and a domain in the cytoplasm. On adhering to this receptor, G-CSF is activated and interacts with JAK2 and Lyn kinase of Src family. It results in the activation of downstream pathway of STATs. The adaptor molecules are phosphorylated by Lyn kinase, which results in interaction of G-CSFR with signaling pathways like PI3K and MAPK/ERK [31,32].

The side effects of G-CSF include fever, chills, musculoskeletal disorders, hypersensitivity, and malaise. Other clinical manifestations might include effusions from pleural cavity and pericardium, increased weight, edema, and capillary leak syndrome. The route of administration of G-CSF is subcutaneous.

Erythropoietin

It is a 34kDa glycoprotein, which constitutes of chains of carbohydrates and residues of sialic acid. These components form a vital part of the glycoprotein and regulate its production, secretion and biological function. The cysteine residues consist of a disulfide bridge in between them, which is pivotal for its function. In fetal stage, this glycoprotein is produced from liver, while in adults, cells of type I renal peritubular region of the renal cortical interstitia in 80% of the adults. In rest of them, it is obtained from hepatic stellate cells of the liver. The chromosomal location 7q11-q22 at chromosome 7 encodes for EPO [33].

The functions of EPO include erythropoiesis, apoptotic inhibition of the progenitor cells of erythrocytes in the bone marrow as well as cell in the neuron [34,35].

The receptor of EPO is EPOR, which is expressed for a specific period from the CFU-E stage to that of pro-erythroblast stage of erythropoiesis. These receptors are less frequently observed with the gradual development of erythroid cells.

The EPO binds to EPOR and hence, enhances cell proliferation, cell survival and differentiation [36, 37]. On adhering to the progenitor cells, it upregulates the EPOR expression, which as a reflex response and increases the EPO response. Along with this, it also enhances the transcription factor expression, which is specific to the process of erythropoiesis [38]. At later stages, this expression is downregulated and there is no further need of EPO to regulate cell survival [39]. Later these precursors produce reticulocytes, which further synthesize hemoglobin. These cells then enter the general circulation and develop into erythrocytes.

Role of Growth Factors as Supportive Treatment in Chemotherapy

Due to the hematopoietic toxicities induced by the chemotherapy regimens, there has always been a constraint to design a safer treatment regimen. Growth factors have emerged as a new class of agents that can help in the accelerated recovery from the hematopoietic side effects of chemotherapy.

Role of G-CSF as a supportive treatment [40]

G-CSF along with its pegylated form (pegfilgrastim) is indicated in the prophylaxis of neutropenia associated with chemotherapy, only if the risk associated with FN increases more than 20%. Figure-2 represents the algorithm for primary prophylaxis of G-CSF.

Dose and route of administration

G-CSF is given subcutaneously as 5μg/kg/day after 24-72 hours have passed since the last chemotherapy until a stable ANC has been achieved.

Pegfilgrastim is given subcutaneously as 100 μg/kg of single dose or an overall dose of 6mg.

Indications

Autologous stem-cell Transplantation

Marrow Transplantation**:**

Initiate G-CSF. The parameters affected by the G-CSF are ANC, fever, and infection. However the infectious mortality as well as the overall survival remains unaffected. The dose recommended for G-CSF is 5μg/kg daily. The regimen can be started after 5-7 days of transplantation.

Peripheral blood stem cell (PBSC) transplantation

The parameters that are affected include fever. ANC is accelerated for a short period, but lacks consistency. Infectious mortality and overall survival remains unaffected.

Allogeneic Marrow transplant

The only parameter affected is recovery of ANC. Data is inconsistent for allo-PBSC. The regimen can be started after 5-7 days of transplantation.

Autologous PBSC

The use of G-CSF along with chemotherapy or without chemotherapy is effective. The dose recommended for G-CSF is 10μg/kg daily. The dose has to be continued for 7 to 10 days before apheresis. The ANC recovery is quite high in this case.

Allogeneic PBSC

The use of G-CSF along with chemotherapy or without chemotherapy is effective. The dose recommended for G-CSF is 10μg/kg daily. The dose has to be continued for 7 to 10 days before apheresis. The ANC recovery is quite high in this case.

AML

The parameters affected include ANC. However, infectious mortality and overall survival remains unaffected.

ALL

The parameters affected include ANC. The parameters that remain unaffected include severe infections, mortality due to infections, hospitalization, and survival. In childhood ALL, an increase in the incidence of secondary leukaemia have been reported after G-CSF administration.

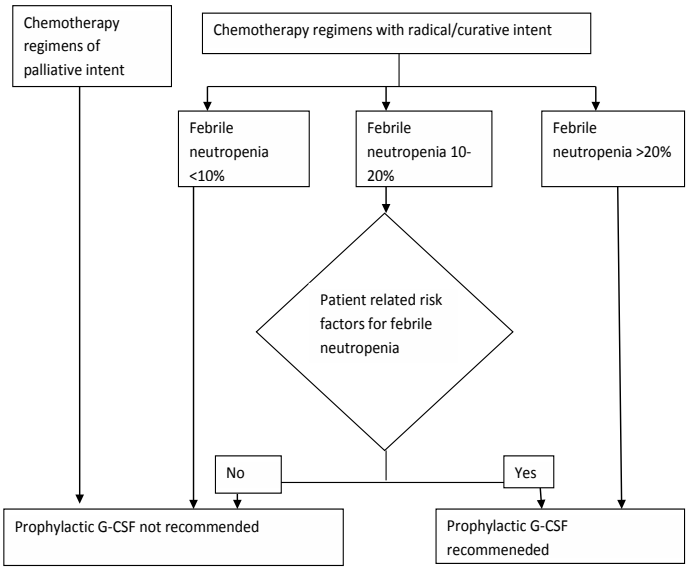

Figure 2. Algorithm for primary prophylaxis of G-CSF [41].

Role of GM-CSF

Sargramostim

Sargramostim is a kind of GM-CSF, which is produced by rDNA technology from the expression system of a yeast, *Saccharomyces Cerevisisae.* It is reported to enhance the rate of recovery of neutrophil after chemotherapy as a supportive therapy for the neutropenia caused by the cytotoxic therapy [42,43].

Sargamostim is utilized as a prophylactic agent after the treatment with chemotherapy. It is indicated in patients with AML, or after bone marrow transplantation. This can be either autologous or allogeneic.

Dose and route of administration

On the day of bone marrow transfusion, GM-CSF should be given, not before 24 hours from the last regimen of chemotherapy and 12 hours from the last radiotherapy regimen. GM-CSF is recommended to be continued until the ANC count sustains at or greater than 1500 cells/μl. This count needs to be maintained for three consecutive days. In case, the ANC count reaches up to or more than 20,000 μl, the GM-CSF dose should be decreased by 50% or can be discontinued. The most widely used GM-CSF dose is 250μg/m^2/day, given subcutaneously, for most of the clinical conditions.

Molgramostim

It is a non-glycosylated GM-CSF, which is indicated in the patients having chemotherapy-induced neutropenia, and hence decreasing the infection-risk and improving the tolerability of the chemotherapeutic regimen. It is also indicated in the pa-

tients that have to undergo bone marrow transplantation in order to enhance the recovery of myeloid.

Dose and route of administration

The dose of molgramostim in cancer chemotherapy is 5-10μg/kg/day, given subcutaneously after 24 hours of the last chemotherapy regimen. This regimen is given for 7-10 days. For bone marrow transplantation, the dose is 10μg/kg/day, given by i.v infusion for a time period of 4-6 hours. This regimen is initiated next day after transplantation and is continued until the ANC count becomes more than 1000/μl. This regimen is given for 30 days [44].

Erythropoietin [45]

EPO is a hormone of glycoprotein nature which is crucial for erythropoiesis regulation. It is usually indicated as a support therapy for the treatment or prevention of anemia induced by chemotherapy in the treatment of malignancies of non-myeloid nature. It is also indicated to reduce the transfusion requirements in patients that will undergo chemotherapy regimens for almost 2 months.

In a study conducted on 100 patients having cisplatin induced anemia, they were subjected to EPO or placebo based treatments. There was an appreciable improvement in the levels of Hb in the EPO group as compared to the placebo. Only 20% of the patients undergoing EPO treatment required transfusions. The data was 56% in the placebo group.

In another study conducted on patients with solid tumors treated with chemotherapy and having chemotherapy –induced anemia, 17 patients were placed in the EPO group while 17 in the placebo group. The dose of EPO was 150 units/kg which had to be administered three times a week. Transfusions were required in only one patient in the EPO group, while 8 patients required transfusions in the placebo group.

Dose and administration

1. Initial Dosing

The dosing of various forms of erythropoietin in chemotherapy-induced anemia are given below. Any one of the following regimens can be followed.

a. Epoietin alfa given as 150 units/kg, 3 times a week via subcutaneous route. The dose can be increased upto 300 units/kg, 3 times a week via subcutaneous route.

b. Epoietin alfa given as 40,000 units/week via subcutaneous route. The dose can be increased upto 60,000 units/kg,/week via subcutaneous route.

c. Darbepoetin alfa given as 2.25μg/kg/week via subcutaneous route. The dose can be increased upto 4.5 μg/kg,/week via subcutaneous route.

d. Darbepoetin alfa given as 500μg/kg every 3 weeks via subcutaneous route.

2. Alternative regimen

Any one of the following alternative regimens can be followed.

a. Darbepoetin alfa given as a fixed dose of 100μg/week via subcutaneous route. The dose can be increased upto 150-200μg/week as a fixed dose via subcutaneous route.

b. Darbepoetin alfa given as a fixed dose of 200μg every 2 weeks via subcutaneous route. The dose can be increased upto 300 μg, every 2 weeks as a fixed dose via subcutaneous route.

c. Darbepoetin alfa given as a fixed dose of 300μg every 3 weeks via subcutaneous route. The dose can be increased upto 500 μg, every 3 weeks as a fixed dose via subcutaneous route.

d. Epoietin alfa given as 80,000 units every 2 weeks via subcutaneous route.

e. Epoietin alfa given as 120,000 units every 3 weeks via subcutaneous route.

For EPO based regimens, the dose adjustment varies from patient to patient and should be maintained at the lowest possible Hb level, which is enough to avoid the transfusion of RBC. In case, the increase in Hb level is more than 1g/dl in a period of 2 weeks, the dose should be decreased by 25-50%.

Conclusion

From the last decade, the hematopoietic growth factors have opened a number of opportunities in the study of science and investigation. The growth factors that are used for the supportive care treatment includes G-CSF, GM-CSF, and erythropoietin. The pegylated form of G-CSF, pegfilgrastim has been used widely in the treatment of neutropenia induced by chemotherapy. GM-CSF has been used. The two forms of GM-CSF, Sargramostim, and Molgramostim is indicated in the treatment of neutropenia induced by chemotherapy for the treatment of AML and also in complications associated with bone marrow transplantation. It is usually indicated as a support therapy for the treatment or prevention of anemia induced by chemotherapy in the treatment of malignancies of non-myeloid nature. Hence, the growth factors can act as an appreciable supportive treatment for chemotherapy. This will help to limit the toxicities of chemotherapy along with improvement in the quality of living.

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