Effects of azelaic acid and its derivatives selection in nanovesicles on cell lines

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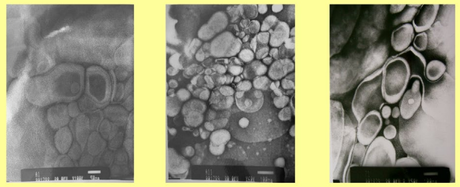
Abstract

New drugs development is difficult rather time consuming in the process, expensive involving preclinical testing, investigational new drug application, clinical trials and FDA approval. Liposomes and niosomes are nanovesicles which have been widely used as drug carriers. The encapsulation of drugs in these vesicular systems offers several advantages, including the modification of the lipophilicity and hydrophilicity, decreasing of toxicity, increasing of stability in circulation time and absorption of the drug. The azelaic acid (AA) nanovesicles and its derivatives compose of AA was modified by complexing AA wicvcdth hydroxypropyl-β-cyclodextrin dAACD) and AA was improved to diethyl azelate (DA) by esterification with Fischer reaction for pharmaceutical was determined. AA AACD and DA were incorporated in liposomes and noisome with the compositions of L-α-dipalmitoyl phosphatidylcholine/cholesterol and Tween 61/cholesterol. AA, AACD and DA and AA, AACD and DA in nanovesicles, using MTT assay in three cancer cell lines (HeLa, KB and $B_{16}F_{10}$) comparing with vincristine, were investigated. AACD showed the highest poteney comparing to AA in HeLa, KB and $B_{16}F_{10}$ AA entrapped in liposomes more potent than the free AA, and less potent than vincristine. When entrapped in bilayer vesicles, DA and AACD were more effective than AA in killing cancer cells. AACD entrapped in liposomes gave the highest anti-proliferation activity in HeLa cell lines with the IC_{50} more potent than vincristine and AA. DA in liposomes demonstrated the IC_{50} less potent than vincristine in KB cell lines, while in $B_{16}F_{10}$ AACD in niosomes showed the IC_{50} less potent than vincristine. This study has suggested that the modification of AA by derivatization and complexation as well as the entrapment in bilayer vesicles can enhance its therapeutic efficacy. However, the cytotoxicity of AA and its derivatives incorporated nanovesicular formulations on mouse epidermal cell lines (JB_6,normal cell lines), using the SRB assay was modest when compared with cisplatin. Plain liposomes and niosomes gave no growth inhibitory effect. AA incorporated nanovesicles has been proved has antiproliferative effect in cancer cell lines. Furthermore, the safety of AA and its derivatives when incorporated in

Transmission electron microscopy (TEM1200S, JEOL, Tokyo, Japan)

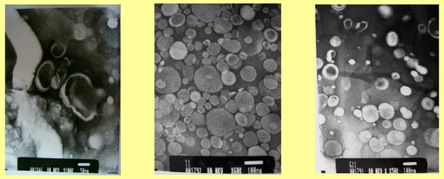


(a) (b) (c)

Pic.1

Negative staining TEM images of liposomes and niosomes entrapped with AA, AACD, and DA

(a) DPPC/CHL (7:3 molar ratio) vesicles containing AA (x100K)
(b) Tween61/CHL (1:1 molar ratio) vesicles containing AA (x60K)
(c) DPPC/CHL (7:3 molar ratio) vesicles containing AACD (x100K)



(d) (e) (f)

Pic.2

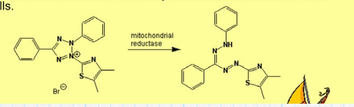
Negative staining TEM images of liposomes and niosomes entrapped with AA, AACD, and DA (continued)

(d) Tween61/CHL (1:1 molar ratio) vesicles containing AACD (x100K)
(e) DPPC/CHL (7:3 molar ratio) vesicles containing DA (x60K)
(f) Tween61/CHL (1:1 molar ratio) vesicles containing DA (x50K)

MTT assay

Principle

- Yellow MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is reduced to purple formazan in the mitochondria of living cells.
- This reduction takes place only when mitochondrial reductase enzymes are active, and therefore conversion is directly related to the number of viable (living) cells.



Anti-proliferative activity assay
Cell culture and treatment
- Human cervical adenocarcinoma (HeLa)
- Human epidermal carcinoma (KB)
- Murine melanoma ($B_{16}F_{10}$)

IC_{50} values of AA, AACD and DA in HeLa, KB, and $B_{16}F_{10}$ cell lines

Table 1

Sample	HeLa			KB			$B_{16}F_{10}$		
	IC_{50} (mM)	Potency (times)		IC_{50} (mM)	Potency (times)		IC_{50} (mM)	Potency (times)	
		$T_{1\,HeLa}$	$T_{2\,HeLa}$		$T_{1\,KB}$	$T_{2\,KB}$		$T_{1\,B16F10}$	$T_{2\,B16F10}$
AA	5.560	0.007	1.000	6.710	1.490×10^{-4}	1.000	6.950	0.005	1.000
AACD	3.750	0.010	1.482	4.200	2.380×10^{-4}	1.600	4.640	0.008	1.500
DA	15.500	0.002	0.359	7.610	1.310×10^{-4}	0.881	15.400	0.002	0.451
Liposome	NA	-	-	NA	-	-	NA	-	-
Liposome entrapped with AA	0.061	0.639	91.150	NA	-	-	NA	-	-
Liposome entrapped with AACD	0.017	2.294	327.060	NA	-	-	NA	-	-
Liposome entrapped with DA	NA	-	-	0.035	0.028	191.710	NA	-	-

IC_{50} values of AA, AACD and DA in HeLa, KB, and $B_{16}F_{10}$ cell lines (continued)

Table 2

Sample	HeLa			KB			$B_{16}F_{10}$		
	IC_{50} (mM)	Potency (times)		IC_{50} (mM)	Potency (times)		IC_{50} (mM)	Potency (times)	
		$T_{1\,HeLa}$	$T_{2\,HeLa}$		$T_{1\,KB}$	$T_{2\,KB}$		$T_{1\,B16F10}$	$T_{2\,B16F10}$
Niosome	NA	-	-	0.090	-	-	0.184	0.201	37.771
Niosome entrapped with AA	NA	-	-	NA	-	-	0.119	0.311	58.403
Niosome entrapped with AACD	NA	-	-	NA	-	-	0.074	0.500	93.919
Niosome entrapped with DA	0.041	0.951	135.610	0.040	0.025	167.750	0.081	0.457	85.802
Vincristine (positive control)	0.039	1.000	142.564	0.001	1.000	6710.000	0.037	1.000	187.838

The values were determined from at least three independent experiments NA, IC50 was not founded even at the highest concentration used
T_1 = potency comparing to vincristine = IC_{50} vincristine/IC_{50} sample
T2 = potency comparing to AA = IC_{50} AA/IC_{50} sample

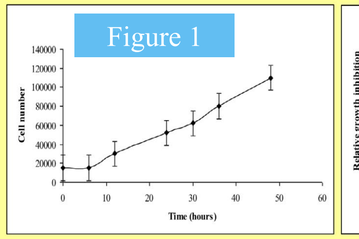
Sulforhodamine B Assay

Principle

- SRB is a bright pink aminoxanthene dye possessing 2 charged SO^{-3} groups capable of electrostatically binding to positive counterions.
- Under mildly acidic conditions, SRB binds to the positive fixed charges of biological molecules.
- In TCA fixed cells, these binding sites are primarily the amino groups of proteins.
- SRB behaves like bromphenol blue, naphthol yellow S, and coomassie blue, which are also used widely as protein stains, but it gives a higher OD and better signal to noise ratio at low cell density than do these other dyes.
- SRB binds to the basic amino acids of cellular macromolecules and the solubilized stain is measured spectrometrically to determine relative cell growth or viability in treated or untreated cells.

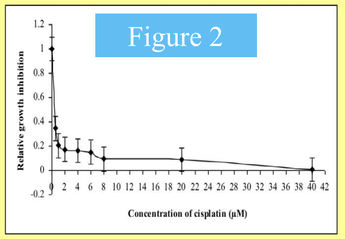
Results

Figure 1



Growth curve of JB6 mouse epidermal cell lines

Figure 2



Dose–response plot of JB6 cells with Cisplatin treatment (24 hours drug exposure)

Figure 3

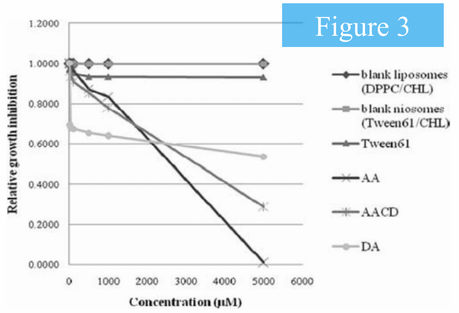


Figure 3 Cytotoxic effect of dose-response plot of JB6 cells with blank liposomes (L-α-dipalmitoyl phosphatidylcholine/cholesterol [DDPC/CHL], 7:3), blank niosomes (Tween 61/CHL, 1:1), Tween 61, azelaic acid (AA), AA β-cyclodextrin complex (AACD) and diethyl azelate (DA) treatments.

Figure 4

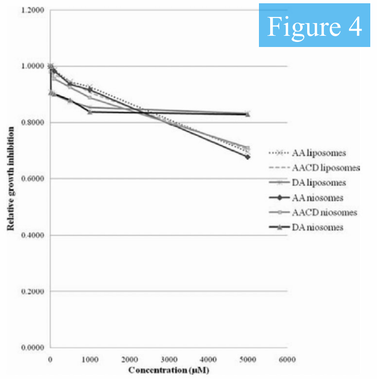


Figure 4 Cytotoxic effect of dose-response plot of JB6 cells with AA liposomes, AACD liposomes, DA liposomes, AA niosomes, AACD niosomes, and DA niosomes treatment.

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Discussion and Conclusion

From other literatures, many cancer drugs were chossed by the targeted cancer cell such as structure, bio marker or energy and food etc. However, in this study was different. AA gave IC_{50} values all three cell lines. However AA was entrapped nano vesicles shows the selective cancer cells that maybe the target of cancer cells can not suitable for drug loaded-nano vesicles. Liposomes loaded drug (AA and AACD) gave IC_{50} in HeLa, in simple AA is the major drug and CD just the complex of AA, CD helps the solubility of AA and the complex was move to the water layer of liposoms. Thus AA and AACD entrapment in liposome which gave the same pattern. However, DA was modified AA to ester structure which gave different pattern, it gave IC_{50} in KB cells it maybe DA move to lipid layer of liposoms. Niosome entrapped AA AACD and DA gave IC_{50} in B_{16} F_{10}. Niosome is produced by surfactant, it maybe solubilize the cell wall of B_{16} F_{10} but not destroy the HeLa and KB. Moreover niosome entrapped with DA gave IC_{50} values all cancerous cells. Thus the selective, they must prove in simple that the nanovesicles entrapped AA DA and AACD must choose two steps to act cell lines. They maybe study in deeply the mechanism of the selection such as Direct killing through the release of harmful products, Direct cytolysis of cancer cells and Indirect killing by recruitment of other immune cells that can lyse the cancer cells. In SRB assay on JB6 normal cells which use for prove safety AA DA and AACD with entrapped or without entrapped drug. From figure 3, 4 gave safety for JB6 cells but AA and AACD gave different because of acidity of drug to destroy cell lines and DA which saturated concentration.