F-251

Post Antibiotic Effect and Sub-MIC Effect of LTX-109 and Mupirocin on *S. aureus* Blood Isolates

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Abstract

BACKGROUND: The development of new synthetic antimicrobial peptides like LTX-109 provides a new class of drugs that can be considered for the treatment of *S. aureus* infections. We evaluated the post antibiotic effects (PAE) of this agent and mupirocin against 10 strains of *S. aureus*. The PAE is defined as the length of time that bacterial growth is suppressed following brief exposure to an antibiotic. The PAE has been recognized as a pharmacodynamic parameter, which may influence optimal dosage intervals. We also determined the sub-MIC effects (SME) of LTX-109, which measures the direct effect of sub-inhibitory levels on strains that have not previously been exposed to antibiotics.

METHODS: Isolates of *S. aureus* were selected from a group of blood stream isolates that included SCC type II (6), IVa (3) and IV (1). These isolates had MICs that ranged from 2-4 mcg/mL for LTX-109 and from 0.06 to >512 for mupirocin. For the PAE, tubes containing Mueller Hinton broth (MHB) and varying concentrations of LTX-109 were inoculated with 5 x 10^6 CFU/ml and incubated at 37°C for varying times. At the end of the exposure period, the cultures were diluted 1:1000 with MHB to remove the antibiotics and re-incubated. For the SME, organism was added to tubes containing 0.2X, 0.3X and 0.4X MIC of LTX-109 and incubated at 37°C. For all cultures, viability counts were taken every hour. The viability counts were used to determine the PAE and SME.

RESULTS: LTX-109 PAEs ranged from 3.3 to 9.3 hours. Mupirocin PAEs were all less than 1.3 hours except for one isolate that had a PAE of 1.9 hours. The range of the LTX-109 SME results are as follows: 0.2XMIC (0.6 to 2.15 hrs), 0.3X MIC (1.3 to 14.4 hrs), 0.4X MIC (2.85 to >24hrs).

CONCLUSIONS: LTX-109 not only possesses activity against mupirocin resistant strains, it demonstrated a prolonged PAE that supports persistence of activity for several hours after the drug is no longer present in the tissue. This is much longer than what was seen with mupirocin.

Introduction

LTX-109 is a new semi-synthetic antimicrobial peptide with a rapid bactericidal mode of action which has been considered for the treatment of bacterial skin infections. LTX-109 kills bacteria quickly and efficiently with a membrane-lysing mode of action which causes ultra rapid membrane disruption.

The post antibiotic effect (PAE) is defined as the length of time that bacterial growth is suppressed following a brief exposure to an antibiotic. The PAE is the period of time before the target organism resumes a normal growth rate after the complete removal of the antibiotic. The PAE is a pharmacodynamic parameter which may be considered in choosing an optimal antibiotic dosing regimen. The sub-MIC effect (SME) measures the direct effect of sub-inhibitory levels of the antibiotic on strains that have not been previously exposed to the antibiotic.

We have done extensive *in vitro* studies of LTX-109 and it has shown excellent activity against *S.aureus* strains resistant to several classes of drugs. In this study we evaluated the PAE of LTX-109 and mupirocin against 10 strains of *S.aureus*. We also determined the SME of LTX-109 against these 10 isolates as these studies may offer another advantage of this agent.

Methods

PAE testing:

- Tubes were prepared which contained 20mls of Mueller Hinton broth and LTX-109 or mupirocin at 2 times the previously determined MIC.
- For each sample we ran a control tube containing antibiotic free broth and a control to determine if the antibiotic removal process was effective, this tube contained the antibiotic at a 1:1000 dilution of the amount used for the sample exposure.
- Tubes were inoculated with the organism to obtain a final concentration of 5 x 10^6 CFU/ml and than incubated in a 37°C shaking water bath for 15 minutes
- After incubation the tubes were washed by centrifugation to remove the antibiotic.
- The PAE was determined by using the following calculation: **PAE=T-C**

  T= time required for the count of CFU in the test culture to increase by 1 log10 above the count observed immediately after drug removal.

  C= time required for the count of CFU in the untreated (antibiotic free) control culture to increase by 1 log10 above the count observed immediately after completion of the same procedure used on the test culture for drug removal.
- LTX-109 is a rapidly bactericidal agent, for this reason we performed the PAE using 2X MIC with a15 minute incubation.

SME Testing:

- Tubes were prepared which contained 20mls of Mueller Hinton broth and LTX-109 at 0.2X MIC, 0.3X MIC and 0.4X MIC
- Tubes were inoculated with the organism to obtain a final concentration of 5 x 10^6 CFU/ml and incubated in a 37°C shaking water bath.
- Every hour tubes were removed from the water bath and a colony count was performed
- The SME was determined by the following calculation: **SME=Ts-C**

  Ts= time required for the culture exposed only to sub-MIC concentrations to increase 1 log10 above the count observed immediately after dilution.

  C= corresponding time for the unexposed control.

Results

	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC							PAE-Hours	SME- Hours LTX-109 Mean			PAE-Hours
																	LTX-109				Mupirocin
ID	LTX-109	LTX-109	MUP	MUP	VAN	VAN	DAP	DAP	LZD	LZD	SCC	PVL	arc (A)	Source	PFGE group	Sample type	Mean	0.2X MIC	0.3X MIC	0.4X MIC	Mean
D 21	4	4	0.06	8	1	1	1	1	2	>8	II	Neg	Neg	Blood	USA-600	MRSA	3.3	1.6	2.5	8.1	0.8
NRS-17	2	2	0.12	16	8	8	4	4	1	4	II	Neg	Neg	Blood	USA-100	VISA	3.3	0.6	1.3	2.85	1.9
D 9	2	4	0.25	16	1	1	1	1	2	>8	II	Neg	Neg	Blood	USA-100	MRSA	3.9	1.1	1.45	4.6	0.95
D 11	2	4	>512	>512	1	1	1	1	2	>8	II	Neg	Neg	Blood	USA-600	MRSA	4.6	1.4	1.7	3.5	Not Done
D 25	4	4	0.25	16	0.5	0.5	0.5	0.5	2	>8	IV	Neg	Neg	Blood	USA-100	MRSA	5.1	1.45	2.85	>24 hrs	1.05
LNS-10	4	4	0.12	16	1	1	0.5	0.5	16	>8	II	Neg	Neg	Blood	No match	LNSSA	5.4	1.5	14.4	>24 hrs	0.9
D 2	2	2	0.12	8	1	1	1	1	2	>8	IVa	POS	POS	Blood	USA 300	MRSA	6.3	1.5	2.75	4.7	-0.6
DNS-6	4	4	0.25	32	2	2	4	4	2	>8	II	Neg	Neg	Blood	USA-100	DNSSA	6.6	1	1.95	3	1.2
DNS-7	4	4	0.12	16	2	2	4	8	2	>8	IVa	POS	POS	Blood	USA-300	DNSSA	8.1	2.15	3.1	17.1	1.2
D 19	4	4	0.12	8	1	1	0.5	0.5	2	>8	IVa	POS	POS	Blood	USA 300	MRSA	9.3	1.5	5.8	>24hrs	1.1
														Average of all Isolates:			5.6	1.38	3.78	11.58	0.85

Conclusions

- The PAE for LTX-109 averaged 5.6 hours versus only 0.85 hours for mupirocin. LTX-109 has been administered three times daily for clinical trials but in view of the prolonged PAE, it may be effective in less frequent administration.
- When the strains were exposed to LTX-109 at sub-MIC levels there was progressive prolongation of the growth curves as compared to the unexposed control. The average delay when exposed to 0.2X MIC was 1.38 hours, 0.3XMIC was 3.78 hours and 0.4XMIC was 11.58 hours.
- The PAE for LTX-109 was longer for USA 300 strains (6.3-9.3 hours) than for USA 100 strains (3.3-6.6 hours) though the number of isolates tested was small.
- In view of the rapid bactericidal activity of LTX-109, its activity against mupirocin resistant strains and the prolonged PAE as compared to mupirocin, LTX-109 demonstrates an *in vitro* and pharmacodynamic advantage over mupirocin.