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ACUTE TOXICITY STUDY OF RECOMBINANT ENFUVIRTIDE IN SWISS ALBINO MICE

Anil Gudipudi*[1], Durga Bhavani Konga[1], Murali K. R. Tummuru[1], M. Lakshmi Narasu[2]

[1*]Virchow Research Center, Hyderabad 500 043, Telangana, India.
[2]Centre for Biotechnology, Institute of Science and Technology, Jawaharlal Nehru Technological University, Hyderabad, 500085, Telangana, India.

ABSTRACT

Recombinant Enfuvirtide is a 36 amino acid peptide which is the first class of drug in fusion inhibitor class. It has antiviral activity against HIV-1 strains at the target site. Enfuvirtide acts through selective inhibition of fusion of the virus with CD4 expressing cells, thus preventing the entry of HIV-RNA into target cells, precluding the initiation of the reverse transcription process and replication cycle of the virus. The present study was performed to investigate the single-dose toxicity of recombinant Enfuvirtide on Swiss Albino Mice. The test compound was administered at 10 times the intended therapeutic dose to two groups of mice (10 (5M+5F)) intravenously (IV) subcutaneously (SC). Likewise, control groups were administered with vehicle control. All the animals were observed for a period of 14 days for mortality and morbidity along with signs of toxicity. At the end of the experimental period, it was found that the single 10X dose administration (IV and SC) of recombinant Enfuvirtide does not exerted any toxic effects in Swiss Albino Mice. No pre-terminal mortality and morbidity were observed during the experimental period. No changes were observed in behavioral pattern, clinical signs, food intake, body weights and organ weights. Further histopathological examination revealed normal architecture and no significant toxic effects observed on vital organs.

KEYWORDS

Amino mice, Histology and Organ weights.

Author for Correspondence:
Anil Gudipudi,
Virchow Research Center,
Hyderabad 500 043, Telangana, India.
Email: anil.gudipudi@gmail.com

INTRODUCTION

Human Immunodeficiency Virus (HIV) affects specific cells of the immune system, called CD4 cells, or helper T cells and destroys these cells over time leading to Acquired Immuno Deficiency Syndrome (AIDS); the compromised immune system makes the individual susceptible to life

threatening infections. To target HIV, the treatment regimen should include drugs that target multiple points in viral entry, replication and release, especially to be used in combination to reduce the risk of resistance to therapy. The drug targets to combat HIV infection include Reverse Transcriptase Inhibitors, polyprotein protease Inhibitors and Fusion Inhibitors. Entry inhibitors are a new family of antiretrovirals presently represented only by one drug, Enfuvirtide[1,2]. Enfuvirtide is approved by US FDA for the treatment of HIV infection in humans[3,4]. Enfuvirtide is a designer peptide that targets multiple sites on gp41 and gp120 making it different to other HIV drugs[3]. Clinical trials with Enfuvirtide proved its superiority in terms of reducing viral loads to undetectable levels in HIV-positive people. Enfuvirtide also called immunodeficiency fusion inhibitor blocks HIV's ability to infect healthy CD4 cells. It can prevent the fresh infection of CD4 cells with HIV thereby promoting the CD4 counts improving the immunity in HIV infected.

Since Enfuvirtide targets the entry of viral genome into the host CD4 cells, it can prevent uninfected cells from becoming infected. Enfuvirtide mimics components of the HIV-1 fusion apparatus and displace them, preventing normal fusion. HIV attaches to the host CD4+ cell receptor using the viral protein gp120; upon binding, gp120 deforms allowing the viral protein gp41 to insert itself into the host cell's plasma membrane. Fusion inhibitors prevent the formation of entry pore through which the viral RNA enters into host cell causing infection, by competitively interacting with gp41. The peptide, Enfuvirtide has the primary sequence Ac-YTSLIHSLIEESQNQQEKNEQELLELDKWASL WNWF-NH2 with a theoretical molecular mass of 4492 Da[2].

MATERIAL AND METHODS

Ethical Clearance

The study was performed in accordance with the 'Guidelines for Laboratory Animals Facility' emerged by the Committee for the Purpose of Control and Supervision of Experiments on Animals(CPCSEA), India[5]. These guidelines promote the humane care of animals used in research by providing specifications that will enhance animals' well being and experimental quality for the advancement of biological knowledge that is relevant to humans and animals. Institutional Animal Ethics Committee (IAEC) had recommended and CPCSEA had approved the project proposal.

Animal Husbandry

The systemic or single dose acute toxicity study is a requirement of worldwide drug regulatory agencies[6-8] for intended use in humans. Mice is a standard rodent species used in toxicology studies based upon the substantial amount of published historical data on the suitability for such studies. The number of animals used in this study is based upon worldwide regulatory guidelines[7]. All the study animals were procured from the National centre for the laboratory animal science (NCLAS), National institute of nutrition (NIN), Hyderabad. Animals were allowed to acclimatize to the experimental conditions for a period of 7days prior to treatment. During the acclimatization period, the animals were observed daily for clinical signs of any disease. Prior to grouping, a detailed physical examination was performed on all the experimental animals. Animals were maintained in a controlled environment room at a temperature of 20±3°C and relative humidity was 30 to 70 percent. Mice were housed (2 or 3) in a cage and were fed *ad libitum* with standard pellet feed.

Route of administration

Recombinant Enfuvirtide which was produced in-house[3] was administered intravenously as well as subcutaneously.

Experimental Procedure

Animals were randomized into four groups (Group I, Group II, Group III, Group IV); test compound was administered to Group II (IV), Group IV (SC) (5 males+5 females) each in single exposure. Whereas Group I and Group III animals were treated with vehicle control (5 males + 5 females) each. All the animals were observed for a period of

14 days for mortality and morbidity. After study duration, all the group animals were sacrificed and observed for gross necropsy and histopathological examinations.

Dosing

High dose (10 times the intended therapeutic dose) of Enfuviritide was administered in single dose (58.5 mg/Kg body weight) through intravenous and subcutaneous routes for two groups (Group II and Group IV) of animals (5 male + 5 females) each.

Observations

There was no mortality and morbidity during the 14 days of observation. No toxic signs and abnormal behavior was observed in the Group II (Intravenous) and Group IV (subcutaneous) animals, which were exposed to the test compound at 58.5mg/kg (10 times the intended therapeutic) dose when compared to the control groups (Group I and III).

RESULTS AND DISCUSSION

Recombinant Enfuvirtide was administered through intravenous and subcutaneous routes to Swiss Albino Mice at dose of 58.5 mg/kg body weight (10 times therapeutic dose) as per the regulatory guidelines. The mice exposed to test compound did not show any behavioral changes during the 14 day study period. The body weight and food intake did not show any significant difference between the groups at any point of time. No mortality or morbidity was observed during the study period. Gross histological evaluation of heart, kidney, Liver, Spleen, Brain, Testis and Ovaries did not show any significant variations between the study and control groups. Histopathological observations were similar among all the groups.

Normal age related increment in the body weight was observed throughout the study period. No significant treatment (Group II and IV) related differences in male mice exposed to the test compound at 58.5mg/kg body weight were observed in comparison with control groups (Group I and III).

Normal age related increment in the body weight was observed throughout the study period. No significant treatment (Group II and IV) related differences in female mice exposed to the test compound at 58.5mg/kg were observed in comparison with control groups (Group I and III).

There was no treatment related significant changes were displayed in food intake pattern at high dose (58.5mg/kg) administered during the study period. Food intake was similar between the treatment and control groups.

No significant differences between the major organ weights of male belonging to various groups. No gross changes in the heart, kidney, liver, spleen and brain were observed in all the animals of treatment groups (Group II and IV) and control groups (Group I and III).

Gross necropsy

Gross observations of systemic organs from control and treatment groups are shown in below Figure No.1.

Histopathology evaluation

Autopsy at the end of the study period did not revealed apparent changes in the Liver, Lung, Heart, Kidney Spleen and thymus from both the control and treatment groups. Microscopic evaluation of organs from the treated and control groups showed no alteration in the cell structure or any unfavorable effect when observed under microscope using multiple magnification power.

STATISTATICAL ANALYSIS

All the results were expressed in Mean ± Standard deviation. One way analysis of variance (ANOVA) was used for the statistical analysis. Student's t-test was used to determine the significance of mean differences.

Table No.1: Mortality of mice exposed to recombinant enfuvirtide

S.No	Days of Observation	Group-I		Group-II		Group-III		Group-IV	
		M	F	M	F	M	F	M	F
1	Base line	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
2	1	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
3	2	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
4	3	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
5	4	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
6	5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
7	6	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
8	7	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
9	8	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
10	9	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
11	10	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
12	11	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
13	12	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
14	13	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
15	14	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

Group I-Control (IV), Group II-Test group (IV), Group III-Control (SC), Group IV-Test group (SC)

Table No.2: Body weights of mice (male) exposed to recombinant enfuvirtide

S.No	Body weights	Groups			
		Group-I	Group-II	Group-III	Group-IV
1	Day0	27.75 ± 3.04 (5)	28.5 ± 3.11 (5)	25.48 ± 2.93 (5)	25.24 ± 2.86 (5)
2	Day1	28.73 ± 3.15 (5)	29.14 ± 3.13 (5)	25.95 ± 3.03 (5)	25.84 ± 2.76 (5)
3	Day3	30.12 ± 2.63 (5)	29.8 ± 2.87 (5)	27.04 ± 2.75 (5)	26.0 ± 2.98 (5)
4	Day7	30.69 ± 3.56 (5)	30.74 ± 3.81 (5)	28.43 ± 2.31 (5)	27.06 ± 3.61 (5)
5	Day11	30.42 ± 4.43 (5)	30.34 ± 4.76 (5)	29.04 ± 3.29 (5)	27.16 ± 3.34 (5)
6	Day14	30.49±5.11 (5)	30.84 ± 5.00 (5)	29.87 ± 4.05 (5)	27.5 ± 4.05 (5)

Values are expressed as Mean ± S.D () No. of animals

Table No.3: Body weights of mice (female) exposed to recombinant enfuvirtide

S.No	Body weights	Groups			
		Group-I	Group-II	Group-III	Group-IV
1	Day0	26.36 ± 1.04 (5)	26.02 ± 1.01 (5)	25.18 ± 1.93 (5)	24.84 ± 1.81 (5)
2	Day1	26.73 ± 1.15 (5)	26.04 ± 0.69 (5)	25.65 ± 1.03 (5)	25 ± 1.33 (5)
3	Day3	26.12 ± 0.63 (5)	26.4 ± 0.86 (5)	26.04 ± 0.75 (5)	26.12 ± 0.57 (5)
4	Day7	27.09 ± 2.56 (5)	26.48 ± 2.11 (5)	26.43 ± 1.31 (5)	26.22 ± 1.49 (5)
5	Day11	26.42±1.43 (5)	26.84 ± 1.28 (5)	25.04 ± 1.29 (5)	25.48 ± 1.62 (5)
6	Day14	27.49±1.11 (5)	27.56 ± 0.95 (5)	26.87 ± 1.05 (5)	26.06 ± 1.09 (5)

Values are expressed in Mean ± S.D () No. of animals

Table No.4: Food intake of mice (male) exposed to recombinant enfuvirtide

S.No	Food Intake	Groups			
		Group-I	Group-II	Group-III	Group-IV
1	Day0	3.63 ± 0.32 (5)	3.72 ± 0.12 (5)	4.23 ± 0.35 (5)	4.26 ± 0.35 (5)
2	Day1	3.84 ± 0.17 (5)	4.2 ± 0.23 (5)	4.26 ± 0.39 (5)	4.44 ± 0.20 (5)
3	Day3	3.78 ± 0.24 (5)	4.17 ± 0.34 (5)	4.38 ± 0.32 (5)	4.47 ± 0.47 (5)
4	Day7	4.05 ± 0.23 (5)	4.29 ± 0.25 (5)	4.26 ± 0.40 (5)	4.05 ± 0.18 (5)
5	Day11	4.23 ± 0.27 (5)	4.41 ± 0.31 (5)	4.38 ± 0.55 (5)	4.17 ± 0.19 (5)
6	Day14	4.31± 0.22 (5)	4.44 ± 0.22 (5)	4.35 ± 0.23 (5)	4.47 ± 0.24 (5)

Values are expressed in Mean ± S.D () No. of animals

Table No.5: food intake of mice (female) exposed to recombinant enfuvirtide

S.No	Food Intake	Groups			
		Group-I	Group-II	Group-III	Group-IV
1	Day0	4.11 ± 0.31 (5)	4.17 ± 0.26 (5)	4.23 ± 0.38 (5)	4.32 ± 0.24 (5)
2	Day1	4.08 ± 0.26 (5)	4.29 ± 0.31 (5)	4.41 ± 0.34 (5)	4.2 ± 0.3 (5)
3	Day3	4.23 ± 0.34 (5)	4.17 ± 0.42 (5)	4.44 ± 0.29 (5)	4.29 ± 0.34 (5)
4	Day7	4.32 ± 0.34 (5)	4.44 ± 0.13 (5)	4.26 ± 0.45 (5)	4.5 ± 0.21 (5)
5	Day11	4.32 ±0.41 (5)	4.56 ± 0.20 (5)	4.38 ± 0.30 (5)	4.29 ± 0.25 (5)
6	Day14	4.23 ±0.22 (5)	4.41 ± 0.39 (5)	4.35 ± 0.28 (5)	4.17 ± 0.34 (5)

Values are expressed in Mean ± S.D () No. of animals

Table No.6: Organ weights of mice (male) exposed to recombinant enfuvirtide

S.No	Groups	Organs						
		Heart	Lung	Liver	Kidney	Spleen	Brain	Testis
1	**Group-I** Control (IV)	155.6 ± 20.3 (5)	201.64 ± 25.4 (5)	1231.3 ± 139.5 (5)	370.43 ± 44.00 (5)	118.4 ± 15.65 (5)	391.3 ± 40.32 (5)	432.5 ± 255.3 (5)
2	**Group-II** Test Compound 10X (IV)	156.0 ± 17.83 (5)	198.4 ± 24.56 (5)	1234 ± 146.31(5)	374.8 ± 43.9 (5)	115.8.8 ± 13.08 (5)	387.4 ± 47.81 (5)	430.0 ± 254.4 (5)
3	**Group-III** Control (SC)	206.5 ± 75.6 (5)	281.56 ± 53.21 (5)	1259.4 ± 300.4 (5)	311.5 ± 61.4 (5)	146.13 ± 13.4 (5)	377.4.4 ± 55.23 (5)	254.76 ± 50.8 (5)
4	**Group-IV** Test Compound 10X (SC)	205.0 ± 85.03 (5)	279.8 ± 49.81 (5)	1268.4 ± 313.3 (5)	310.8 ± 60.2 (5)	147.0 ± 3.5 (5)	372.0 ± 54.03 (5)	244.4 ± 44.38 (5)

Values are expressed in Mean ± S.D () No. of animals

Table No.7: Organ weights of mice (female) exposed to recombinant enfuvirtide

S.No	Groups	Organs						
		Heart	**Lung**	**Liver**	**Kidney**	**Spleen**	**Brain**	**Testis**
1	**Group-I** Control (IV)	157.43 ± 38.93 (5)	240.43 ± 34.2 (5)	1318.3 ± 130.43 (5)	329.04 ± 28.75 (5)	168.03 ± 120.43 (5)	357.5 ± 9.83 (5)	390.43± 120.32 (5)
2	**Group-II** Test Compound 10X (IV)	157.8 ± 41.70 (5)	236.0 ± 52.85 (5)	1325 ± 128.1 (5)	337.0 ± 46.57 (5)	178.8 ± 126.8 (5)	368.4 ± 8.86 (5)	382.2 ± 121.75 (5)
3	**Group-III** Control (SC)	140.6 ± 36.5 (5)	204.3 ± 54.54 (5)	1210.43 ± 210.4 (5)	300.3 ± 83.5 (5)	129.03 ± 50.03 (5)	380.4 ± 62.12 (5)	420.04 ± 108.21 (5)
4	**Group-IV** Test Compound 10X (SC)	139.0 ± 41.19 (5)	203.5 ± 75.78 (5)	1220.6 ± 208.3 (5)	303.4 ± 85.04 (5)	130.2 ± 48.03 (5)	378.0 ± 68.03 (5)	418.6 ± 106.2 (5)

Values are expressed in Mean ± S.D () No. of animals

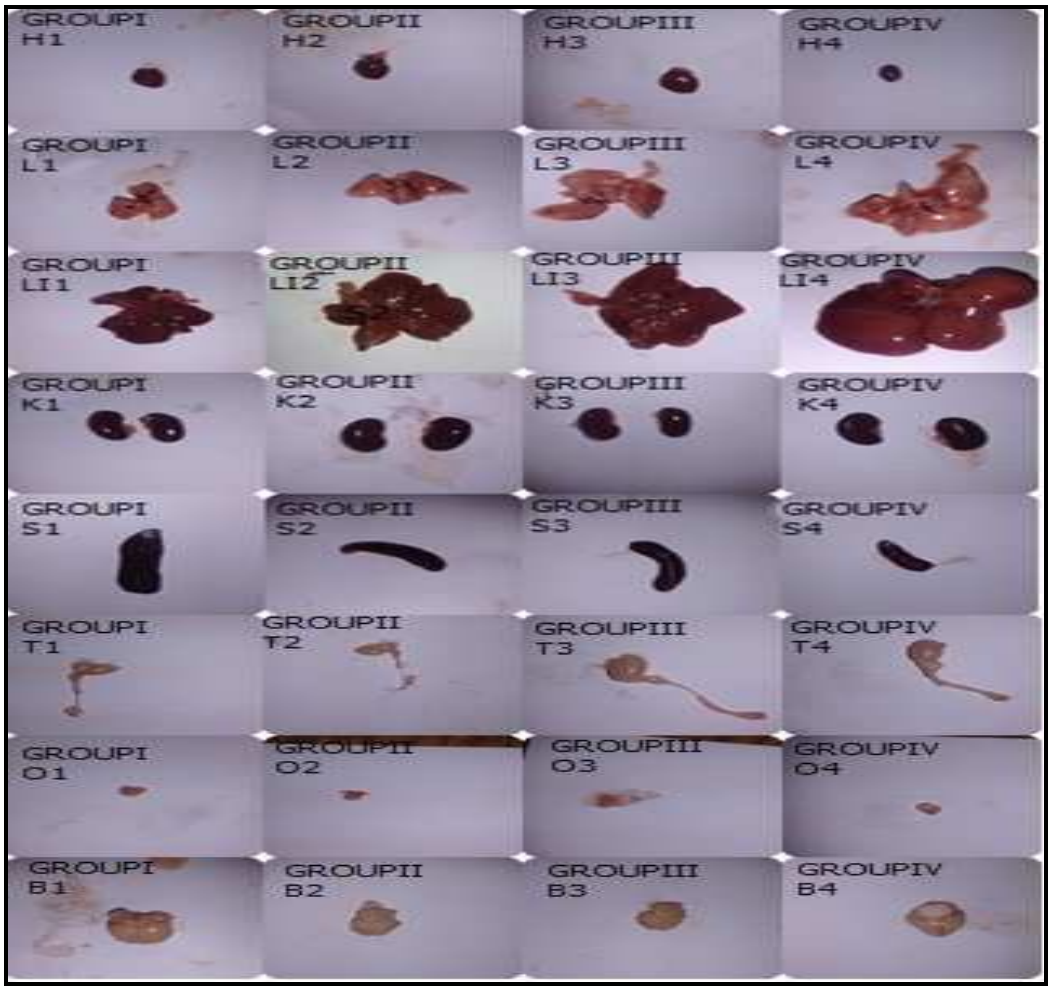

Figure No.1: Gross observations of systemic organs Heart (H1, H2, H3 and H4), Liver (LI1, LI2, LI3 and LI4), Lung (L1, L2, L3 and L4) Kidney (K1, K2, K3 and K4), Spleen (S1, S2, S3 and S4), Brain (B1, B2, B3 and B4), Testis (T1, T2, T3 and T4), Ovaries (O1, O2, O3 and O4)

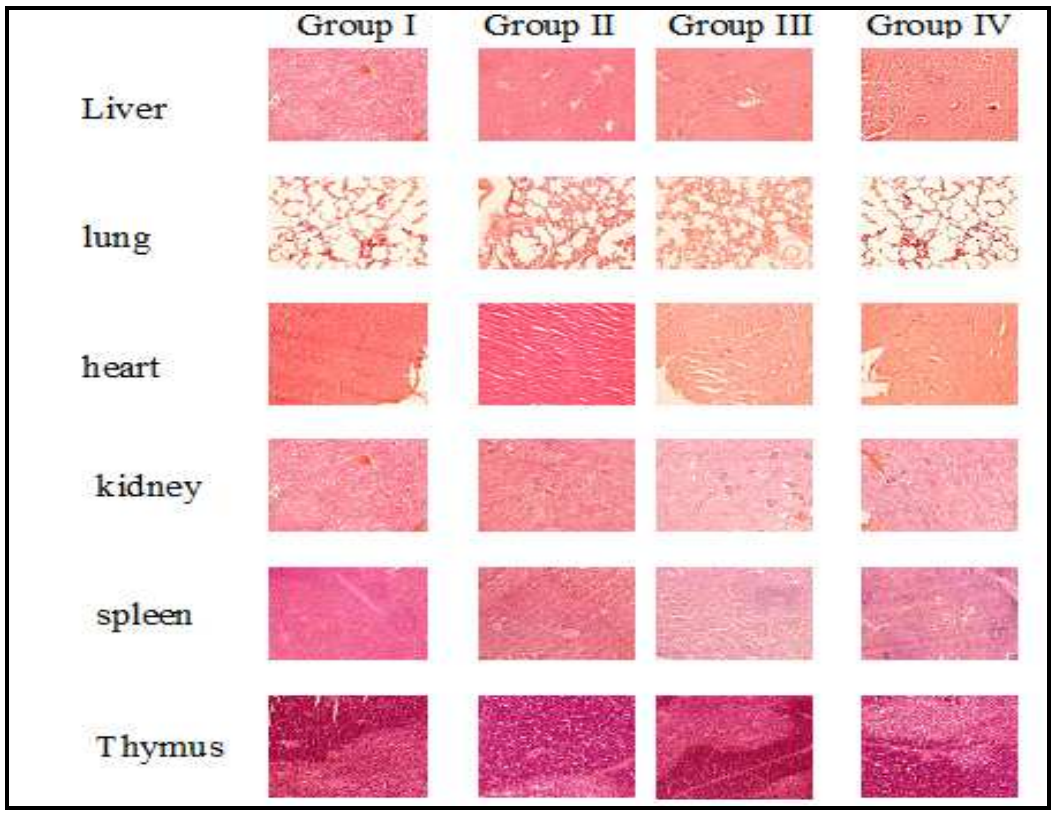

Figure No.2: Histopathological observations of systemic organs Liver, Lung, Heart, Kidney, Spleen and Thymus

CONCLUSION

From the experimental data it was concluded that single dose toxicity of recombinant human Enfuvirtide was well tolerated in both male and female Swiss albino mice at the 58.5mg/kg (10X intended human therapeutic dose). No adverse effects were detected at the highest dosage (10X) administered. No treatment related changes were detected for Body weights, Food intake and histology and Organ weights.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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