Systemic Safety Evaluation of Central Nervous System Function in the Rat by Oral Administration of Linezolid

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Summary

This study was aimed to describe the pharmacokinetic and safety of Linezolid in assessment of central nervous system parameters in rodents at three different dose levels followed by oral administration. Non-clinical safety pharmacology evaluation of candidate drugs includes the 'core battery' assessment of vital organ functions in the ICH S7A guidance document. The functional observation battery (FOB) is a systematic evaluation of nervous system function in the rat comprising more than 30 parameters across autonomic, neuromuscular, sensorimeter & behavioural domains. Overnight fasted/fed male wistar rat, swiss albino mice strains were used. Each parameter was tested at 3 dose levels (oral route) with the vehicle control group and reference standard drug. Pharmacokinetic parameters of Linezolid by oral route at 3 different doses were studied in male wistar rats. An attempt was made to correlate the levels of Linezolid within the body with effects observed on general behaviour (IRWIN TEST) according to the standardized observation battery order. Linezolid does not show any potential interaction with PTZ & theophylline compared to vehicle groups. Drug was also devoid of pro-convulsive potential when studied in electroshock model. Linezolid did not show any proconvulsive potential after I.C.V. administration. The value of carrying out FOB as part of safety pharmacology 'core battery' is emphasized by the fact that Linezolid, an Oxazolidinone antibiotic was devoid of any potential interaction in assessment of parameters in FOB. This preclinical study data of linezolid may help to anticipate the most frequently required "follow up" studies.

Key Words: Linezolid, Central Nervous System, Core battery, Safety Pharmacology

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1. Introduction

Infection is more prevalence all around the world; wide use of antibiotics comes into existence. Most commonly used types of antibiotics are Penicillins, Fluoroquinolones, Cephalosporins, Macrolides, Tetracylines and Aminoglycosides. Some of these mention classes of antibiotics have severe adverse effects, such as long course of treatment and development of resistance, which limits their use.

In response to the emergence of resistant bacteria, efforts are being made to develop antimicrobial agents that are active against these resistant organisms.

Linezolid is the first of a new oxazolidinone class of antimicrobial agents. It has excellent activity against virtually all important gram-positive pathogens. The oxazolidinones are inhibitors of bacterial ribosomal protein synthesis. The response appears to combinations of linezolid with other antibiotics to be additive or neutral and rarely antagonistic or synergistic. Development of resistance is rare but has occurred in patient with indwelling prostheses who received long term linezolid therapy [Richard Sadovsky and Moellering, 2003].

Safety pharmacology studies investigate the potential undesirable pharmacodynamic effects of a substance on physiological functions in relation to exposure in the therapeutic range and above. Investigations may include the use of isolated organs or other test systems not involving intact animals [Anon, 2000].

The study includes effect of Linezolid on general behaviour – Irwin test, Pro-convulsive potential- interaction with PTZ, direct intra-cerebro-ventricular drug administration (I.C.V.). Pentobarbitone induced hypnosis were studied in rats. Potentiation of electroshock induced seizures and pro-convulsive potential interactions with theophylline were studied in mice, this study was aimed to describe pharmacokinetic parameters and its correlation in assessment of linezolid safety for CNS parameters in rodents at three different doses.

2. Materials and methods

2.1 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.

2.2 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.

2.3 Experimental study plan (method)

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.

2.4 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.

Linezolid was administrated orally as a 1% Tween suspension in a dosing volume of 2.5ml/kg in overnight fasted wistar rats. Each rat was serially bled from retro orbital plexus at predetermined time points of 0.0, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12 & 24 hrs after drug administration. The blood samples were centrifuged at 10,000 rpm for 10 min and resulting serum obtained was pooled from three rats at each time point & then immediately stored at - 60°C until analyzed on LC/MS.

2.5 Assessment of CNS parameters

2.5.1 Behavioural IRWIN test & Effect on body temperature following single oral administration in the rat

The effects of Linezolid on general behaviour were studied in male wistar rats each weighing 200-220gm. Linezolid was administered by oral route at doses of 50, 100 & 200-mg/kg body weight. 1Caffeine (20mg/kg per oral) and chlorpromazine (10mg/kg per oral) were used as reference standard drugs. Observation was made at 1, 2 and 3 hours post-dosing (Table 2). Animals were observed for potential neurobehavioural effect using a standard observation battery, which allows assessment of both peripheral & central nervous system activities. Potential effect on body temperature was also assessed (Table 3). Methods were adapted from those described by Irwin (1968) for detecting behavioural effects in mice. Results were presented as semi-quantitative or quantitative values & the occurrence of effect was characterized by a statistical analysis in accordance with the method described by Mattson (1996) for functional observation battery (FOB) [Mattson, J.L. *et.al*., 1996].

2.5.2 Evaluation of proconvulsive effect following single oral administration in the rat or mice

2.5.2.1 Pro-convulsive potential- interaction with PTZ in Rats

Animals were infused with a solution of pentylenetetrazole that induces seizures at about 20-25 minutes after dosing in control rats. Pre-treatment with substances which possess proconvulsant properties leads to rapid onset of seizures. Animals were observed

for seizures with cut of time of 30 minutes [File, S.E., *et al*., 1998 and Mirski, M.A.Z., *et al*., 1994]. The time of occurrence of seizures was noted. Caffeine was used as positive reference drug [Enginar, N., *et al*., 1991] (Table 4).

2.5.2.2 Potentiation of electroshocks induced seizures in mice

In this test Swiss male albino mice were subjected to sub-threshold corneal electroshock (9 mA ×0.3 sec) and incidence of tonic extension and death were recorded, whereas mortality was recorded over 24 hrs observation (Table 5). Linezolid was administered orally 30 min before corneal electroshock whereas Theophylline was injected intraperitoneally 15 min prior to corneal electroshock [Woodbury, L.A and Davenport, V.O., 1952].Theophylline was used as a positive control drug.

2.5.2.3 Pentobarbital induced hypnosis

Rats were injected with pentobarbitone, dose 40mg/kg which induces the hypnosis after oral administration. Test substance which possessing hypnosis property will increase the latency of recovery of righting reflex in pentobarbitone pretreated rats. Chlorpromazine was used as reference standard drug at dose of 5mg/kg per oral (Table 6).

2.5.2.5 Pro-convulsive potential- interaction with theophylline

Test was done in fasted swiss male albino mice weighing 25-30g each. Linezolid was administered orally 30 min prior to the Theophylline administration. Theophylline was administered by I.P route at a dose of 150 mg/kg/i.p. Mice showing incidence of tonic extensor was recorded during 3-hour observation period whereas death was recorded over 24 hrs post dosing.[De Sarro, A and De Sarro, G.B *et al.*, 1991] Ofloxacin was used as positive control drugs (Table 7).

3. Results and Discussion

3.1. Pharmacokinetic overview

Pharmacokinetic parameters of linezolid in wister rats at different doses by oral administration the results are given in table 1. The pharmacokinetic data showed that linezolid is rapidly and extensively absorbed after oral dosing.

Table 1: Pharmacokinetic parameters of Linezolid in Wistar Rat at different doses by oral route.

	Linezolid		
	50 mg/kg	100 mg/kg	200 mg/kg
N	6	6	6
AUC (µg.hr/ml)	137.48±0.295	385.09±3.746	743.5±29.504
Cmax (µg/ml)	29.22±4.511	63.43±0.345	99.40±8.976
Tmax (hrs)	1±0.00	2±0.00	2±0.00
Cl (L.hr/kg)	0.3563±0.003	0.26±0.003	0.26±0.006
Vd (L/kg)	0..4486±0.345	0.7238±0.034	0.18±0.042
$T_{1/2}$ (hrs)	1.77±0.245	1.93±0.110	4.69±1.010

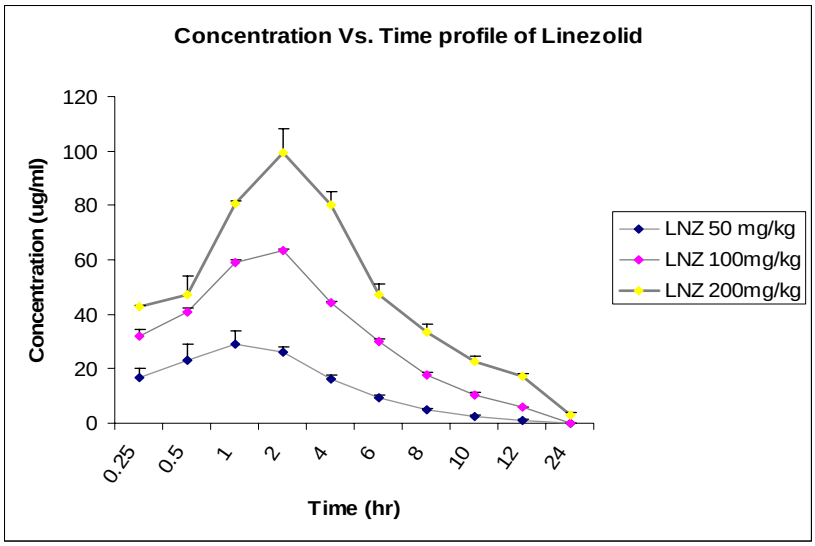

Figure 1: Pharmacokinetic profile of Linezolid

Maximum plasma concentration was reached within 1 to 2 hrs after dosing & the absolute bioavailability is 100%. Linezolid may be administered without regard to the timing of meals. High fat food affectsC_{max} in such a way that it is delayed from 1.5 hrs to 2.2 hrs (17%). However the total exposure measured as AUC values similar under both

conditions. The plasma protein binding is approx 31% and is concentration dependent. The Vd of linezolid at steady state averaged 40 to 50 liters in healthy adult volunteers.

In clinical data, following oral administration of a single 600 mg dose linezolid is rapidly absorbed the mean (SD) Pharmacokinetic parameters were AUC = 91.40 (39.30) μg/ml; C_{max} = 12.70 (3.96) μg/ml; T_{max} = 1.28 (0.66) hours. Linezolid primarily metabolised by oxidation of the morpholine ring. Preclinical study data of linezolid is not available in public domain; therefore safety study for linezolid was conducted to evaluate its safety parameters. The study was planned by following ICH S7A guidelines which is the current GLP for performing safety pharmacological studies.

3.2 Effect of linezolid on general bahaviour (Irwin test)

Table 2: FOB outcomes arranged in standardized order

Activity(Normal score)	Chlorpro-mazine	Linezolid 50	Linezolid 100	Linezolid 200	Caffeine	Vehicle
Arousal (4)	2.66	4	4	6	6	4
Finger Approach (4)	0.66	3.33	2.6	3.3	2	3.3
Head touch (4)	0	4	3.3	3.3	7.3	4
Unusual behaviour (0)	1	0	0	0	1	0
Catalepsy(0)	1	0	0	0	0	0
Position passivity (4)	5	4	4	4.67	5	4
Visual placing(4)	2	4	4	4	2	4
Fear(4)	0	4	2	2	0	3.3
Grooming(0)	0	0	0	0.6	0	0
Aggressive Irritability (0)	0	0	0	0	0	0

Abnormal vocalization (0)	0.6	0	0.6	0	0.6	0
Body position(4)	4	4	4	4	4	4
Spontaneous locomotor activity (4)	0	4	3.3	4	0	4
Rearings(4)	0	2.6	0.6	2.6	0	2
Ataxic gait(0)	NE	0	0	0	NE	0
Twitche(0)	0	0	0	0	0	0
Seizures(0)	0	0	0	0	0	0
Startle response (4)	3.3	4	0	0	3.3	4
Tremors(0)	0	0	0	0	0	0
Sensitivity to pinching the tail (4)	2	4	4	3.3	2	4
Hypotonic gait (0)	4	0	0	0	4	0
Grip strength (4)	2	4	4	4	2	4
Body tone (4)	4	4	4	4	4	4
Abdominal tone (4)	4	4	4	4	4	4
Limb tone (4)	2	4	4	4	2	4
Corneal reflex (4)	4	4	4	4	4	4
Pinna reflex (4)	0	4	3.3	4	0	4
Hind limb reflex (4)	0	4	4	4	0	4

Righting reflex on ground (0)	1.3	0	0	0	1.3	0
Air Righting reflex (0)	1.6	0	0	0	1.6	0
Skin color (4)	4	4	4	4	4	4
Cyanosis (0)	0	0	0	0	0	0
Writhing symtomps (0)	0	0	0	0	0	0
Tail position(0)	0	0	0	0	0	0
Piloerection (0)	0	0	0	0	0	0
Salivation(4)	4	4	4	4	4	0

Table 3: Body Temperature: mean (in degree Fahrenheit)

Time	LNZ 50	LNZ 100	LNZ 200	Chlorpromazine	Vehicle	Caffeine
1hr	101.06	101.2	99.78	101.25	103.18	101.9
2hr	101.65	99.65	99.65	100.63	101.6	102.6
3hr	101.51	100.88	99.30	101.15	101.35	102.55

Oral doses of 50, 100 and 200 mg/kg of Linezolid were administered to six rats in each dose group. Body temperature was found normal at all the doses. No signs of tremors, convulsions, irritability, salivation, diarrhoea and no loss of righting reflex were observed at all the doses. The fear-eye closure to rapid finger approach, decrease in rearing, startle response delayed reaction was observed at dose of 100 and 200 mg/kg. At a 10-mg/kg dose, chlorpromazine produced a piloerection in some animals, loss of righting reflex and decrease in spontaneous motor activity as well as alertness were observed at 2 hr and 3hr post dosing. Animals were found to be lethargic and had a relaxed body posture. [Irwin, S., 1968]

3.3 Pro convulsive potential of linezolid

Table 4: Interaction with PTZ in I.V. infusion rat model

Compound	Dose (mg/kg, P.O.)	Onset of clonus(min)	% mortality
Control (N=8)	------	29.41±0.429	62.5
Linezolid (N=6)	50	26.99±1.937	30
Linezolid (N= 6)	100	29.17±1.020	30
Linezolid (N=6)	200	29.0±2.453	66.66
Caffeine (N= 6)	150	20.16±0.155*	100

*Statistically different from control at $p<0.05$ (ANOVA with Newman-Keuls Multiple Comparison Test), Linezolid does not show any potential interaction with PTZ.

Table 5: Pro-convulsive potential of Linezolid in conjunction with electroshock test compound/ doses

Compound	Dose (mg/kg), *p.o.*	Incidence of tonic extension	Incidence of Death
Control	--	0/6	0.0
Linezolid	50	0/6	0.0
Linezolid	100	0/6	0.0
Linezolid	200	0/6	0.0
Theophylline (*i.p.*)	150	6/6***	100.0***

Statistically different from control at***$p<0.01$ (One way Anova using Newman-Keuls Multiple Comparison Test)

In mice subjected to corneal electroshock, Theophylline (150 mg/kg) caused severe tonic hind limb extension and death whereas doses of Linezolid up to 200 mg/kg, did not affect any incidence of convulsions and death.

Each value represents the number of mice which exhibit tonic extension or death/number of mice tested. Linezolid is devoid of pro-convulsive potential when given before electroshock.

Table 6: Effect of Linezolid on pentobarbital-induced hypnosis

Compound	Dose (mg/kg) *p.o.*	Sleep onset (min)	Righting reflex (min)
Pentobarbitone (Vehicle Control) (*i.p.)(12)*	45	3.80±0.26	54.58±4.31
Linezolid (6)	50	4.73±0.15	59.03±6.16
Linezolid(6)	100	3.99±0.31	56.57±1.94
Linezolid(10)	200	3.45±0.297	89.87±8.761*
Chlorpromazine(9)	5	2.64±0.26**	111.14±11.81**

** Statistically different from control at p<0.001 &*p<0.01 (One way Anova using Newman-Keuls Multiple Comparison Test)

Each value represents the Mean ± SEM of duration of sleep (min) after pentobarbital and drug administration. Linezolid potentiate pentobarbital-induced hypnosis at the dose 200mg/kg.

Table 7: Drug Interaction study of Linezolid and Theophylline

Compound	Dose (mg/kg),p.o.	Incidence of tonic extension	% Mortality
Vehicle Control	-	0/8	0.0
Linezolid	50	0/8	0.0
Linezolid	100	0/8	0.0
Linezolid	200	2/8	25.0
Ofloxacin	300	3/6*	50.0
Ofloxacin (i.p.)	100	6/6***	100.0

Statistically different from control at *** p<0.001 and * p<0.05 (One way Anova with Newman-Keuls Multiple Comparison Test)

Each value represents the number of mice, which exhibit tonic extension or death/number of mice tested**.** Linezolid up to 100 mg/kg does not show any incidence of

convulsions and death when co-administered with theophylline. However Linezolid at 200 mg/kg dose, showed convulsions and death in 25 % animals. Ofloxacin (100 mg/kg *i.p.*)/(300mg/kg p.o.) showed convulsions, tremors and death when co-administered with theophylline. Linezolid didn't show any potential interaction with Theophyline.

The effect on CNS, a modified Irwin test was used at three time point of each 1 hour into wistar rats. In this test the linezolid had no effect on behavioural changes, co-ordination and body temperature compared to vehicle control where as chlorpromazine & caffeine produced significant effects on above parameters compared to vehicle controls group.

In pentobarbital induced hypnosis, linezolid 200 mg/kg per oral showed significant effect. The proconvulsive potential of linezolid was checked with the PTZ induced convulsion in rats, interaction with theophylline in mice, electroshock induced convulsions, in mice and was found to be no significant effect compared to vehicle control group.

The value of carrying out FOB as part of safety pharmacology 'core battery' is emphasized by the fact that Linezolid, an Oxazolidinone antibiotic was devoid of any potential interaction in assessment of parameters in FOB. This preclinical study data of linezolid may help to anticipate the most frequently required "follow up" studies.

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