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Short Communication

Synergic Antibacterial Effect of Curcumin with Ampicillin; Free Drug Solutions in Comparison with SLN Dispersions

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Abstract

Purpose: This study was designed to investigate benefit of using nanotechnology on increasing of synergic antibacterial effect of natural and chemical antibacterial agents.

Methods: At first the MIC and MBC of Curcumin and Ampicillin as selected antibacterial agents was determined, after that Solid Lipid Nanoparticles (SLNs) of each active ingredients as well as Curcumin-Ampicillin loaded SLNs were prepared using high pressure homogenization technique. Characterization of prepared SLNs was done, then MIC, MBC and contact killing time were investigated for Curcumin-Ampicillin loaded SLNs in comparison with free Curcumin and Ampicillin solutions as well as Ampicillin and Curcumin SLNs.

Results: Based on results nanoparticles with the size of 150 nm show much more decreased MIC and MBC when Ampicillin and Curcumin were loaded together on SLNs than solutions in which free Ampicillin and Curcumin were mixed.

Conclusion: It seems that using nanotechnology could cause decrease the dosage of antibiotics and risk of having antibiotic resistance bacteria strains.

Introduction

Todays increased resistance to antibiotics and decreasing of effective antibiotics has resulted in a challenge in the treatment of infections in the world.[1]

Curcuma longa as a plant of Zingiberaceae family has antimicrobial effect against many microorganisms with different mechanisms.[2-3] Ampicillin as a β-lactam could inhibit bacteria cell wall synthesis to kill the bacteria.[4] Beta-lactam antibiotics are the most preferred antibiotic group in the treatment of infections due to their wide spectrum and minimal side effects but bacteria go to be resistance against them.[5] Some studies have previously demonstrated that curcumin potentiates the effects of chemical antibiotics against bacteria strains. [6]

Our previous studies showed that loading of antibiotics on SLNs could cause to decrease MIC and MBC against S.aureus, Ecoli and P.aeroginosa significantly.[7,8]

The aim of this study was to investigate the synergistic effect of curcumin and ampicillin against P.aeroginosa, S.aureus, Ecoli, C.diphtheria, B.Subtilis and MRSA as well as to determine the antibacterial activity of their combination when loaded on SLNs.

Materials and Methods

Preparation of Solutions

Solution A

A stock solution of free ampicillin was prepared in sterile water that was further diluted in Muller–Hinton broth to reach a concentration of 0.125 μg/mL.

Solution B

A stock solution of free curcumin was prepared in sterile water that was further diluted in Muller–Hinton broth to reach a concentration of 0.125 μg/mL.

Solution C

Then a stock solution of free ampicillin and curcumin was prepared by mixing of 50-50% of the two above solutions.

Preparation of SLNs

Curcumin and ampicillin loaded SLNs were prepared separately using high pressure homogenization method based on the Varshosaz et al method in which hot oily phase was added to aqueous phase under homogenization.[9] Table 1 shows the formulations.

Table 1. SLN formulations.

Formulation name	Amount of curcumin(mg)	Amount of Ampicillin(mg)	Amount of cholesterol(mg)	Amount of tween 80(mg)	Distilled water(ml)	Ethanol/acetone (ml)	Homogenization (minutes)
A	726	----	80. 605	1.5	121	22/7.5	7
B	----	218	605	1.5	121	22/7.5	7
C	726	218	605	1.5	121	22/7.5	7
D	363	109	605	1.5	121	22/7.5	7
E	Mixture of formulation A and B in 50-50% ratio, formulation E was designed to compare that is it better that active ingredients load together on SLNs or each of them load separately and then prepared drug loaded SLNs mix in 50-50% ratio (comparison of formulation D and E).						

Determination of particle size of SLNs

All SLNs, were investigated to determine particle size distribution by Malvern instrument.

Morphology studies

Morphology of the nanoparticles was characterized by scanning electron microscopy.

Antibacterial studies
Inhibitory zone Determination

The "well diffusion test" was carried out using P.aueroginosa (ATCC 27853), S.aureus (ATTC 25923), Ecoli (ATCC 25922), C.diphtheria (ATCC 39255), B.Subtilis (PTCC 1720) and MRSA 252.[8]

MIC and MBC Detection

The conventional broth macrodilution tube method was used to determine MIC and MBC of all above solutions and formulations with respect to P.aueroginosa, S.aureus, Ecoli, C.diphtheria, B.subtilis and MRSA in vitro.[7,10]

killing contact time Determination

In this procedure, 0.1 ml of bacteria suspension equivalent to 0.5 McFarland standards were added to tubes contain 9.9 ml Muller-Hilton broth with MIC concentration of formulations A, B, C, D and E Muller-Hilton broth as control, and incubation was done at 37°C. Then samples were taken from growing bacteria cultures in desired times and spread on Muller-Hilton agar and colonies were count after incuation.

Results and Discussion
Particle size of SLNs

Results (Z-average) showed that the particle size of formulations A,B,C and D were 159, 149, 163 and 145 nm respectively. Poly Dispersity Indexes were less than 0.5 for all formulations.

Morphology studies

All desired SLN formulations were spherical.

Antibacterial studies
Determination of inhibition zone

Table 2 shows the result of inhibition zone of formulation A-D. Results show that although MRSA and C.diphtheria are resistant against curcumin SLNs (formulation A) but these SLNs could increase inhibition zone when is combine with ampicillin on SLNs (formulation C), so a synergy could be seen. There are not significant differences between formulation D and E for none of bacteria strains except S.aureus, MRSA and P.aueroginosa in which seems that it will be better if drugs load together in formulation process (formulation D) instead of preparing of each formulation (A and B) and then mix them in 50-50% ratio (formulation E).

Table 2. Results of measurement of inhibition zone of formulations A-E for six bacterial strains

Bacteria strain	Formulations				
	A	B	C	D	E
S.aureus	1.5 cm	2.5 cm	3 cm	3 cm	2.5 cm
Ecoli	0.5 cm	3 cm	2 cm	1.5 cm	1.5 cm
B.Subtilis	1 cm	1.5 cm	2.5 cm	2 cm	2 cm
P.aueroginosa	0	1 cm	1 cm	0.75 cm	0.5 cm
C.diphtheria	0	2 cm	2.25 cm	2 cm	2 cm
MRSA	0	1.5 cm	2 cm	1.5 cm	1.25 cm

MIC and MBC studies

Results were shown in Tables 3 and 4. The enhancement of antibacterial effect when curcumin used with ampicillin was obtained as reported in Table 3 for free drugs solutions especially for MIC regarding to B.subtilis, C.diphteria and MRSA. Comparisons of data series of Table 3 and 4, show that MIC and MBC of ampicillin and curcumin which were loaded together on SLNs (formulation C) were much more decreased than solution C in which free ampicillin and curcumin was mixed together. About B.subtilis MIC of formulation C is practically the same as solution C but the resistancy of bacteria to free drugs in MBC was broken by using SLNs. P.aueroginosa is resistant against free drug

mixture of ampicillin and curcumin but formulation C is effective on this strain and MBC is two times larger than MIC in this situation. For C.diphteria, no significant changes were observed in MIC of Solution C and formulation C but MBC decreased 4 times when drugs loaded on SLNs in comparison with free drugs. About MRSA MIC decreased 2 times with using SLNs and the resitancy of MRSA about MBC was broken. The increasing of antibacterial effect of ampicillin SLNs in comparison with free ampicillin against Ecoli, S.aureus and P.aueroginosa was reported by Alihoseyni et al.[8] Previously the efficacy of SLNs to decrease MIC and MBC of Curcumin was demonstrated.[11]

Killing contact time

Table 5 shows killing contact time results. Formulation C after 10 hours contact with S.aureus, Ecoli, P.aueroginosa, C.diphtheria, B.subtilis show the highest killing percentage in comparison with formulations A, B, D and E. The efficacy of all formulations against all bacteria strains is time dependent and the most bacteria killing percentage was reported after 10 hours, may be due to sustained drug release profiles.

Presented results demonstrated the probability of benefits of using nanotechnology to decrease the dosage of antibiotics and decrease risk of having antibiotic resistance bacteria strains.

Table 3. MIC and MBC results for desired solutions against six bacteria strains

Bacterial Strains	MBC (µg/ml)			MIC (µg/ml)		
	Solutions			Solutions		
	A	B	C	A	B	C
S.aureus	8	Resistance	7.5 (ampi) 125 (cur)	2	125	2(ampi) 31.25(cur)
Ecoli	30	Resistance	30 (ampi) 500(cur)	8	500	7.5(ampi) 125(cur)
B.Subtilis	Resistance	Resistance	Resistance	30	Resistance	15(ampi) 250(cur)
P.aueroginosa	Resistance	Resistance	Resistance	Resistance	Resistance	Resistance
C.diphtheria	30	Resistance	30 (ampi) 500(cur)	8	250	7.5(ampi) 125(cur)
MRSA	Resistance	Resistance	Resistance	30	Resistance	15(ampi) 250(cur)

Table 4. MIC and MBC results for desired formulations against six bacteria strains

Bacterial Strains	MBC (µg/ml)				MIC (µg/ml)			
	Formulations Name				Formulations Name			
	A	C	D	E	A	C	D	E
S.aureus	250	4(ampi) 62.5(cur)	4(ampi) 62.5(cur)	4(ampi) 62.5(cur)	125	0.5(ampi) 7.8 (cur)	4(ampi) 62.5(cur)	1 (ampi) 15.62 (cur)
Ecoli	500	8(ampi) 125(cur)	16(ampi) 250(cur)	16(ampi) 250(cur)	250	4(ampi) 62.5(cur)	16(ampi) 250(cur)	8(ampi) 125(cur)
B.Subtilis	1000	32(ampi) 500(cur)	64(ampi) 1000(cur)	32(ampi) 500 (cur)	500	16(ampi) 250(cur)	32(ampi) 500(cur)	16(ampi) 250 (cur)
P.aueroginosa	resistant	32(ampi) 500(cur)	64(ampi) 1000(cur)	32(ampi) 500 (cur)	resistant	16(ampi) 250(cur)	32(ampi) 500(cur)	16 (ampi) 250 (cur)
C.diphtheria	500	8(ampi) 125(cur)	16(ampi) 250(cur)	16(ampi) 250(cur)	250	8(ampi) 125	16(ampi) 250(cur)	8(ampi) 125 (cur)
MRSA	resistant	16(ampi) 250(cur)	32(ampi) 500(cur)	32(ampi) 500(cur)	resistant	8(ampi) 125(cur)	32(ampi) 500(cur)	8(ampi) 125(cur)

Table 5. Results of killing contact time studies for SLNs

Bacteria Strain	Formulation Name	Percentage of decreasing on bacteria count (%)		
		4 hrs	8 hrs	10 hrs
S.aureus	A	80	86	96
	B	0	0	100
	C	88	100	100
	D	50	70	100
	E	75	95	100
Ecoli	A	68	60	81
	B	66	73	83
	C	50	73	85
	D	0	11	72
	E	50	70	83
P.aueroginosa	A	0	12	25
	B	9	18	54
	C	0	25	62.5
	D	0	9	45
	E	0	20	60
C.diphtheria	A	0	50	70
	B	66	66	66
	C	60	85	93
	D	25	60	70
	E	0	20	60
B.Subtilis	A	0	0	0
	B	0	70	70
	C	83	80	100
	D	25	75	100
	E	150	50	70
MRSA	A	0	33	86
	B	0	28	64
	C	44	72	83
	D	0	53	76
	E	25	65	90

Ethical Issues
Not applicable.

Conflict of Interest
The Authors report no declaration of interest.

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