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Research Article

Pre Clinical Toxicity Evaluation of Recombinant Pegylated Granulocyte Colony Stimulating Factor (Rh PEG-GCSF)

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ABSTRACT

Recombinant protein technology produces drugs for human therapy in unprecedented quantity and quality. Pegfilgrastim is in a class of medications called colony stimulating factors. It works by helping the body make more neutrophils.

The aim of the present study is to carry preclinical toxicity study on Acute toxicity and Skin sensitization study by using recombinant Pegylated Granulocyte Colony Stimulating Factor in rats and mice-Acute toxicity study and Guine pigs – skin sensitization test. The objective is to assess the safety profile of rh PEG-GCSF in Swiss albino mice and Sprague Dawley rats and guinea pigs for the treatment of cancer based on the guidelines of EMEA & DBT.

In the present study the preclinical toxicity study is carried by conducting Acute Toxicity Study & Guinea Pig Skin Maximisation Test.

There was no mortality or morbidity observed during the 14 days study period. No toxic signs and abnormal behaviour was observed in the Group III (Intravenous) and Group IV (subcutaneous) animals, which were exposed to the test compound at 10 times of intended therapeutic dose.

There was no mortality or morbidity observed during the 14 days study period. No toxic signs and abnormal behaviour was observed in the Group I (Intravenous) and Group II (subcutaneous) animals, which were exposed to the test compound at 10 times of intended therapeutic dose.

On the basis of the results, interpreted according to GPMT Magnusson/Kligman and OECD TG 406, the skin sensitization test in guinea pigs, the substance "rh G-CSF" –pegfilgrastim must be considered as non sensitizer for skin.

Key-words: Recombinant protein technology, Pegfilgrastim, Pegylated, Acute Toxicity Study, Guinea Pig Skin Maximisation Test.

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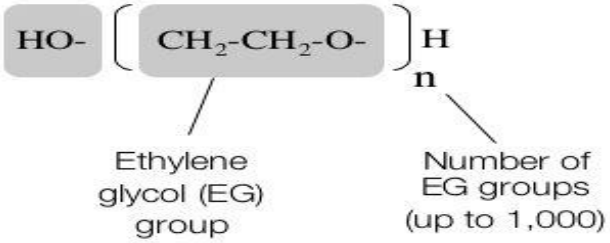
INTRODUCTION:-

Recombinant protein technology produces drugs for human therapy in unprecedented quantity and quality. Research is now focusing on the relationship between pharmacokinetic and pharmacodynamic properties of molecules, with the aim of engineering proteins that possess enhanced therapeutic characteristics in contrast to being used as simple replacements for the natural equivalent. The addition of a polyethylene glycol (PEG) moiety to filgrastim resulted in the development of pegfilgrastim. Granulocyte colony-stimulating factor (G-CSF) is known for its role in regulating granulocytopoiesis

Pegfilgrastim is used to reduce the chance of infection in people who have certain types of cancer and are receiving chemotherapy medications that may decrease the number of neutrophils (a type of blood cell needed to fight infection). Pegfilgrastim is in a class of medications called colony stimulating factors. It works by helping the body make more neutrophils.

Biochemical nature of drug



Improve stability and solubility

- Reduce immunogenicity and proteolysis
- Slow clearance from the body, less frequent dosing
- Improve clinical effects
- Prolong patent protection
- More competitive in market with an increase commercial opportunity
- PEGylated proteins are marketed since 1990

PEGfilgrastim:

- Has no toxicity problems
- Has extended half-life
- Produces neutrophilia in normal mice, rats, and primates
- Reverses therapy-induced neutropenia in mice and primates
- Has a “self-regulating” feature

Pegfilgrstim is a glycoprotein molecule, Polyethylene glycol (PEG) conjugation to proteins has emerged as an important technology to produce drug molecules with sustained duration in the body. PEG is a water-soluble, biocompatible polymer that is commonly utilized as an additive in protein formulations, and to facilitate crystallization of proteins (Cleland and Jones 1996; Kerwin *et al.* 2002). Historically, conjugation of protein therapeutics with PEG has been performed to improve the half-life of the protein in the blood serum because the large size of the PEG-conjugated molecule slows down renal clearance (Molineux 2002). PEG conjugation has also been used in protein encapsulation systems, where it improved the encapsulation efficiency and lowered the initial rate of release (Hora *et al.* 1990; Al-Azzam *et al.* 2005). However, there was no systematic correlation between the extent of protein aggregation and improvements in the above processes. It has been previously documented that PEG conjugation retards protein precipitation from the liquid state (Katre 1990; Kim and Park 2001);

The effects of PEG conjugation to the aggregation properties of a therapeutic protein, granulocyte colony stimulating factor (GCSF) have been investigated. GCSF is a ~18 kDa four-helix bundle protein that belongs to the family of hematopoietic cytokines (Hill *et al.* 1993; Chaiken and Williams 1996; Kolvenbach et al. 1997; Brems 2002; Raso *et al.* 2005). It regulates the growth and differentiation of hematopoietic progenitor cells to functionally activate the formation of mature neutrophils.

PEGylated Drugs in the Market

Present Study

In the present study the preclinical toxicity study is carried by conducting:

(i) ACUTE TOXICITY STUDY:

Acute toxicity describes the adverse effects of a substance that result either from a single exposure or from multiple exposures in a short space of time (usually less than 24 hours). To be described as acute toxicity, the adverse effects should occur within 14 days of the administration of the substance.

In most acute (short-term) toxicity test, a single dose of a test substance is given to an animal. One measure of acute toxicity is the lethal dose 50 (LD50), or the dose of a substance that kills 50 percent of the animals tested. The acute toxicity study of pegfilgrastim is carried in swiss albino mice and sprague dawley rats by both intravenous and subcutaneous routes – this study is carried for 14 days

(ii) GUINEA PIG SKIN MAXIMISATION TEST:

Skin sensitization resulting in allergic contact dermatitis is a common occupational and environmental health issue. Many hundreds of chemicals have been implicated as skin sensitizers, and allergic contact dermatitis is without doubt the most common manifestation of immunotoxicity in humans. It is important need for the accurate identification and characterization of chemicals that have the potential to cause skin sensitization. Initially guinea pig methods were favoured for the purposes of hazard identification, the most widely applied being the Guinea Pig Maximization Test

The Guinea pig skin maximisation test (GPMT) in this the animals are exposed intradermally to the test material, along with an adjuvant to enhance the immune reaction of the guinea pig. The guinea pigs are then a short while later exposed to a lower concentration of the test material, and their allergic reaction, if any of rh Peg-GCSF. The skin sensitization test of pegfilgrastim is carried out in guinea pigs through intradermal route – this study is carried for 50 days

Human granulocyte colony-stimulating factor (G-CSF) is produced by recombinant DNA technology. In comparison with natural products, its bioactivity is similar in vivo & in vitro. RHuG-CSF is one of the main cytokines modulating the granulocytic hematopoiesis in bone marrow. It acts on the hematologic progenitor cells of granulocyte selectively, promoting its proliferation and differentiation. It enhances the function and counts of granulocyte in peripheral blood as well.

In vitro, G-CSF stimulates growth, differentiation and functions of cells from the neutrophil lineage. It also has blast cell growth factor activity and can synergize with IL-3 to shorten the Go period of early hematopoietic progenitors. Consistent with its in vitro functions, G-CSF has been found to play important roles in defense against infection, in inflammation and repair, and in the maintenance of steady state hematopoiesis. PEGylated GCSF is a prescription medication called a white cell booster that helps the body to produce more white blood cells to reduce the risk of infection.

This present study is taken up to develop one's own immune system , and avoid the infection to the body after the drug administration .The pegfilgrastim is a white cell booster , helps to fight against the infection ,repair the body , maintain the steady state level of the blood cells

Bioviz Technologies Private Limited has recently developed GCSF and PEGylated GCSF using recombinant DNA technology with an intention to promote it for treatment of cancer. So it becomes mandatory to undertake its safety evaluation as per the guidelines of DBT and Schedule Y of DCGI for recombinant products.

Study & Observation:-

Test compound:

Recombinant PEG-GCSF internally coded as BV02 is produced by recombinant DNA technology. Filgrastim is produced by Escherichia coli (E. coli) bacteria into which has been inserted the human granulocyte colony stimulating factor gene. The protein comprising 175 amino acids has an amino acid sequence that is identical to the natural sequence predicted from human DNA sequence analysis, except for the addition of a methionine at the N-terminus.

Formulation details:
(i) Form: Prefilled syringe
(ii) Strength: 0.3mg / PFS (0.3mg/ml)
(iii) Dose: Recommended clinical dose 0.3mg of drug (Until the ANC reached 10,000/mm^3).
(iv) Vehicle: Water for Injection
(v) Route of Administration : Subcutaneous, Intravenous
(vi) Stability: The product is stable for up to 2 yrs

i) ACUTE TOXICITY STUDY:

Test details: The acute toxicity tests have been undertaken with single exposure of test compound by intravenous and subcutaneous route in 10 times of intended recommended therapeutic concentration, the study is carried for 14 days.

Methodology: All the animals were observed for activity and lethality in addition to bi–weekly monitoring of live phase, cage side and physical observations. All the animals were euthanized on 15th day of post exposure i.e., after test compound exposure.

Experimental methodology:

i) Test system & Strain Mice (Swiss albino), Rats (Sprague dawley)
ii) No. of animals
Mice: 28 (14♂ + 14♀)
Rats: 28 (14♂ + 14♀)
iii) Dose level High dose (10 times of intended therapeutic dose)
iv) Routes of administration Intravenous and Subcutaneous

Swiss albino mice:

GROUP I: The safety of test compound was determined in 10 (5M+5F) mice aged 4-6 weeks and weighing 20-25 gms after a single exposure of 10 times of recommended therapeutic dose. Water for Injection was administered to vehicle control group animals (2M + 2F). Route of administration is intravenous (IV).

GROUP II: The safety of test compound was determined in 10 (5M+5F) mice aged 4-6 weeks and weighing 20-25 gms after a single exposure of 10 times of recommended therapeutic dose. Water for Injection was administered to vehicle control group animals (2M + 2F). Route of administration is subcutaneous (SC).

In Sprague dawley rats:

Experimental details:

GROUP III: The safety of test compound was determined in 10 (5M+5F) rats aged 6-8 weeks and weighing 200 – 220 gms after a single exposure of 10 times of recommended therapeutic dose. Water for Injection was administered to vehicle control group animals (2M + 2F). Route of administration is intravenous (IV).

GROUP IV: The safety of test compound was determined in 10 (5M+5F) rats aged 6-8 weeks and weighing 200 – 220 gms after a single exposure of 10 times of recommended therapeutic dose. Water for Injection was administered to vehicle control group animals (2M + 2F). Route of administration is subcutaneous (SC).

Experimental design:

Group	Targeted dose levels	ROA	Dose (mcg /Kg)	Test species	No. of animals	Study duration @ (Days)

I	HD (10 X TD)	IV	390	Mice	5M+5F	14
II		SC	390	Mice	5M+5F	
III		IV	270	Rats	5M+5F	
IV		SC	270	Rats	5M+5F	
VEHICLE CONTROL						
I	VC	IV	-	Mice	2M+2F	14
II		SC	-	Mice	2M+2F	
III		IV	-	Rats	2M+2F	
IV		SC	-	Rats	2M+2F	

Duration of exposure: Single exposure, ROA: Route of administration

Experimental procedure:

Mice: Two groups of five males and five females of Swiss albino mice were selected. Another two groups of two males and two females were selected for Vehicle control groups. Animals were conditioned for one week. Before test compound administration, mice were placed in a restrainer. Group I received a single intravenous injection of high dose (HD) before IV administration, animal tail was warmed to dilate the veins and make the procedure easier and Group II received a single subcutaneous injection of high dose (HD). Volume of administration was according to the body weight. Mice were observed for morbidity, mortality over a period of 14 days.

Rats: Two groups of five males and five females of Sprague dawley rats were selected. Another two groups of two males and two females were selected for Vehicle control groups. Animals were conditioned for one week. Before test compound administration, mice were placed in a restrainer. Group III received a single intravenous injection of high dose (HD) before IV administration, animal tail was warmed to dilate the veins and make the procedure easier and Group IV received a single subcutaneous injection of high dose (HD). Volume of administration was according to the body weight. Rats were observed for morbidity, mortality over the period of 14 days.

II) Skin sensitization test in Guinea pigs: (GUINEA PIG MAXIMIZATION TEST)

Recombinant PEG-GCSF internally coded as BV02 is produced by recombinant DNA technology. Filgrastim is produced by Escherichia coli (E. coli) bacteria into which has been inserted the human granulocyte colony stimulating factor gene. The protein comprising 175 amino acids has an amino acid sequence that is identical to the natural sequence predicted from human DNA sequence analysis, except for the addition of a methionine at the N-terminus.

Formulation details:

(i) Form : Prefilled syringe
(ii) Strength : 0.3mg / PFS (0.3mg/ml)
(iii) Dose : **Recommended clinical dose 0.3mg**
(iv) Vehicle : Water for Injection
(v) Stability : The product is stable for up to 2 yrs

Principle of the test method: The test animals will be initially exposed to the test substance by intradermal injections (Induction exposure). Following a rest period of 10 to 14 days (Induction period), during which an immune response may develop, the animals are exposed to a challenge dose. The extent and degree of skin reaction to the challenge exposure in the test animals is compared with that demonstrated by control animals, which undergo sham treatment during induction and receive the challenge exposure.

EXPERIMENTAL METHODOLOGY

TEST METHOD

Species : Albino Guinea pigs
Strain : NIH Hartley
No. : 20
Sex : 10M+10F
Weight : 250-350 (gm) at the beginning of the test

Guinea pigs were caged in polypropylene cages. 3 animals were accommodated per group. Each group was caged in a cage. Room temperature and humidity were regulated by a conditioning plant and were monitored daily. Recordings of the housing conditions are being retained in Virchow biotech PCT files. The cages and the housing room were cleaned before the animals were accommodated, then cleaning and disinfecting were performed periodically. The cages and water bottles will be cleaned with hot water, dried and then sterilized. The animals were fed with standard pellet complete diet supplied by Sainath agencies. Filtered tap water was supplied ad libitum. Ear punching will individually identify each animal. Each cage will be identified with cage label showing Unique ID No. of the animal it contains, Study No, Test Substance Code, Group No, Dose, Cage No and Animal No. Before being used in this study, the animals were kept in quarantine for one week. During this period they were observed daily. At the end of the quarantine week, the animals were evaluated for their suitability in the study.

Test groups:

I. Treatment group : 5 males and 5 females
II. Control group : 5 males and 5 females

Materials:

The following material was provided for this study and prepared as indicated below:

i) Test Article : rh PEG-GCSF
ii) Storage Conditions : 2-4^{o} C

S.No	Group	No. of animals
1	I-Control (Saline)	10 (5M+5F)
2	II-Test group (Normal saline + rhPEG-GCSF)	10 (5M+5F)

Dose levels: The **highest dose** (concentration) administered in induction phase is to be well tolerated systemically, and should be high enough to cause mild-to-moderate skin irritation; the challenge dose used is the highest non-irritating concentration.

Experimental procedure

Induction: Intradermal Injection

Day 0-Treated group

Three pairs of intradermal injections of 0.1 ml volume are given in the shoulder region which is cleared of hair so that one of each pair lies on the each side of the midline.

i) Injection 1: a 1:1 mixture (v/v) FCA (Freund's Complete Adjuvant) & normal saline.
ii) Injection 2: the test substance in vehicle (Normal saline)
iii) Injection 3: the test substance formulated in a 1:1 mixture of (v/v) FCA & normal saline

Day 0 control group:

Three pairs of intradermal injections of 0.1 ml volume are given in the same as in the treated animals.

i) Injection 1: a 1:1 mixture (v/v) FCA (Freund's Complete Adjuvant) & normal saline.
ii) Injection 2: Undiluted vehicle (Normal saline)
iii) Injection 3: a 50% of formulation vehicle in a 1:1 mixture (v/v) FCA & normal saline.

Induction: Topical application

Day 5-7 -treated and control groups

Approximately twenty –four hours before the topical induction application, if the substance has not shown any skin irritation, the test area, after close-clipping is painted with 0.5ml of 10% sodium lauryl sulphate in Vaseline, in order to create a local irritation.

Day 6-8 - treated groups

The test area is again cleared of hair. A filter paper (2 x 4 cm) will be fully-loaded with test substance in normal saline and applied to the test area and held in contact by an occlusive dressing for 48 hours.

Day 6-8 - control groups

The test area is again cleared of hair. The vehicle only will be applied in a similar manner to the test area and held in contact by an occlusive dressing for 48 hours.

Challenge: treated and control groups
(Day 20-22): Treated and control groups

The flanks of treated and control animals are cleared of hair, a patch or chamber loaded with the test substance is applied to one flank of the animals and the patches are held in contact by an occlusive dressing for 24 hours.

Observations – treated and control groups:

Observation and Grading: treated and control groups

- After 21 hours of removing the patch, the challenge area was cleaned and closely clipped.
- After 3 hours (approximately 48 hours from the start of the challenge application) the skin reaction was observed and recorded according to the grades shown in appendix.
- 24 hours after the first observation a second observation (72 hours) was done and once again recorded.
- Blind reading of test and control animals was done.
- All skin reactions and any unusual findings, including systemic reactions, resulting from induction and challenge procedures was observed and recorded according to the grading scale of Magnusson/Kligman.

Magnusson and Kligman grading scale for the evaluation of challenge patch test reactions:

Evaluation	Grade
0	No visible change
1	Discrete or patchy erythema
2	Moderate and confluent erythema
3	Intense erythema and swelling

Observational data:

I) Treatment group:

Time after removal of patches	Number of the animal									
	TG1	TG2	TG3	TG4	TG5	TG6	TG7	TG8	TG9	TG10
48 hours	0	0	1	0	0	0	1	0	0	2
72 hours	0	0	1	0	0	0	2	0	0	2

In the treated group after the removal of the patch ,after 48 hours of the challenge both male and female animals ,out of the 10 animals 2 animals showed discrete or patchy erythema and 1 animal showed moderate and confluent erythema .

After 72 hours of the challenge both the male and female animals- out of the 10 animals 1 animal showed discrete or patchy erythema and 2 animal showed moderate and confluent erythema

II) Control group:

Time after removal of patches	Number of the animal									
	CG1	CG2	CG3	CG4	CG5	CG6	CG7	CG8	CG9	CG10
48 hours	0	1	0	0	0	0	0	0	1	0
72 hours	0	1	0	0	0	0	0	0	1	0

In the control group vehicle is applied, at 48 hours out of 10 - 2 animals and at 72 hours out of 10 -2 animals showed grade 1 hypersensitivity.

RESULTS AND DISCUSSION:-

I) ACUTE TOXICITY STUDY IN MICE:

There was no mortality or morbidity observed during the 14 days study period. No toxic signs and abnormal behaviour was observed in the Group I (Intravenous) and Group II (subcutaneous) animals, which were exposed to the test compound at 10 times of intended therapeutic dose.

- PEG-GCSF was well tolerated upon acute administration to mice at the highest dosage (390 mcg/Kg body weight) administered.
- No untoward effects or mortality occurred following acute intravenous and subcutaneous administration of PEG-GCSF to Swiss albino mice

ACUTE TOXICITY STUDY IN RATS:

There was no mortality or morbidity observed during the 14 days study period. No toxic signs and abnormal behaviour was observed in the Group III (Intravenous) and Group IV (subcutaneous) animals, which were exposed to the test compound at 10 times of intended therapeutic dose.

- PEG-GCSF was well tolerated upon acute administration to rats at the highest dosage (270 mcg/Kg body weight) administered.
- No untoward effects or mortality occurred following acute intravenous and subcutaneous administration of PEG-GCSF to Sprague dawley rats.

Both animal's rats and mice are initially evaluated for their white blood cell (WBC) and absolute neutrophil count (ANC) in response to pegfilgrastim. As the WBC count and the ANC significantly increased on day +11 compared with pre-treatment values (p = 0.0002 and p = 0.033, respectively).

II) GUINEA PIG SKIN MAXIMISATION TEST

In treatment group after 48 hours of the challenge, 2/10 animals showed discrete or patchy erythema and 1/10 showed moderate and confluent erythema. At 72 hrs 1/10, 2/10 showed discrete and confluent erythema respectively. 2/10 control animals showed grade 1 (delayed contact hypersensitivity) at 48 and 72 hrs.

DISCUSSION:-

Neutropenia continues to be a challenge in the treatment of neoplastic disease and results in impairment of delivery of chemotherapy on time as well as at the prescribed dose intensity. The development of filgrastim has revolutionized the ability of oncologists to support patients who received cytotoxic chemotherapy and has allowed patients to avoid the potential complications of low neutrophil counts, particularly infection.Within this context, the use of filgrastim requires daily administration, which necessitates a high degree of compliance in a population of patients who can be and often times are severely ill and debilitated.

This trial has indicated the benefits of a single injection of pegfilgrastim for the reduction of chemotherapy-induced neutropenia . Investigations into other settings of neutropenia (eg, congenital neutropenia) or stem-cell support (eg, mobilization ofCD34_ cells) will be of interest to assess the efficacy inthese contexts.

Filgrastim is produced in *E. coli* cells, the molecule is non-glycosylated and therefore, differs from G-CSF isolated from a human cell. Pegfilgrastim is made by attaching a polyethylene glycol (PEG) to filgrastim. Pegfilgrastim is used to reduce the incidence of infection, as manifested by febrile neutropenia, in patients with nonmyeloid malignancies receiving myelosuppressive anticancer drugs. By stimulating the production of neutrophils and neutrophil precursors that, in turn, clear pegfilgrastim from the circulation, presumably by granulocyte colony-stimulating factor receptor.

Pegfilgrastim binds to the high-affinity G-CSF receptors and regulates the maturation and proliferation of neutrophils within the bone marrow. The addition of polyethylene glycol increases the size of the molecule, resulting in a significant decrease in renal clearance. The reduced clearance greatly increases the circulating half-life of the drug in the human body resulting in sustained effects of the drug. It is conceivable that the rh G-CSF formulations currently available for clinical use differentially affect WBC number and function. The present study aimed to address whether pegfilgrastim given as prophylaxis for chemotherapy-induced neutropenia affects the number and function of immune cells, a finding with potential implications for the treatment of cancer patients. This is a type of Immunotherapy that helps to develop your body's own immune system and help to fight cancer.

The pre clinical toxicity study was carried out on both rats and mice by conducting Acute toxicity study through IV and SC route of administration and skin sensitization study in guinea pigs .by the above study mortality ,lethality and sensitization activity of the pegylated rh GCSF was observed.

The pegfilgrastim 0.3 mg/ml was given to both rats and mice through intravenous and subcutaneous route according to their body weight to all the four groups (I, II, III, IV).the study observation was carried for 14 days , all the animals were observed during the study , the test group animals are compared with the control group animals ,no lethality and no mortality was observed during the acute toxicity study.

The pharmacokinetic properties of the substance was observed , absorption of the substance was noted, the peak serum concentration of pegfilgrastim occurs at 16 to 120 hours after dosing. The distribution of pegfilgrastim was limited to the plasma compartment.Serum concentrations of pegfilgrastim are maintained during the period of neutropenia after myelosuppressive chemotherapy.

The metabolic rate of filgrastim has not been fully determined and it is not known whether the drug is metabolized or how it is eliminated from the body. It has been suggested that the level of circulating neutrophils in the body may affect the half-life and clearance of filgrastim, decreasing and increasing, respectively, as neutrophil counts increase.

By stimulating the production of neutrophils and neutrophil precursors that, in turn, clear pegfilgrastim from the circulation, presumably by granulocyte colony-stimulating factor receptors, pegfilgrastim exhibits self regulated clearance. During chemotherapy-induced neutropenia, clearance of pegfilgrastim is significantly reduced and its concentration is maintained, but as neutrophils recover, the clearance of pegfilgrastim is accelerated. Pegfilgrastim is cleared from the body

a. By hepatic metabolism
b. Simultaneously through hepatic- and neutrophil-mediated routes
c. By rapid excretion by the kidneys and by neutrophils
d. Through a primarily neutrophil-mediated route by hepatic metabolism

Simultaneously through erythrocytes, neutrophils, and eosinophils in the bloodstream by rapid excretion by the kidneys, followed by neutrophils, Through a primarily neutrophil-mediated route.

After the drug administration highest dose 10 times of the intended dose by both IV and SC routes to all the four group of animals of both male and female animals, the animals are observed for 14 days for the activity of the substance, the body weight of animals was noted , cage side behavior was observed for all the animals. the ANC (absolute neutrophil count) was found by the formula , ANC should reach 10,000/mm3

The treated group is compared with the control group of animals , no mortality and no lethality was observed in all the groups.

Skin sensitization is a skin response to a hapten (a foreign, low molecular weight substance)-pegfilgrastim that acts like an allergen. In some individuals, certain haptens can induce a type IV (delayed, cell-mediated) hypersensitivity response of the skin. The hapten (allergen) must be able to penetrate the skin, combine with skin proteins, and then produce an immune response (hapten-specific T cells are primed in lymph nodes by dendritic (Langerhans) cells that emigrate from the skin). The initial exposure is called the *sensitization phase* and has no clinical symptoms. The delayed skin response from a later exposure to the allergen is called the *elicitation phase*. Clinical symptoms include erythema (redness), vesicles/bullae, papules, scaling, and pruritus (itching). Common examples of substances that can induce a skin sensitization reaction in certain individuals—also known as allergic contact dermatitis

The skin sensitization test was conducted in guinea pigs to find the substance irritant or non irritant,10 male and 10 female animals are taken , drug is applied to the treated group as a patch after the hair is removed on the animals and observed for the reaction, the control group only vehicle is applied.

By observing the results, in treatment group of animals after 48 hours of the challenge, 2 out of 10 animals showed discrete or patchy erythema and 1 out of 10 showed moderate and confluent erythema. At 72 hrs 1 out of 10, 2 out of 10 showed discrete and confluent erythema respectively. 2 out of 10 control animals showed grade 1 (delayed contact hypersensitivity) at 48 and 72 hrs, interpreted according to Magnusson/Kligman and , the test substance “rh G-CSF”-pegfilgrastim has showed some allergic reactions on skin ,so it was considered as non sensitizer for skin.

CONCLUSION

Taken together, the experimental evidence of Acute toxicity study herein presented indicates that the administration of pegfilgrastim to help in the neutrophil recovery and reduce the risk of infection, Recombinant Granulocyte Colony Stimulating Factor (rh PEG-GCSF) administered at ten times of intended therapeutic dose by intravenous and subcutaneous routes under experimental Conditions did not cause any mortality till 14th day in mice and rats .On the basis of the results obtained after the substance administration to all the animal groups, ANC level has reached 10,000/mm3 till the 11th day, no mortality was seen in all animal groups.

On the basis of the results, interpreted according to GPMT Magnusson/Kligman and OECD TG 406, the skin sensitization test in guinea pigs, the substance "rh G-CSF" –pegfilgrastim must be considered as non sensitizer for skin.

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