Chapter-6

Acute Toxicity Study for Tozasertib in Rats

6.1 Introduction

Aurora kinase are a serine/threonine class of enzyme that regulates various cellular activities including mitosis, proliferation, apoptosis, etc. [209]. Inhibitors of aurora kinase are being explored against a variety of diseases and disorders including cancer, metabolic, immunomodulatory, infectious and neurological origin [210]. Alignment studies have revealed that in between human and rat aurora kinase there was 82.9% primary sequence identity similarity [140]. In this relevance, studies are being conducted on rat models to explore the role of aurora kinase enzyme in biological and pathological signaling. Tozasertib is a potent inhibitor of this enzyme with an affinity toward all three isoforms i.e. aurora kinase A [(0.6 nM(Ki app)], B [18 nM(Ki app)] and C [4.6 nM(Ki app)] [211]. Tozasertib targets the ATP binding site of aurora kinase with immediate vicinity to amino acids Leu139, Lys162, Leu210, Tyr212, Thr217, Lys224, Leu263, and Asp274 [140]. The compound was under clinical trial for its anticancer activity but due to adverse effects such as QT prolongation and susceptibility to viral infections such as herpes zester [211,212]. $T_{1/2}$ of the drug is approximately 7 hours and it is primarily metabolized by the liver using Cyp3A4, Cyp2C8 and flavin monooxygenase [213]. Recent studies have emerged suggesting the role of tozasertib in various disorders by targeting cellular pathways such as apoptosis, mast cell granulation, inflammation, kinesins, etc [140,168,174,214]. The idea behind these investigations is to target pan aurora kinase with a lower dose of tozasertib that is not cytotoxic and devoid of side effects.

There are several reports published in the past decade suggesting the role of tozasertib against various neurological disorders. One report has suggested that tozasertib inhibits neuronal apoptosis via DLK/JIP3/MA2K7/JNK pathway in the early brain injury model of rats [214]. Further, studies have demonstrated that tozasertib inhibits RIPK1 dependent cell death cascade [168]. RIPK1 signaling is critically involved in neurodegenerative disorders including amyotrophic lateral sclerosis, multiple sclerosis, stroke, and traumatic injury suggesting the role of tozasertib and its analogs in the development of novel therapeutic targets [215]. In previous chapters we have demonstrated the potent effect of tozasertib against chronic pain. The highest dose that we have investigated was 40mg/kg *i.p.* which produces significant analgesia in rodents with nerve injury and the CFA model of chronic pain. It is very important to screen the toxic effect of dose regimens of tozasertib before performing future studies using this compound against different experimental paradigms. Thus, here we have performed the acute toxicity studies of tozasertib using a single-dose administration to rationalize the use of lower dose selection as compared to the cancer modalities.

6.2 Experimental design

The protocol was designed in accordance with Organization for Economic Co-operation and Development (OECD) 420 fixed-dose method with some modifications as per the previously published literature [157,158]. Experimental animals were randomly assigned into two groups with 6 animals each. The first group received the vehicle and the second group received tozasertib at the single dose of 40 mg /kg intraperitoneally (*i.p.)*. The detailed cage-side observations were conducted including changes in eyes and mucous membranes, skin and fur, respiratory, circulatory,

autonomic, and central nervous systems, somatomotor activity, and behavior patterns with aforementioned time points. Further, body weight, food water consumption, hematological analysis, biochemical screening, organ weight recordings and histopathological analysis was performed.

6.3 Results and discussion

6.3.1 Tozasertib administration did not affect the on gross behavior in rats

Monitoring of the rats performed post-tozasertib 40mg/kg *i.p.* administration to observe any abnormal response or behavior elicited by the animals. There was no change observed in the gait, skin color, lacrimation, sleepiness, writhing, convulsion, tremor, diarrhea, salivation after 30 min, 2 hrs, 4 hrs, 24 hrs of tozasertib administration. Moreover, no mortality was observed in rats treated with saline and tozasertib 40mg/kg *i.p.*

6.3.2 Tozasertib did not altered body weight and food-water consumptions in rats

Tozasertib treatment 40mg/kg i.p. have no effects in rats body weight. Analysis with t-test has suggested that there was no significant effect across the groups on body weight of animals (Figure 6.1A). The effect of tozasertib 40mg/kg *i.p* administration on food and water consumption was recorded on daily basis starting from day 0 up to day 14. No statistical difference was found for food and water consumption between the saline and tozasertib group illustrating the fact that tozasertib 40mg/kg has no effect on the daily food (Figure 6.11B) and water (data not shown) intake of rats.

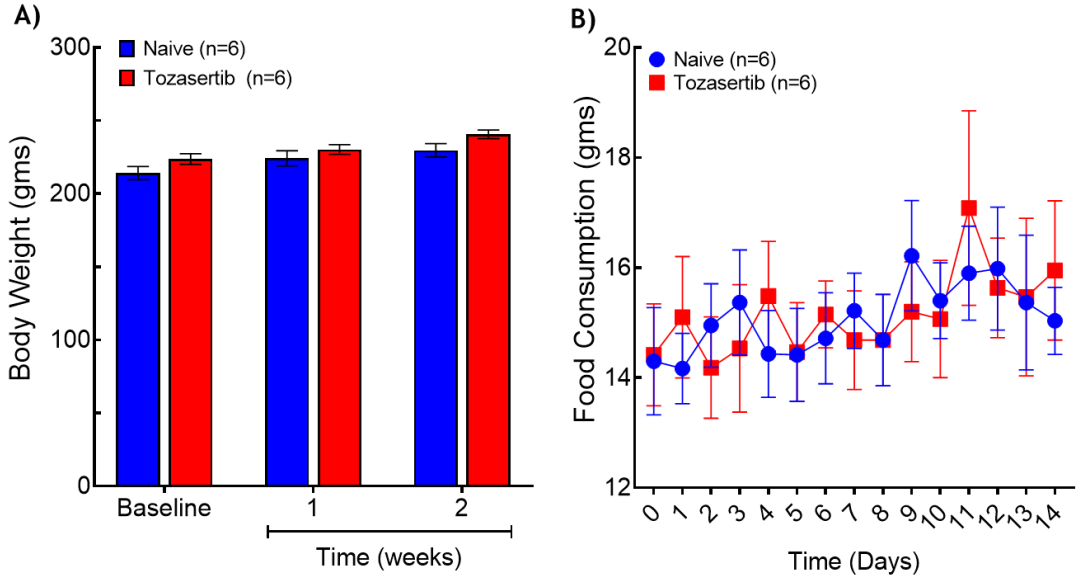

Figure 6.1 Effect of tozasertib single dose administration on body weight and food consumption of rats. A. Body weights of rats for the course of experiment **B.** Food consumption recordings for rats treated with saline and tozasertib. Data is expressed as mean ± SEM and significance was set at $p<0.05$.

6.3.3 Hematology index

The complete profiling of blood samples collected from the female rats was performed to detect any effect of tozasertib administration over the various components of blood (Table 6.1). The hematological analysis revealed that there were no significant changes observed in the levels of WBC, lymphocytes, monocytes, granulocytes, RBC, hemoglobin, hematocrit, MCV, MCH, MCHC, RDW, platelet count, thrombocytocrit (PCT) between the tozasertib and saline-treated groups.

Table 6.1. Effect of tozasertib on hematological profiling in rats

S.N.	Parameter	Units	Historical control range	Naïve	Tozasertib (40mg/kg i.p.)
1	WBC	10^3 cells/µL	4-6	5.12 ± 0.31	5.296 ± 0.21 **(NS)**
2	Lymphocyte	10^3 cells/µL	2-5	3.83 ± 0.12	4.063 ±0.19 **(NS)**
3	Monocytes	million/µL	0.05-0.10	0.082 ± 0.01	0.075 ±0.01 **(NS)**
4	Granulocytes	cells/µL	0.6-1.1	0.58 ± 0.16	0.673 ±0.05 **(NS)**
5	RBC	million/µL	6-8	7.41 ± 0.13	7.0466 ±0.24 **(NS)**
6	Hemoglobin	g/dl	120-150	145.74 ± 3.51	144.066 ±1.64 **(NS)**
7	Hematocrit	L/L	35-45	39.45 ± 0.36	39.3916 ±1.21 **(NS)**
8	Mean corpuscular volume	fL	55-65	55.58 ± 1.24	56.83 ± 1.95 **(NS)**
9	Mean corpuscular hemoglobin	g/dl	17-21	19.48 ± 0.32	19.221 ±0.19 **(NS)**
10	Mean corpuscular hemoglobin concentration	g/dl	270-340	322.167 ± 3.62	316.88 ±5.19 **(NS)**
11	Red blood cell distribution width	fL	10-15	13.94 ± 0.26	14.416 ±0.43 **(NS)**
12	Platelet count	mcL	900-1100	1033.66 ± 16.62	1030.571 ±6.85 **(NS)**
13	Mean Platelet volume	fL	5-7	6.40 ± 0.33	6.256 ±0.06 **(NS)**
14	Platelet distribution width	%	15-17	15.32 ± 0.21	14.923 ±0.16 **(NS)**
15	Thrombocytocrit (PCT)	%	0.4-0.8	0.56 ± 0.016	0.576 ±0.03 **(NS)**

6.3.4 Tozasertib did not affected the blood biochemical profile of rats

Biochemical analysis in the blood samples provides information regarding the multiple parameters associated with metabolism. Statistical analysis suggested that there was no significant effect of tozasertib on tbil, DBIL, tp, ALB, GLO, ALT, AST, ALP, TG, TCH, GLU, BUN, CR, UA, LDH, CK of rats as compared to the saline

treated rats (Table 6.2). These results indicate that tozasertib as 40mg/kg *i.p.* do not produce biochemical toxicities.

Table 6.2. Effect of single dose administration of tozasertib 40mg/kg *i.p* on biochemical parameters of rats.

S.N.	Parameter	Unit	Historical control range	Naïve	Tozasertib (40mg/kg i.p.)
1	Total Bilirubin (tbil)	mg/dl	0.4-0.8	0.72 ± 0.015	0.66 ± 0.022 **(NS)**
2	Direct bilirubin (DBIL)	mg/dl	1-2	1.79 ± 0.041	1.58 ± 0.029 **(NS)**
3	Total Protein (tp)	g/dl	40-48	45.01 ± 0.67	43.74 ± 0.33 **(NS)**
4	Serum albumin (ALB)	g/dl	20-26	23.39 ± 0.31	23.17 ± 0.39 **(NS)**
5	Globulin (GLO)	g/dl	20-30	23.08 ± 0.53	23.22 ± 0.59 **(NS)**
6	Alanine aminotransferase (ALT)	U/L	25-35	27.34 ± 0.49	27.36 ± 0.14 **(NS)**
7	Aspartate aminotransferase (AST)	U/L	130-180	170.62 ± 2.29	167.69 ± 7 **(NS)**
8	Alkaline phosphatase (ALP)	U/L	90-140	129.23 ± 1.55	131.06 ± 0.36 **(NS)**
9	Triglyceride (TG)	mg/dl	0.5-1.0	0.79 ± 0.03	0.84 ± 0.02 **(NS)**
10	Total cholesterol (TCH)	mg/dl	1.0-2.0	1.81 ± 0.05	1.69 ± 0.07 **(NS)**
11	Blood sugar (GLU)	mmol/L	4-8	6.45 ± 0.22	6.25 ± 0.07 **(NS)**
12	Urea nitrogen (BUN)	mg/dl	5-10	7.8 ± 0.47	7.42 ± 0.12 **(NS)**
13	Creatinine (CR)	mg/dl	25-30	26.96 ± 0.53	26.41 ± 0.19 **(NS)**
14	Uric acid (UA)	mg/dl	0.05-0.07	0.06 ± 0.01	0.06 ± 0.01 **(NS)**
15	Lactate dehydrogenase (LDH)	U/L	1200-1800	1593.68 ± 33.41	1550.44 ± 55.76 **(NS)**
16	Creatine kinase (CK)	U/L	1100-1700	1636.94 ± 30.18	1519.26 ± 56.55 **(NS)**

6.3.5 Gross necroscopy

The macroscopic examination was performed to observe the presence of any tumor, inflammation, atrophy, swelling, hypertrophy. The architecture of all the organs was found to be intact with no signs of edema or inflammation in tozasertib and saline-treated groups.

6.3.6 Tozasertib single dose administration has no effect on organ body weight of rats

The weight of different organs isolated from female rats was measured post day 14. The weight of all the organs including heart, adrenal gland, kidney, lungs, liver, brain, pancreas, stomach, uterus and ovary was statistically non-significant in tozasertib (40mg/kg *i.p.*) treated rats as compared to the saline-treated group (Table 6.3). These findings suggest that there was no toxic effect of tozasertib at 40mg/kg *i.p.* on organ weight.

Table 6.3. Effect of tozasertib single dose administration on body organ weight of rats

S.NO	Weight (gms)	Naïve	Tozasertib 40 mg/kg i.p.
1	Heart	0.50 ± 0.03	0.58 ± 0.017 **(NS)**
2	Adrenal Right	0.13 ± 0.01	0.12 ±0.03 **(NS)**
3	Adrenal left	0.13 ± 0.01	0.13 ± 0.02 **(NS)**
4	Kidney Right	0.65 ± 0.02	0.70 ± 0.02 **(NS)**
5	Kidney Left	0.68 ± 0.03	0.67 ± 0.06 **(NS)**
6	Lungs	1.53 ± 0.06	1.57 ± 0.04 **(NS)**
7	Brain	1.62 ± 0.08	1.65 ± 0.06 **(NS)**
8	Liver	5.85 ± 0.05	5.93 ± 0.08 **(NS)**
9	Pancreas	0.65 ± 0.06	0.60 ±0.04 **(NS)**
10	Stomach	2.58 ± 0.05	2.05 ± 0.05 **(NS)**
11	Uterus	0.61 ± 0.10	0.580 ± 0.069 **(NS)**
12	Ovary	0.33 ± 0.08	0.31 ± 0.063 **(NS)**

6.3.7 Histopathology

Histopathological analysis for the liver and kidney of rats have revealed that there was no presence of abnormal structures in tozasertib as well as saline treated rats. The organs were observed to be intact in both the groups with normal distribution of tissue architecture (figure 2). Liver tissue was normally distributed including hepatocytes and Kupffer cells in both the groups. Architecture of kidney including shape of glomerular tubule, intestine and blood vesicles in tozasertib 40mg/kg *i.p.* and saline treated rats.

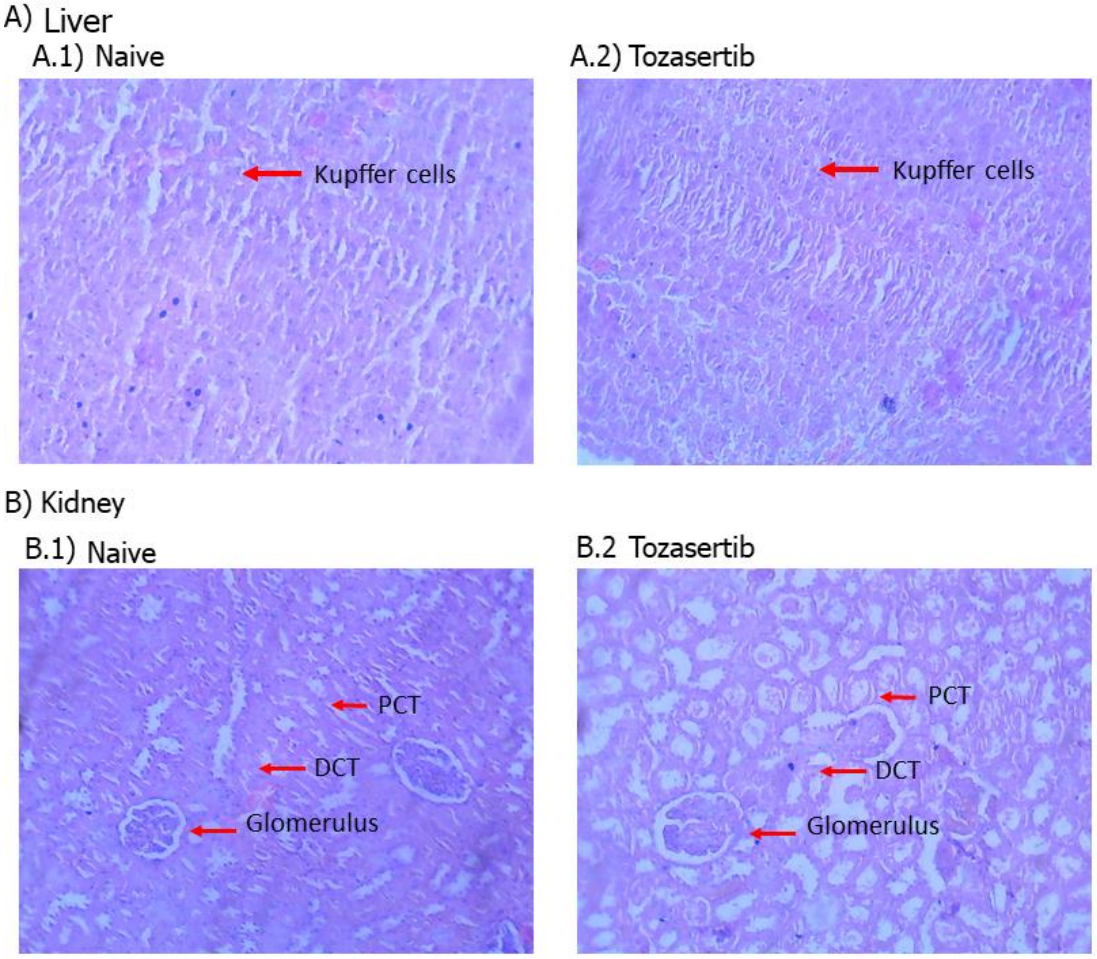

Figure 6.2 Effect of tozasertib on histopathological architecture of liver and kidney of rats. A) liver B) Kidney architecture was found to be intact in both tozasertib and saline treated animals. The imaging was performed on 10X magnification.

Tozasertib is a pan aurora kinase inhibitor with demonstrated efficacy against various type of cancer and promising potential against neurodegenerative, somatosensory, immune system and metabolism related disorders. A recent trend is being shifted toward its application in several pathophysiological condition with the use of a lower dose regimen to reduce the side effects and achieve potent efficacy. To rationalize the research utilizing tozasertib in preclinical setup we have performed acute toxicity study of this compound in Sprague Dawley rats. Rats were administered with 40mg/kg *i.p.* tozasertib or saline and detailed behavioral, molecular and histopathological observations were performed. We have observed that tozasertib 40mg/kg *i.p.* dis not altered the behavioral responsiveness in rats including gait, skin color, lacrimation, sleepiness, writhing, convulsion, tremor, diarrhea, salivation as compared to the saline treated rats. Next, we have found that tozasertib did not affected body weight, food-water consumption in rats as examined at different time points. Further, the hematological and biochemical studies have revealed that tozasertib at 40mg/kg did not produce any significant changes in blood profile of rats as compared to the saline treated rats. Finally, the histopathological analysis of liver and kidney was performed in both the groups and our findings demonstrated that tozasertib 40mg/kg *i.p.* did not show any sign of necrosis or inflammation in rat organs. Our preliminary study suggests that single dose administration of tozasertib at 40mg/kg *i.p.* is safe in rats and could be utilized as an effective tool to modulate the aurora kinase activity in different experimental paradigms.

6.4 Outcomes

Highest dose used in this thesis work was found to be safe in acute toxicity studies. Low and moderate dose of tozasertib can be used during chronic pain and other pathophysiological conditions. However, future studies are required to investigate the effect of this molecule during chronic repeated dose treatment.