

Plasma Bactericidal Titres of NXL103, a Novel Oral Streptogramin, after Single Dose Administration of 0.5g, 1g and 1.5g to Healthy Volunteers.

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ABSTRACT

Background: NXL103 consists of a 30:70 ratio of PI/PII RPR202868 (PI) and RPR132552 (PII) which exhibit bactericidal synergism against common respiratory tract infection (RTI) organisms. Single oral dose studies of NXL103 have been performed in human volunteers. The objective of this study was to examine whether bactericidal activity was present in the plasma of the subjects who had received single 0.5g, 1g, or 1.5g doses of NXL103, as well as to examine the magnitude and longevity of the effect.

Methods: Post-dose plasma samples from 8 volunteers per dose group were serially diluted in 50% growth medium/50% pooled control plasma and inoculated with *S. pneumoniae* or *S. aureus* at 5x10⁸cfu/ml. After incubation 10µl was streaked onto solid agar medium. The reciprocal of the highest dilution of plasma which suppressed 99.9% of the original inoculum was the Plasma Bactericidal Titre. Plasma samples from placebo dosed volunteers were also tested.

Results: Human volunteer mean C_{max} values of PI+PII were 0.75, 1.37 and 1.85 µg/ml for the 0.5, 1, and 1.5g doses respectively. Elimination half life ranged from 2-3 hours for PI and 4-6 hours for PII. NXL103 achieved bactericidal titres in the majority of subjects at all doses tested. There was a dose dependant response. In the highest dosed group (1.5g), plasma samples from 8 out of 8 subjects were bactericidal against *S. aureus*. Titres reached a maximum of 8 in 3 individuals at 2-4 hours post-dose, and lasted for up to 6 hours. Against *S. pneumoniae*, volunteer plasma was bactericidal in 6 subjects of 8 dosed with 1.5g. Maximum titres of 4 were achieved between 2-4 hours and duration was 4 hours in two cases. Placebo treated plasma samples were not bactericidal.

Conclusion: Orally dosed NXL103 achieved significant bactericidal titres in the plasma of healthy volunteers. Further dose optimisation will make NXL103 a potential therapy for oral treatment of community acquired RTI and SSTI.

INTRODUCTION

NXL103 (formerly XRP 2868) is a novel semi-synthetic oral streptogramin that consists of a 30/70 (w/w ratio) association of a pristinamycin IA (PI) derivative and a pristinamycin IIB (PII) derivative. NXL103 is being developed for the treatment of respiratory tract and skin and skin structure infections.

A single escalating oral doses phase I study has been performed. This was a double blind, randomized, placebo-controlled study in healthy adult male volunteers. In each of 6 dose groups, 10 healthy male subjects were enrolled and received either NXL103 (8 subjects) or placebo (2 subjects).

Due to differing pharmacokinetic properties of the PI and PII components the administered 30:70 ratio is not maintained constantly in human plasma. However it has been shown *in vitro* that a broad range of ratios maintain a synergistic bactericidal effect. The objective of this study was to examine the titre and duration of bactericidal activity present in the plasma of volunteers who had received 0.5g, 1g, 1.5g doses of NXL103 by the oral route. By a serial dilution of plasma samples the activities against *S. pneumoniae* and *S. aureus* were measured.

MATERIALS AND METHODS

Strains used
Strains used were selected from the culture collection of Novexel. *S. aureus* 011HT18 (Smith), *S. pneumoniae* 030MV1 were both fully susceptible to penicillins and macrolides.

Susceptibilities of strains to NXL103
Susceptibilities of strains were determined in appropriate liquid medium. MBCs were determined in the absence and in the presence of 50% human plasma.

MBC (µg/ml)	<i>S. aureus</i> 011HT18	<i>S. pneumoniae</i> 030MV1
Without human serum	0.12	0.12
With 50% human serum	0.25	0.5

Test Medium and inoculum preparation
S. aureus 011HT18 (Smith) was cultured overnight in Mueller Hinton broth and then diluted in growth medium to give inoculum of around 5x10⁸ cfu/ml. *S. pneumoniae* 030MV1, overnight culture in blood agar in CO₂, was diluted in brain heart plus 4% red blood cell extract to give an inoculum of around 5x10⁸ cfu/ml.

Establishment of plasma bactericidal activity test conditions

50% plasma/50% growth broth was used as the test medium. Preliminary experiments with the test strains confirmed that the strains selected tolerated up to 50% plasma in the growth medium. Due to the dilution factor this method results in a limit of detection of 2-fold the MBC. That is to say, a drug concentration in the original sample plasma equivalent to 1 x MBC will not be detected.

Preparation of the dilution series
Equal volumes of test plasma were mixed with pooled human control plasma and 2 fold serial dilutions were carried out. An equal volume of growth medium containing the test strain was then added to each well.

Plasma bactericidal activity
After overnight incubation all wells were sampled and 10µl streaked onto solid agar medium. The highest dilution of plasma which suppressed 99.9% of the original inoculum was defined as the plasma bactericidal activity. Therefore in relation to the original inocula, growth of 5 colonies or less was considered as bactericidal.

RESULTS

FIGURE 1: The bactericidal titres in the plasma of human volunteers after single oral dosing of NXL103 at 0.5g (subjects 301-310), 1g (subjects 501-510), and 1.5g (subjects 701-710)

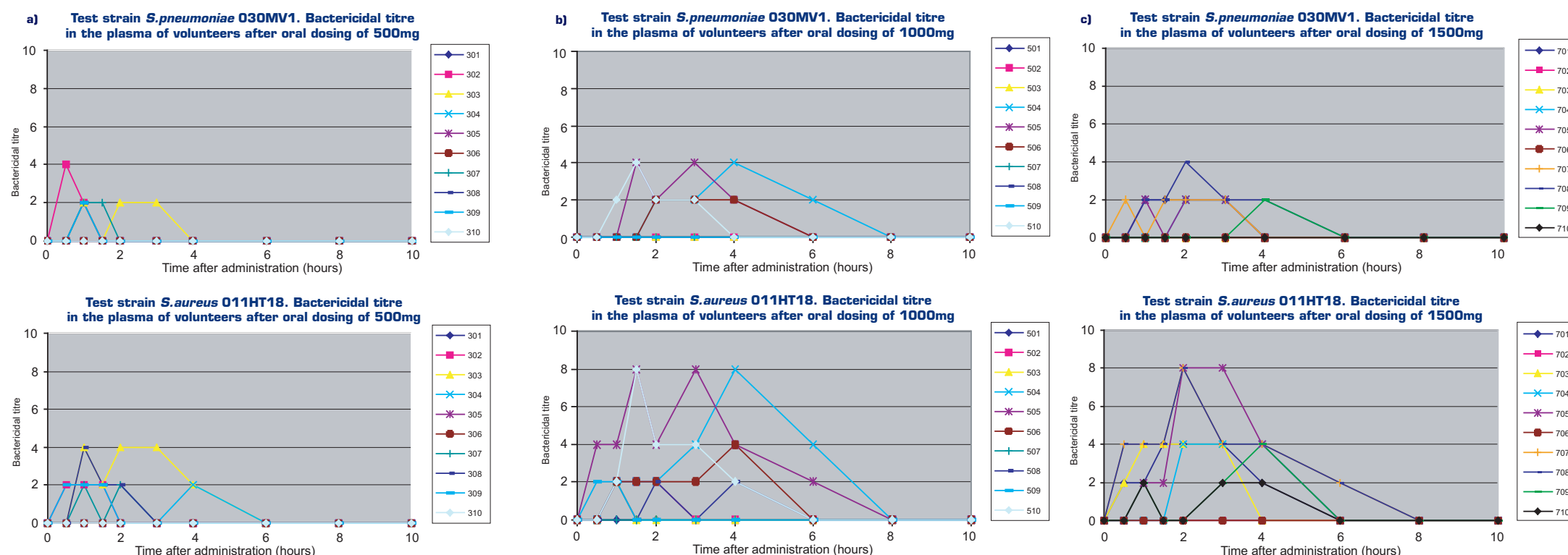


TABLE 1: Individual plasma samples showing bactericidal activity against *S. pneumoniae* (red) and *S. aureus* (blue). Total concentrations of PI + PII in volunteer plasma are in ng/ml. 0 represents non detectable, or placebo dosed volunteer

a) Test strain <i>S. pneumoniae</i> 030MV1. Bactericidal titre in the plasma of volunteers after oral dosing of 500mg										
Time (h)	301	302	303	304	305	306	307	308	309	310
0	0	0	0	0	0	0	0	0	0	0
0.5	0	0	0	0	0	0	0	0	0	0
1	443	641	660	199	0	333	514	615	466	0
1.5	387	586	491	294	0	356	536	589	303	0
2	304	380	682	238	0	383	460	551	248	0
3	163	179	832	316	0	158	198	476	98	0
4	148	175	950	856	0	141	232	291	55	0
6	72	92	379	253	0	67	100	114	27	0
8	36	44	201	115	0	30	43	61	8	0
10	16	27	111	50	0	11	18	34	0	0
12	12	13	72	29	0	7	11	17	0	0
15	8	11	36	15	0	0	7	8	0	0
24	7	7	10	5	0	0	0	6	0	0

b) Test strain <i>S. pneumoniae</i> 030MV1. Bactericidal titre in the plasma of volunteers after oral dosing of 1000mg										
Time (h)	501	502	503	504	505	506	507	508	509	510
0	0	0	0	0	0	0	0	0	0	0
0.5	0	218	33	308	716	169	0	280	732	1024
1	0	895	574	669	844	561	0	552	610	2181
1.5	0	673	267	562	1372	901	0	366	399	1968
2	0	664	381	717	1626	1138	0	695	310	1677
3	0	386	408	1321	2080	1575	0	454	541	1688
4	0	209	631	1764	1432	1247	0	616	367	1097
6	0	90	456	1012	873	404	0	215	160	492
8	0	40	322	416	480	147	0	84	74	253
10	0	20	143	177	288	62	0	37	38	148
12	0	9	60	100	182	31	0	24	25	93
15	0	7	23	49	92	18	0	9	14	51
24	0	0	0	12	18	0	0	5	8	13

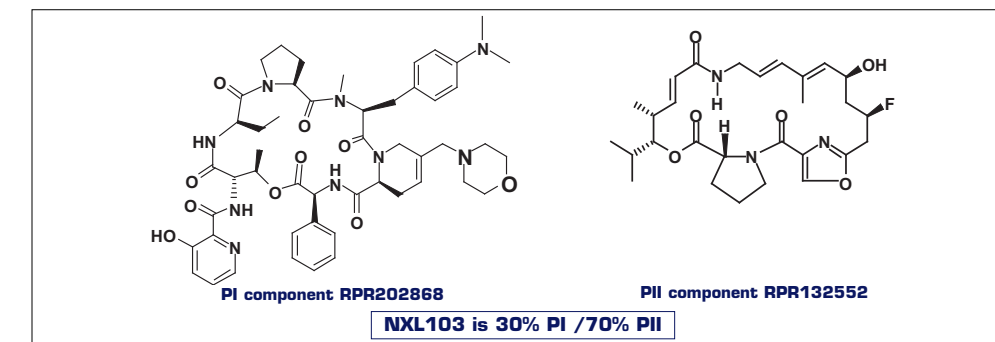
c) Test strain <i>S. pneumoniae</i> 030MV1. Bactericidal titre in the plasma of volunteers after oral dosing of 1500mg										
Time (h)	701	702	703	704	705	706	707	708	709	710
0	0	0	0	0	0	0	0	0	0	0
0.5	1052	0	511	360	333	0	971	1427	260	75
1	2097	0	973	305	1395	0	1422	2091	507	730
1.5	1699	0	991	530	1090	0	1068	1602	370	564
2	1627	0	1342	961	2156	0	1590	1663	361	289
3	2040	0	1280	1205	2701	0	1422	1423	440	687
4	833	0	660	942	1805	0	1019	1160	1440	960
6	421	0	288	324	981	0	526	724	433	209
8	144	0	249	118	725	0	193	339	453	69
10	66	0	123	46	328	0	81	168	184	34
12	38	0	58	28	212	0	42	101	78	19
15	20	0	42	16	113	0	28	50	27	8
24	8	0	11	6	24	0	8	11	12	0

a) Test strain <i>S. aureus</i> 011HT18. Bactericidal titre in the plasma of volunteers after oral dosing of 500mg										
Time (h)	301	302	303	304	305	306	307	308	309	310
0	0	0	0	0	0	0	0	0	0	0
0.5	361	1387	69	233	0	107	165	276	471	0
1	443	641	660	199	0	333	514	615	466	0
1.5	387	586	491	294	0	356	536	589	303	0
2	304	380	682	238	0	383	460	551	248	0
3	163	179	832	316	0	158	198	476	98	0
4	148	175	950	856	0	141	232	291	55	0
6	72	92	379	253	0	67	100	114	27	0
8	36	44	201	115	0	30	43	61	8	0
10	16	27	111	50	0	11	18	34	0	0
12	12	13	72	29	0	7	11	17	0	0
15	8	11	36	15	0	0	7	8	0	0
24	7	7	10	5	0	0	0	6	0	0

b) Test strain <i>S. aureus</i> 011HT18. Bactericidal titre in the plasma of volunteers after oral dosing of 1000mg										
Time (h)	501	502	503	504	505	506	507	508	509	510
0	0	0	0	0	0	0	0	0	0	0
0.5	0	218	33	308	716	169	0	280	732	1024
1	0	895	574	669	844	561	0	552	610	2181
1.5	0	673	267	562	1372	901	0	366	399	1968
2	0	664	381	717	1626	1138	0	695	310	1677
3	0	386	408	1321	2080	1575	0	454	541	1688
4	0	209	631	1764	1432	1247	0	616	367	1097
6	0	90	456	1012	873	404	0	215	160	492
8	0	40	322	416	480	147	0	84	74	253
10	0	20	143	177	288	62	0	37	38	148
12	0	9	60	100	182	31	0	24	25	93
15	0	7	23	49	92	18	0	9	14	51
24	0	0	0	12	18	0	0	5	8	13

c) Test strain <i>S. aureus</i> 011HT18. Bactericidal titre in the plasma of volunteers after oral dosing of 1500mg										
Time (h)	701	702	703	704	705	706	707	708	709	710
0	0	0	0	0	0	0	0	0	0	0
0.5	1052	0	511	360	333	0	971	1427	260	75
1	2097	0	973	305	1395	0	1422	2091	507	730
1.5	1699	0	991	530	1090	0	1068	1602	370	564
2	1627	0	1342	961	2156	0	1590	1663	361	289
3	2040	0	1280	1205	2701	0	1422	1423	440	687
4	833	0	660	942	1805	0	1019	1160	1440	960
6	421	0	288	324	981	0	526	724	433	209
8	144	0	249	118	725	0	193	339	453	69
10	66	0	123	46	328	0	81	168	184	34
12	38	0	58	28	212	0	42	101	78	19
15	20	0	42	16	113	0	28	50	27	8
24	8	0	11	6	24	0	8	11	12	0

Structure



RESULTS

- 500 mg dose (Fig 1a, Table 1a)**
Bactericidal concentrations were achieved against *S. aureus* in 6 out of 8 volunteers, and 5 out of 8 for *S. pneumoniae*. A titre of 4 was reached and maximum duration of effect was 4 hours.
- 1000 mg dose (Fig 1b, Table 1b)**
Bactericidal concentrations were achieved against *S. aureus* in all volunteers, and 4 out of 8 for *S. pneumoniae*. A titre of 8 was reached and maximum duration of effect was 6 hours.
- 1500 mg dose (Fig 1c, Table 1c)**
Bactericidal concentrations were achieved against *S. aureus* in all volunteers, and 6 out of 8 dosed for *S. pneumoniae*. A titre of 8 was reached and maximum duration of effect was 6 hours.

CONCLUSIONS

- Plasma concentrations which were bactericidal to both *S. aureus* and *S. pneumoniae* were achieved in the majority of subjects, particularly with 1g and 1.5g dosage.
- Although the dose response was not strictly linear, increasing doses from 0.5g to 1g or 1.5g of NXL103 resulted in more volunteers attaining bactericidal concentrations of PI and PII in plasma, and also resulted in a longer duration of effect.
- Further formulation/dose optimisation is ongoing. This will make NXL103 a potential therapy for oral treatment of community acquired RTI and SSTI.