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Effect of Sub-Acute Intravenous Injection of Endothelin Receptor Agonist IRL-1620 in Mice

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Abstract: The present study was conducted to evaluate effect of sub-acute intravenous injection of ET_B receptor agonist IRL-1620 in mice. Swiss albino mice (n = 24, weighing 20-25 g) were divided into two groups (12 animal each), control group (0 µg kg⁻¹) and IRL-1620 treated group (1 µg/kg/min, i.v., for 30 days). Each group was further subdivided into two groups depending on sex of mice having 6 animals each. No significant change in animal body weight, hemogram, liver function test and renal function tests was observed on treatment with IRL-1620. Histological examination of liver was normal and Kidneys showed mild congestion. In conclusion, sub-acute dose (1 µg/kg/min, i.v.) of endothelin receptor agonist ET_B IRL-1620 did not cause toxic effect on liver and kidney.

Key words: Endothelin, IRL-1620, ET_B, ET_A, toxicity

INTRODUCTION

Since, the first description of endothelins in 1988, it has become evident that endothelins play important role in many physiologic functions. Endothelin is synthesized in and produced by variety of normal cells, including endothelial cells, vascular smooth muscle cells and variety of endothelial tissues (Penna *et al.*, 2006; Wesson, 2006). In mammals, endothelin family comprise of three endogenous isoforms ET₁, ET₂ and ET₃. All the three are 21 amino acid residues peptides containing disulfide bridges (Yanagisawa *et al.*, 1999). These peptides mediate their actions through two receptors types ET_A and ET_B (Sakurai *et al.*, 1990).

The ET axis is known to participate in growth and progression of variety of tumors. IRL-1620 (M. wt. 1820.95) is a linear ET analog specific for ET_B receptor (Watakabe *et al.*, 1992). The IRL-1620 stimulates ET_B receptor causing dilation of tumor blood vessels. The selective enhancement of tumor blood flow resulted in greater percentage of drug reaching tumor. Than normal tissue (Rajeshkumar *et al.*, 2005, 2007; Takai *et al.*, 1992). Rai and Gulati (2003) reported that IRL-1620 caused significant increase in blood flow to breast tumor tissue than normal tissue in normal rats. There was significant increase in blood perfusion to tumor tissue. The increase in blood perfusion was attenuated by BQ788 a specific antagonist of ETB receptor suggesting an ETB receptor stimulation leads to vasodilation in tumor tissues. Gulati and Rai (2004) reported that ETB receptor stimulation with endothelin 1 increased tumor blood flow by 153%, decrease in vascular resistance (147%) and tumor blood perfusion

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by 176% in breast tumor rats. Increased delivery of cytotoxic agents to tumor will have advantage of enhancing the benefits of cancer chemotherapy, while minimizing systemic toxicity. It has been reported that endothelins have a short half-life of 7-8 min. As an analog of ET-1, IRL-1620 may have a similar half-life (Rubin and Levin, 1994) and this is of advantage for therapeutic uses.

No change in pharmaco-kinetic profile of chemotherapeutic agents were observed when given along with IRL-1620 (Rai *et al.*, 2005). Such an approach may also lower the required dose and decrease the adverse effects. Keeping the therapeutic importance of IRL-1620 in view, the present study was planned to evaluate safety aspect of sub-acute dosing of ET_B receptor agonist IRL-1620 in mice.

MATERIALS AND METHODS

Swiss albino mice (n = 24, weighing 20-25 g) procured from Haffkine Institute, Mumbai, India in 2006 and were divided into two groups (12 animal each), control group and IRL-1620 treated group (1 µg/kg/min, i.v., for 30 days). Each group was further subdivided into two groups depending on sex of mice having 6 animals each. Mice were housed in polypropylene cages under hygienic conditions and acclimatized to the laboratory conditions for a period of seven days prior to initiation of dosing. Animals were kept in 12 h light and dark cycle and received food (Amrit Feeds Limited, Calcutta, India) and water *ad libitum*. The cages were placed randomly on the racks and their positions changed daily to avoid any bias or any influence due to the specific location of the cage. The groups were, control male mice group (CMM), IRL-1620 treated male mice group (IRL-1620-MM), Control Female Mice group (CFM) and IRL-1620 treated female mice group (IRL-1620-FM). Animals were given freshly prepared intravenous injection of IRL-1620 for 30 days. Control group was injected with distilled water only. All the animals were observed for clinical signs and were monitored for mortality. The body weight were monitored weekly throughout the study.

Overnight fasted animals were sacrificed on 31st day, blood and tissues samples were collected. The Institutional animal ethics committee of Ramnarain Ruia College Matunga, Mumbai (India) had approved the study protocol.

Physical Parameters

The general behavioral symptoms and any local reactions were observed during the treatment period. Mortality, if any, during the course of the experiment was recorded.

Hematological Parameters

Hemoglobin, Erythrocyte Sedimentation Rate (ESR), Red Blood Cell count (RBC), Total Leukocyte Count (TLC) and platelet counts were recorded on automated cell counter from Coulter.

Biochemical Parameters

Serum Glutamate Oxaloacetate Transferase (GOT), Glutamate Pyruvate Trans aminase (GPT), alkaline phosphatase (ALP) activities, Blood Urea Nitrogen (BUN), serum creatinine, total proteins, triglycerides, cholesterol and plasma sugar levels were performed using diagnostic kits (Merck India Ltd., Mumbai, India) on semi-auto analyzer STATFAX3300-1207(Ark Diagnostics, Mumbai, India).

Sodium, potassium and chloride were estimated on electrolyte analyzer (Roche Diagnostics, Mumbai, India).

Histopathological Examination

Liver and kidneys were removed from the sacrificed animals and were preserved in 10% buffered formalin for histological examination.

Statistical Analysis

The results are expressed as Mean±SD. Student's t-test was used for the statistical evaluation of data and $p < 0.01$ was accepted as significant.

RESULTS

General Symptoms

No significant change in behavior was noticed in the IRL-1620 treated mice of either sex as compared to respective control groups.

Mortality Rate

No mortality was recorded in any group throughout the dosing period.

Body Weight

Increase in body weight was comparable in treated and control group animals.

Hematology Parameters

There were no significant variations in the hemoglobin levels in both the IRL-1620 treated groups as compared to respective control groups. In treated groups of either sex, there were no significant variations in the Red Blood Cell (RBC), Total Leucocyte Count (TLC) and platelet count as compared to respective control groups. No significant variations in the ESR was observed in all IRL-1620 treated groups as compared to respective control group animals (Fig. 1).

Clinical Biochemistry Parameters

Liver Function Tests

No significant change in serum GOT, GPT, ALP activities (Fig. 2) and bilirubin and total protein levels were observed in IRL-1620 treated groups of either sex with respect to their respective control groups (Table 1).

Kidney Function Tests

In treated mice groups of either sex, no significant change in BUN, creatinine levels, Na, K and Cl levels were observed as compared to respective control groups.

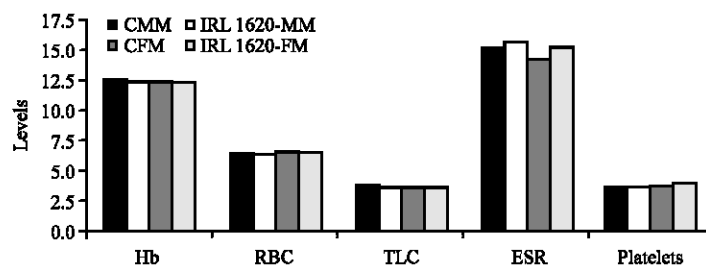


Fig. 1: Effect of sub-acute dosing of IRL-1620 on hemogram in mice

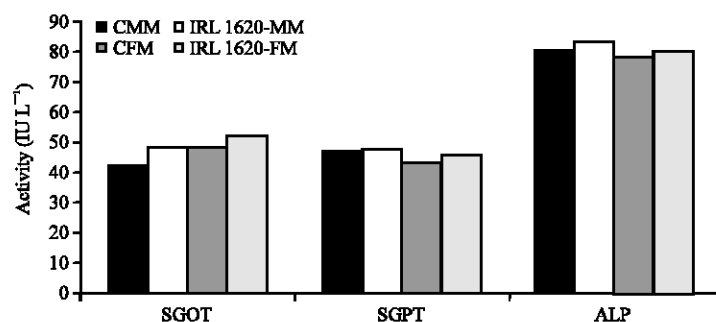


Fig. 2: Effect of sub-acute dosing of IRL-1620 on serum GOT, GPT and ALP activities (IU L^{-1}) in mice

Table 1: Effect of sub-acute dosing of IRL-1620 on biochemical parameters in mice

Biochemical parameters	Control male mice group (CMM)	IRL-1620 Treated male mice group (IRL-1620-MM)	Control female mice group (CFM)	IRL-1620 Treated female mice group (IRL-1620-FM)
Glucose (mg dL^{-1})	109.89±20.12	111.00±21.25	108.90±28.23	110.26±25.31
BUN (mg dL^{-1})	18.56±5.21	19.08±1.25	18.87±2.32	20.03±1.25
Creatinine (mg dL^{-1})	1.08±0.13	1.10±0.08	1.06±0.12	1.09±0.15
Na (mEq L^{-1})	130.00±1.32	129.97±2.01	121.00±2.61	120.00±3.21
K (mEq L^{-1})	4.10±0.12	4.15±0.15	4.50±0.15	4.52±0.12
Cl (mEq L^{-1})	92.43±2.50	92.21±3.12	94.21±2.16	94.16±2.15
Bilirubin (mg dL^{-1})	1.01±0.01	1.09±0.04	1.06±0.05	1.11±0.07
Proteins (g dL^{-1})	5.66±0.21	5.22±0.22	5.62±0.16	5.15±0.27
Triglycerides (mg dL^{-1})	234.00±44.21	233.01±25.21	228.23±25.32	228.00±32.12
Cholesterol (mg dL^{-1})	205.41±32.10	215.36±21.32	215.00±15.21	223.50±18.25

Data is presented as Mean±SD (n = 6 in each group)

No significant change in glucose, cholesterol and triglycerides levels were observed in IRL-1620 treated groups as compared to respective control groups (Table 1).

Histopathology

In the microscopic evaluation of the histological sections of the liver of IRL-1620 treated animals were found to be of normal morphology. Kidneys showed mild vascular congestion.

DISCUSSION

Blood flow rate is a major factor influencing delivery and distribution of drugs to tumors and their therapeutic outcome. Attempts has been made to influence tumor blood flow rate using vasoactive agents like IRL-1620 (Rajeshkumar *et al.*, 2007) and formulation developed from it is under Phase I clinical trial. As per our information and literature review this is the first publication regarding sub acute toxicity of IRL-1620 and very limited information is available regarding IRL-1620.

This study was conducted to evaluate the safety aspect of sub-acute dosing of IRL-1620 in mice. On intravenous injection of IRL-1620, no significant change in the body weights of mice were observed as compared to respective control group animals. This could be because of normal food and water intake through out the dosing period which were not altered during the treatment time. These results correlated well with recent reports. Rats treated with normal saline and IRL-1620 showed comparable increase in body weights (Rajeshkumar *et al.*, 2007). IRL-1620 safety was further established it was not pruritic and actually inhibited responses to histamine and ET-1, where as ET1 caused pruritis (Trentin *et al.*, 2006).

Endothelin 1 and its receptor antagonists are reported to alter various hematological parameters. Bosentan, endothelin receptor antagonist caused decrease in hemoglobin levels in patients with severe heart failure (Packer *et al.*, 2005). Halim *et al.* (1993) reported that on continuous infusion of endothelin 1 in rabbits caused decrease in platelet counts. In contrast to published reports regarding ET1 and ET receptor antagonists, in the present study IRL-1620 treatment did not cause significant change in hematological parameters suggesting IRL-1620 being safer than ET1 and endothelin antagonists.

Liver has intricate micro vascular system that allows homogeneous perfusion throughout the organ. Endothelin induced intra hepatic disturbance may play certain role in drug induced toxicity. Endothelin 1 was found to induce flow redistribution to the deeper and hilar portions of liver (Masuda, 2006). There are conflicting reports of effect of endothelin on aminotransferases. Cozzi *et al.* (2006) reported that endothelin receptor antagonist caused increase in aminotransferases whereas, Halim *et al.* (1993) reported that bolus injection of ET1 causes increase in activity of aminotransferases in rabbits. Safety of IRL-1620 was further established in our study where IRL-1620 (1 µg/kg/min, i.v.) did not cause significant increase in aminotransferase activities in mice.

Endothelin also known to have pro-inflammatory and pro-fibrotic properties. It is closely related with normal renal physiology and pathology (Neuhofer and Pittrow, 2006). The ET-1 exerts a wide variety of biological effects, including constriction of cortical and medullary vessels, mesangial cell contraction, stimulation of extracellular matrix production and inhibition of sodium and water reabsorption along the collecting duct. In addition, ET-1 is causally linked to renal disorders characterized by increased renal vascular resistance, including acute ischaemic renal failure, calcineurin inhibitor toxicity, hepatorenal syndrome and others (Neuhofer and Pittrow, 2006; Kohan, 1997). In contrast, no significant alteration in BUN, serum creatinine, sodium, potassium and chloride levels were observed on treatment with IRL-1620 (1 µg/kg/min, i.v.) suggesting normal renal functions. Our biochemical observation correlated well with histological examination of liver and kidney which showed normal morphology after the treatment with endothelin in mice of either sex.

In conclusion, sub-acute dose (1 µg/kg/min, i.v.) of endothelin receptor agonist ET_B IRL-1620 did not cause toxic effect on liver and kidney.

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