Synergy of Polyphenon E® with Cefuroxime against 11 *Staphylococcus aureus* (MRSA)

E-1466

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Abstract

Background: Catechins, specifically epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG), are polyphenols present in green tea. Polyphenon E® (PE) is a partially purified fraction of green tea leaves from *Camellia sinensis* and consists of 85-95% catechins which includes 55-72% EGCG. In addition to clinical trials for various indications, the antimicrobial effect of PE is also under investigation. The *in vitro* synergistic activity of PE in combination with cefuroxime (CEF) was assessed using a time kill method against MRSA (ATCC 33592) and 10 MRSA clinical isolates. **Methods:** Study strains were tested for PE and CEF MICs by CLSI broth microdilution. Synergy testing was performed by time kill method with PE at 32 µg/mL and cefuroxime at 4 µg/mL. Bacterial counts were determined at 0, 8 and 24 h and the colony forming units (CFU)/mL from PE + CEF were compared to PE and CEF alone and to CFU/mL at time 0. **Results:** The PE MICs were 128 µg/mL for all MRSA and CEF MICs ranged from 16->256 µg/mL. There was >2 log(10) decrease in CFU with PE + CEF compared with the most single active agent at 8 and 24 h for all MRSA. The CFU decreased >2 log from the initial bacterial concentration for 9 of 11 strains at 8 h and 6 of 11 at 24 h. The differences in CFU/mL between PE + CEF and the single agent and initial counts [log(10)] were:

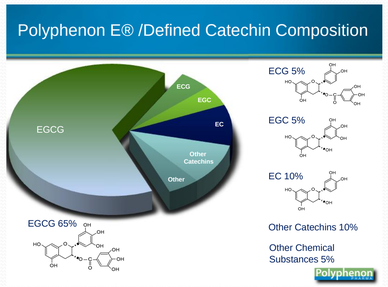
Strain	Compared to most active single agent		Compared to initial CFU/mL	
	8 h	24 h	8 h	24 h
ATCC 33592	4.89	4.31	2.84	1.36
MRSA 1	3.97	4.81	2.90	1.61
MRSA 2	6.48	3.44	4.40	0.48
MRSA 3	7.04	4.62	4.30	1.64
MRSA 4	3.60	3.30	1.17	-0.12
MRSA 5	5.48	7.36	3.98	4.28
MRSA 6	3.54	6.52	2.23	4.69
MRSA 7	6.43	7.40	4.22	4.52
MRSA 8	3.52	5.09	1.73	2.63
MRSA 9	5.00	5.66	3.65	3.32
MRSA 10	4.24	5.39	2.95	3.27

Conclusions: When CEF at a concentration of 4 µg/mL was combined with PE at ¼ MIC concentration of 32 µg/mL, a significant reduction in MRSA CFU/mL was achieved in comparison to PE and CEF alone and in comparison to initial CFU/mL. These data support further investigation for potential of PE in combination therapy against MRSA.

Introduction

Polyphenon E® is a partially purified fraction of green tea leaves from *Camellia sinensis* and is a mixture of catechins and other green tea components. Catechins constitute 85-95% of the total drug substance of which 55-72% by weight is EGCG and other catechins, epicatechin, epigallocatachin and epcatechin gallate, primarily comprise the rest. In addition to the known catechins, PE also contains gallic acid, caffeine and theobromine which together constitute about 2.5%.

The anti-bacterial activity of PE has been studied recently and synergy was observed with oxacillin or cefuroxime and PE against *Staphylococcus aureus* (MRSA) ATCC 33592. The objective of this study was to reproduce the synergy results obtained with cefuroxime and PE against MRSA 33592 and to extend the study to a set of clinical MRSA strains.



Materials & Methods

Antimicrobial Agents
Polyphenon E®, Polyphenon Pharma, Lot #PE070830
Cefuroxime, Sigma, Lot #067K0487

Microorganisms
S. aureus ATCC 33592 (methicillin and gentamicin resistant, mecA positive)
S. aureus ATCC 29213 (for MIC QC only)
10 *S. aureus* (MRSA) – clinical isolates collected in 2009 from University Hospital, Cleveland, Ohio

Media
Cation Adjusted Mueller Hinton Broth (CAMHB), Becton Dickinson, Sparks, MD

Methods

- Minimum inhibitory concentration (MIC) determinations were performed according to the CLSI broth microdilution procedure
- Synergy testing was performed according to time kill method based on the CLSI bactericidal procedure and was defined as >2 log difference from most active single component and at least a 2 log kill from the initial growth control.
- All strains were tested at a PE concentration of 32 µg/mL and a cefuroxime concentration of 4 µg/mL (average peak serum concentration).

Results

- Synergy was detected with MRSA 33592 using PE at ¼ the MIC and cefuroxime at 4 µg/mL, the average maximum serum concentration (Table and Figure 1).
- Synergy, based on at least a 2 log decrease in bacterial concentration compared to the most active single agent, was demonstrated with all 10 clinical MRSA after 8 and 24 hours incubation with the PE-cefuroxime combination (Table and Figures 2 – 4).
- The bacterial concentration decreased at least 2 logs after 8 hours incubation compared to the initial growth control for 7 of the 10 MRSA, nearly a 2 log reduction for 2 of the 10 MRSA and a 1.4 log reduction for MRSA #4 (which did have very high cefuroxime MIC of >256 µg/mL).
- The bacterial concentration remained at least 2 log(10) lower than the initial growth control for 6 of the 10 strains after 24 hours incubation.

Table: MIC and synergy results (tested at PE 32 µg/mL and cefuroxime 4 µg/mL) for all study strains

SA Strain	MIC (µg/mL)		Difference in CFU [log(10)]			
			Compared to CFU of most active single agent		Compared to initial CFU	
	Cefuroxime	PE	8 hrs.	24 hrs.	8 hrs.	24 hrs.
ATCC 29213	2	128	-	-	-	-
ATCC 33592	>256	128	4.89	4.31	2.84	1.36
MRSA 1	>256	128	3.97	4.81	2.9	1.61
MRSA 2	128	128	6.48	3.44	4.4	0.48
MRSA 3	64	128	7.04	4.62	4.3	1.64
MRSA 4	>256	128	3.6	3.3	1.17	-0.12
MRSA 5	16	128	5.48	7.36	3.98	4.28
MRSA 6	32	128	3.54	6.52	2.23	4.69
MRSA 7	32	128	6.43	7.4	4.22	4.52
MRSA 8	128	128	3.52	5.09	1.73	2.63
MRSA 9	128	128	5	5.66	3.65	3.32
MRSA 10	128	128	4.24	5.39	2.95	3.27

>2 log(10)

Figure 1: Time kill curve of *S. aureus* ATCC 33592

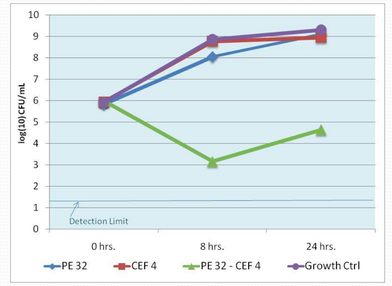


Figure 2: Time kill curve of MRSA 1

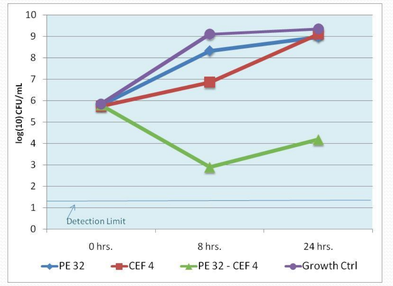


Figure 3: Time kill curve of MRSA 5

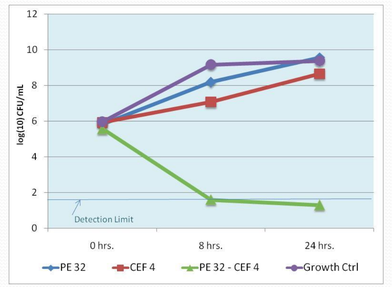
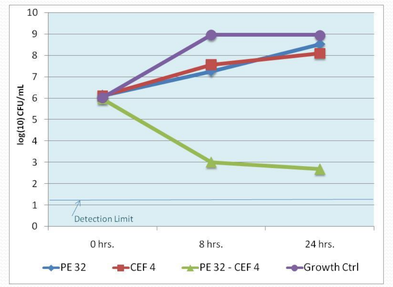


Figure 4: Time kill curve of MRSA 10



Conclusions

- Prior results demonstrating synergy with PE and cefuroxime against *S. aureus* ATCC 33592 have been confirmed in this study
- Time kill assays on 10 recent clinical MRSA isolates demonstrates *in vitro* activity of PE/cefuroxime against MRSA.
- Further testing and validation in an *in vivo* model is warranted

References

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The 49th Interscience Conference on Antimicrobial and Chemotherapy (ICAAC) 12-15 September 2009, San Francisco, CA, USA

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