Systemic Safety Evaluation of Gastro Intestinal Tract, Urinary System and Central Vascular System in the Rat by Oral Administration of Linezolid

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Summary

This study was to assess the safety of linezolid in gastrointestinal effect, urine biochemical parameters, cardiovascular system in rodents at three different dose levels administered by orally. Overnight fasted/fed male wistar rat, swiss albino mice weighing around 200-250g, 25-30g respectively. Animals were divided in to 5 different groups, each groups comprises of 6 animals. First 3 groups are 3 different dose levels (50, 100, 200 mg/kg body weight of Linezolid) vehicle control group and standard drugs. The effects of Linezolid on gastrointestinal tract, CVS and urinary biochemical changes were evaluated in this study. Wister rats, doses of Linezolid, 50, 100, 200mg/kg body weight did not have any effect on the GIT motility. But dose of Linezolid at 100mg/kg ($P<0.05$) reduce the gastric emptying. All the different doses of Linezolid urine biochemical parameters were highly significant when compared to control group of animals. No remarkable changes in cardio vascular system at three different doses of Linezolid. The value of during this study as a part of safety pharmacology core battery is emphasized by the fact that linezolid, an oxazolidinone antibiotic has no significant effect on GIT, urine biochemical parameters and CVS. Linezolid 100mg/kg group animals showed significant effect of gastric emptying.

Key Words: Linezolid, Central Vascular System (CVS), Gastro Intestinal Tract (GIT), Safety Pharmacology

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Introduction

Antimicrobial drugs are the greatest contributors to the therapeutics in recent era's for effective diseases. Their advent changed the outlook of physician about the power drugs can have on diseases. In response to the emergence of resistant bacteria, effects are being made to develop antimicrobial agent against this resistant organism (1,2).

Pharmaceutical companies generally asses the effects on vital systems such as CNS, GIT, CVS and urinary system, as a part of their non-clinical safety pharmacological evaluation of Linezolid and this was included as a part of the core battery in the ICH S7A safety pharmacology guidance document (3).

Safety pharmacology studies should do reveal any functional effects on the major physiological systems. Investigation may include the use of isolated organs or other system not involving intake animals. These studies may allow for a mechanistically based explanation specific organ toxicity which should be considered carefully with respect to human use and indication.

Linezolid is as a first antimicrobial agent (4) having excellent activity against virtually all important gram positive pathogens. The oxazolidinones are the inhibitors (5) of the protein synthesis. Oxazolidinone antibiotic (6,7) has no significant effect on GIT, urine biochemical parameters and CVS. This study aimed to describe the safety of linezolid in assessment of GIT, CVS and urinary biochemical parameters in rodents at three different doses. So far there is no preclinical safety information available in scientific domain.

Materials and methods

Materials

Linezolid was procured from Symed labs while Chlorpromazine HCl and Ofloxacin (USP27) were purchased from La-Pharmaceuticals and the Jang pharma respectively Sodium CMC (USP/NF) was purchased from Signet chemical corporation, Mumbai. Pentylenetetrazole was procured from sigma chemical Co. USA. Caffeine 99%, CAS 58-08-2 was purchased from Lancaster, England while Theophylline anhydrous was

procured from Sigma chemical Co. USA. All the other chemicals used were of high analytical grade.

Animals

The study was carried out in overnight fasted/ fed male wister rat, swiss albino mice weighing around 200-250g, 25-30g respectively (Wockhardt Animal house facility). Animals were identified individually by body marking. Animals were housed in cages of standard dimensions with sawdust bedding. The animals were kept in standard laboratory conditions. Feed and water was given in *ad libitum* quantity to the rats and mice.

Experimental study plan (method)

For assessment of GIT and Urine biochemical parameters

Thirty male Wistar rats were randomly divided into five groups each group comprises of six animals each. Group I received vehicle of Linezolid. Group II received Linezolid at dose of 50 mg/kg, Group III received Linezolid at 100 mg/kg, Group IV received Linezolid at 200 mg/kg and Group V received reference drug by oral route.

For assessment of CVS parameters

Fifteen male Guinea pigs were randomly divided into five groups each group comprises of three animals each. Group I received vehicle of Linezolid. Group II received Linezolid at dose of 15 mg/kg, Group III received Linezolid at 30 mg/kg, Group IV received Linezolid at 60 mg/kg and Group V received reference drug by oral route.

Dose ranges & exposures

The doses were selected by extrapolating human therapeutic dose of 600mg to rat on the basis of body surface area. Lower dose i.e. 50mg/kg is equivalent to human therapeutic dose. Middle and high doses were selected in multiples of 2 of lower dose. Pharmacokinetics study was carried out in rat at these doses for estimation of drug levels and its possible correlation with effects observed during safety pharmacological assessment. Homogeneity of groups was validated on the criterion of body weight measured on the day of the study. The number of animals per group was the minimum

number enabling an accurate assessment of the studied pharmacological effect and comparison using standard statistical tests.

Assessment of GIT parameters

The effect of Linezolid on gastrointestinal motility and gastric emptying was assessed in male Wistar rats. The day prior to the study, animals were put on to a water-only fast for 14-16 hours. On the day of the study, animals were dosed by oral route with Linezolid (50, 100 & 200 mg/kg) and its vehicle in volume of 2.5 ml/kg and with Atropine in a volume of 10 ml/kg. 1 hour after dosing, a 10% aqueous suspension of activated charcoal in 2.5% sodium carboxymethylcellulose was administered orally in a volume of 2 ml/animal. 15 minutes later, animals were euthanased, the stomach was ligatured at the level of cardia and pylorus, and then the stomach and the intestines were removed from the pylorus to the extremity of the caecum. The total length of the intestine and the distance covered by the charcoal were measured immediately afterwards. The distance the charcoal meal travels was expressed as the percentage of the total length of the gastrointestinal tract from pyloric end to ileoceacal junction. The stomach was weighed full, then opened and rinsed, and weighed empty to determine gastric emptying.

Assessment of Urinary biochemical parameters

Two days prior to the study, animals were acclimatized in metabolic cages. On day three Linezolid at dose of 50, 100 and 200 mg/kg and its vehicle was administered orally to fasted Wistar rats and again kept in metabolic cages. The urine samples were collected after 8 hours of drug administration. The following parameters were assessed by Uriscan Pro. Urinary volume, Specific gravity, pH, Ketone, Urobilinogen, bilirubin Blood, Proteins, Nitrites, glucose, Leucocytes and Vitamin C content.

Assessment of Cardio Vascular effect

Cardiovascular effects of Linezolid were studied in urethane (1.25g/kg) anaesthetized male guinea pigs weighing 400 to 450gms. A pressure transducer was connected to carotid artery for measurement of systolic and diastolic blood pressure. Linezolid was infused for 15 min through Jugular vein at a dose of 15,30 and 60 mg/kg. Blood samples were withdrawn from carotid artery for measurement of concentrations of

Linezolid at predetermined time intervals (15min & 2hrs). Serum concentration of Linezolid was determined by validated HPLC method. Lead II ECG was recorded on Powerlab polygraph system (ADInstruments) for calculation of heart rate and QT interval measurement.

QT_c was calculated using the following formula:

(Bazzet HC 1920): – $QT_c = QT/RR^{1/2}$ ($msec/sec^{1/2}$) [60]

(Fridericia LS 1920): –$QT_f = QT/RR^{1/3}$ ($msec/sec^{1/3}$) [61]

Results and Conclusions

Gastrointestinal Effects:

Test compound / Doses:

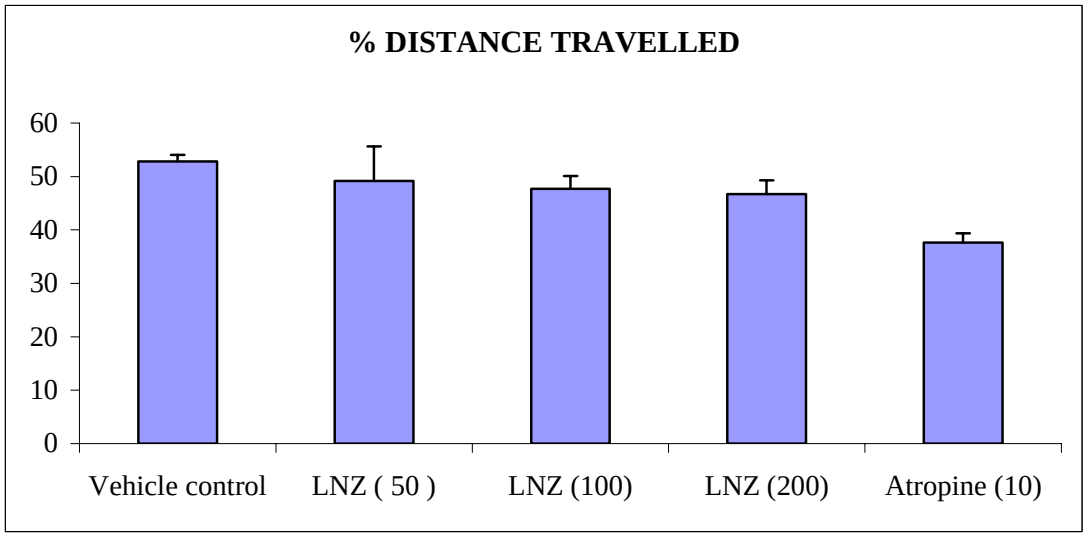
Linezolid: 50,100,200mg/kg, *p.o. Vol.-(0.5ml/200g)*

Atropine: 10 mg/kg, *p.o. Vol.-(2ml/200g)*

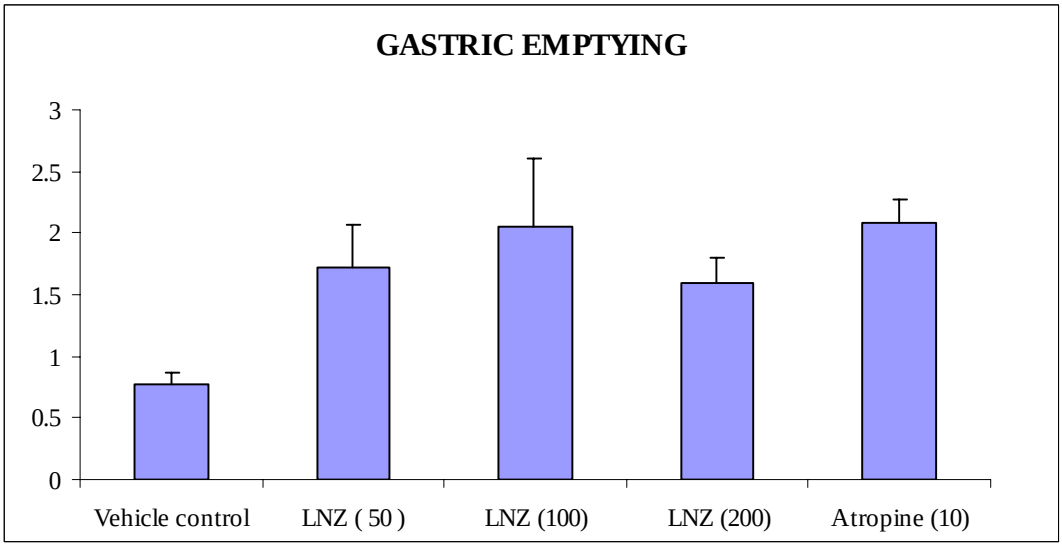
Table – Effect of Linezolid on GIT motility of Wistar Rats

Compound	Dose (mg/kg)	% Distance traveled	Gastric emptying
Vehicle control (n=11)	---	52.83±1.24	0.77±0.10
Linezolid (n=10)	50	49.16±6.47	1.72±0.35
Linezolid (n=9)	100	47.73±2.38	2.06±0.54*
Linezolid (n=7)	200	46.72±2.58	1.60±0.20
Atropine (n=9)	10	37.65±1.74 **	2.09±0.19*

Significantly different from vehicle control ** ($p<0.01$) & * ($p<0.05$) (One way ANOVA using Newman-Keuls Multiple Comparison Test)



Y-axis = % distance traveled.



Y-axis = Weight in Gram.

In Wistar rats, doses of Linezolid 50,100 200 mg/kg did not have any effect on the GIT motility. However, dose of linezolid at 100 mg/kg ($p<0.05$) reduced the Gastric emptying. As expected, Atropine significantly decreased the GIT motility as evident by the decreased distance traveled by the charcoal meal and Gastric-emptying rate.

Urine Biochemical Parameters:

Two days prior to the study, animals were acclimatized in metabolic cages. On day three, Linezolid was administered orally at a dose of 50, 100 and 200 mg/kg to fasted Wistar rats. After dosing animals were again kept in metabolic cages for collection of urine. A separate vehicle group was also maintained to rule out the effect of vehicle if any on urinary parameters of Wistar rats. The urine samples were collected after 8 hours of drug administration. The following parameters were assessed by Uriscan.

Linezolid 50 mg/kg:

Sr.No.	**Parameter**	**Unit**	**Animal no.**		
			1	**2**	**3**
1	Blood		-ve	-ve	-ve
2	Bilirubin	mg/dl	-ve	-ve	-ve
3	Urobilinogen	mg/dl	Nor	Nor	Nor
4	Ketone	mg/dl	5	10	5
5	Protein	mg/dl	100	100	100
6	Nitrites	mg/dl	+ve	+ve	-ve
7	Glucose	mg/dl	-ve	-ve	-ve
8	pH		6.0	5.5	6.0
9	Specific Gravity		1.025	1.025	1.025
10	Leucocytes	WBC/µl	-ve	10	-ve
11	Vit.C	mg/dl	10	25	50

Linezolid 100 mg/kg:

Sr.No.	Parameter	Unit	Animal no.		
			1	**2**	**3**
1	Blood		-ve	-ve	-ve
2	Bilirubin	mg/dl	-ve	-ve	-ve
3	Urobilinogen	mg/dl	Nor	Nor	Nor
4	Ketone	mg/dl	5	-ve	10
5	Protein	mg/dl	100	100	100
6	Nitrites	mg/dl	-ve	-ve	-ve
7	Glucose	mg/dl	-ve	-ve	-ve
8	pH		6.0	5.5	6.0
9	Specific Gravity		1.025	1.025	1.025
10	Leucocytes	WBC/µl	-ve	-ve	-ve
11	Vit.C	mg/dl	50	10	10

Linezolid 200 mg/kg:

Sr.No.	Parameter	Unit	Animal no.		
			1	**2**	**3**
1	Blood		-ve	-ve	-ve
2	Bilirubin	mg/dl	-ve	0.5	-ve
3	Urobilinogen	mg/dl	1	Nor	1
4	Ketone	mg/dl	5	-ve	10
5	Protein	mg/dl	300	100	100
6	Nitrites	mg/dl	-ve	-ve	-ve
7	Glucose	mg/dl	-ve	-ve	-ve
8	pH		5.0	6.5	6.0
9	Specific Gravity		>=1.030	1.020	1.025
10	Leucocytes	WBC/µl	10	-ve	-ve
11	Vit.C	mg/dl	50	10	10

Control (1 % Tween 80):

Sr.No.	Parameter	Unit	Animal no.		
			1	**2**	**3**
1	Blood		-ve	-ve	-ve
2	Bilirubin	mg/dl	-ve	-ve	-ve
3	Urobilinogen	mg/dl	Nor	Nor	5
4	Ketone	mg/dl	-ve	-ve	5
5	Protein	mg/dl	30	100	100
6	Nitrites	mg/dl	-ve	+ve	-ve
7	Glucose	mg/dl	-ve	-ve	-ve
8	pH		6.0	8.0	7.5
9	Specific Gravity		1.02	1.02	1.01
10	Leucocytes	WBC/µl	-ve	-ve	-ve
11	Vit.C	mg/dl	-ve	10	-ve

Linezolid up to 200 mg/kg didn't produce any significant effect on renal parameters measured by Uriscan-pro compared to control. pH was between 5.0 to 8; Specific Gravity was between 1.01to 1.025. Proteins content, Ketone, Urobilinogen, Leucocytes, Vitamin C content was found to be within normal range compared to control. Blood, Bilirubin & Nitrites were almost absent in both Linezolid & vehicle treated animals.

Cardiovascular effects:

Effect of Linezolid on Heart rate and ECG in anaesthetized Guinea pig.

.**Doses**: 15, 30 and 60 mg/kg i.v infusion for 15 min

Vehicle control: Vehicle used was 150 µl Pharmasol (15 %) + 100 µl Ethanol + 700 µl Polyethylene glycol (6%) + 0.1N NaOH. pH – 5.0

Heart Rate: (B.P.M) (n=3)/ RR (msec)

Time points	**Vehicle Control**	**LNZ 15 mg/kg i.v.**	**LNZ 30 mg/kg i.v**	**LNZ 60 mg/kg i.v**
0 min	0.21±0.04	0.24±0.01	0.28±0.00	0.21±0.03
15 min	0.21±0.03	0.24±0.01	0.24±0.01	0.21±0.03
30 min	0.22±0.04	0.26±0.02	0.25±0.01	0.20±0.02
1 hr	0.24±0.03	0.25±0.04	0.23±0.01	0.21±0.02
1.5 hr	0.22±0.01	0.27±0.03	0.29±0.06	0.21±0.02
2 hr	0.25±0.03	0.28±0.04	0.31±0.07	0.22±0.01

The values are expressed as Mean ± SEM. Statistically non significant compared to vehicle control (Two way ANOVA by using Bonferroni posttests)

QT Interval in msec: (n=3)

Time points	Vehicle Control	LNZ 15 mg/kg i.v.	LNZ 30 mg/kg i.v	LNZ 60 mg/kg i.v
0 min	197.50±2.51	166.75±2.76	149.50 ±6.02	182.75±13.29
15 min	179.00±8.02	164.25± 0.25	150.50±1.50	190.75±21.81
30 min	180.75±13.79	161.00±10.03	153.50±0.00	178.75±12.79
1 hr	168.75±8.78	156.00±4.01	158.00±9.53	183.50±10.53
1.5 hr	168.00±6.02	155.50±3.51	153.25±10.78	175.00±7.52
2 hr	170.00±7.52	144.00±7.52	152.75±9.28	179.95±12.99

The values are expressed as Mean ± SEM. Statistically nonsignificant compared to vehicle control (Two way ANOVA by using Bonferroni posttests)

QTCF: (Corrected QT interval as per Fridericia formula $QT_f = QT/ RR^{1/3}$ $(msec/sec^{1/3})$**) (n=3)**

Time points	Vehicle Control	LNZ 15 mg/kg i.v.	LNZ 30 mg/kg i.v.	LNZ 60 mg/kg i.v.
0 min	333.58±15.74	265.56 ± 3.13	220.24±0.24	308.61±34.83
15 min	301.36±26.29	261.07±0.65	242.45±5.64	327.10±49.39
30 min	314.57±33.03	252.56±22.65	251.10±5.15	304.38±27.63
1 hr	272.00±22.98	247.66±17.02	257.78±18.89	311.44±26.67
1.5 hr	277.86±13.52	233.62±22.28	234.35±32.25	294.03±19.12
2 hr	276.69±18.37	219.91±19.36	227.85±29.83	299.06±28.15

The values are expressed as Mean ± SEM. Statistically nonsignificant compared to vehicle control (Two way ANOVA by using Bonferroni posttests)

QTCB:(Corrected QT interval as per Bazett's formula $QT_f = QT/RR^{1/2}$ (msec/sec$^{1/2}$) **(n=3)**

Time points	Vehicle Control	LNZ 15 mg/kg i.v.	LNZ 30 mg/kg i.v.	LNZ 60 mg/kg i.v.
0 min	434.14±33.44	335.16±3.16	272.39±1.25	401.36±53.35
15 min	391.32±42.40	329.17±1.49	307.77±9.20	428.58±72.51
30 min	445.56±118.41	316.45±32.70	311.10±0.30	396.87±40.65
1 hr	345.48±34.78	312.78±27.65	329.31±26.27	405.89±40.48
1.5 hr	357.49±19.76	291.26±33.39	295.23±44. 50	381.225±29.01
2 hr	342.81±35.82	271.90±28.78	279.07±46.42	385.865±40.76

The values are expressed as Mean ± SEM. Statistically nonsignificant compared to vehicle control (Two way ANOVA by using Bonferroni posttests)

Linezolid up to 60 mg/kg didn't have any significant effect on heart rate and QT interval compared to vehicle control

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