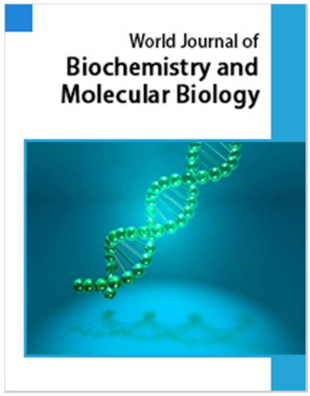
World Journal of Biochemistry and Molecular Biology
2017; 2(3): 12-15
http://www.aascit.org/journal/wjbmb

American Association for Science and Technology



Keywords
Exosome,
Nanoparticles,
Anti-Ovarian Cancer,
Plant Extract

Received: June 12, 2017
Accepted: August 1, 2017
Published: September 8, 2017

Exosome-Loaded Plant Extract as an Anti-ovarian Cancer Drug Delivery System

Ukwubile Cletus Anes[1, *], Nettey Henry[2]

[1]Department of Science Laboratory Technology, Biology Unit, School of Science and Technology, Federal Polytechnic, Bali, Nigeria
[2]Department of Pharmaceutics and Microbiology, University of Ghana School of Pharmacy, University of Ghana, Legon Accra, Ghana

Email address

doccletus@yahoo.com (U. C. Anes)

[*]Corresponding author

Citation

Ukwubile Cletus Anes, Nettey Henry. Exosome-Loaded Plant Extract as an Anti-ovarian Cancer Drug Delivery System. *World Journal of Biochemistry and Molecular Biology.* Vol. 2, No. 3, 2017, pp. 12-15.

Abstract

Exosomes are nanoparticles that play a crucial role in intercellular communication. This research investigated the role of exosome as drug carrier in ovarian cancer therapy. Exosomes were isolated by ultracentrifugation of blood from the liver. Morphology of exosome-plant complex was studied using scanning electron and atomic force microscopes. Under optimal conditions for cancer therapy, exosome-loaded plant extract showed higher anti-ovarian cancer efficacy such as reduced viable cell, high inhibitory concentration, induced apoptosis, and prevention of metastasis of cancer cells than doxorubicin anticancer drug. Our results showed that exosome-loaded anti-ovarian cancer plant extract is a promising nanosized drug formulation for ovarian cancer therapy.

1. Introduction

Exosome are cell-derived vesicles that are present in many and perhaps all biological fluids including blood, urine and cultured medium of cell cultures. They are a class of membrane secreted lipid vesicles formed intracellular by invaginations of the multi-vesicular body limiting membrane and then fused out of the cell plasma membrane [1]. These small vesicles range from 30-100 nm and are of endocytic origin that were first proposed to function as a way for reticulocytes to eradicate the transferrin receptor while maturing into erythrocytes, were later named exosomes. As intercellular communicators, exosomes have received much attention as not only a basic natural characteristic, transporting mRNA and proteins among cells, but also a possible alternatives to traditional nanoparticle approaches as drug delivery vehicles with certain advantages: a. biological and small drugs can be loaded into exosomes due to the natural presence of proteins and genetic materials; b. exosome derived drug delivery vehicles have broader distribution in biological fluids, likely producing longer circulating time and possibly better efficacy; c. Exosomes derived from tissue-specific cells can cross the physiological barrier and target the tissue via their natural surface proteins [2-4]. Exosomes potentially have advantages over polymer and liposome based nano-particle delivery systems, with a better safety profile and better selectivity [5].

The plant *Camelia sinensis* is a taxon of dicotyledonous flowering plants found in the tropics. In Nigeria, the plant is found in Ngoruje (Sarduana Local Government Area) Taraba State, where it is locally called “Tea” by the Fulanis, and used to produce the

famous green tea by The Mambila Beverages Company Ltd, Kakara, Taraba State. This study was carried out to investigate the application of exosomes-loaded plant extract of *C. sinensis* as a carrier of anti-cancer drug in cancer drug delivery system.

2. Materials and Methods

2.1. Materials

The materials used in this study were: air-dried leaves of *Camelia sinensis*, ovarian cancer cell line, centrifuges model 800D, oven, buffer solution, Scanning Electron microscope, X-ray diffractometer, Bovine serum albumin, penicillin plus streptomycin solution GC-MS (Agilent 7820A), methanol (analytical grades), PBS solution, MTT reagent, CO_2 incubator, filter paper, scanning biological microscope, magnetic stirrer, among others.

2.2. Methods

Collection, identification, preparation and extraction of plant.

Fresh leaves of *C. sinensis* (Theaceae), were collected from Kankara, Sarduana Local Government Area, Taraba State, and was authenticated by a biologist. The leaves were air-dried and reduced into fine powder using electronic blender. 2000 g of the powder was defatted in 2500 mL petroleum ether and then extracted with 5 Liters of methanol by Soxhlet apparatus. The filtrate was concentrated *in vacuo* at room temperature and further fractionated successively using solvents in increasing order of their polarities from the eluotropic series. Final weight of methanol leaf extract was calculated. Fractions of extracts were bio-guided by anti-ovarian cancer activity *in vitro*. Fraction with best biological activity (anti- ovarian cancer) was used for the study. Purification of the compound was done using a 60 cm long glass column and 60-120 mesh size silica gel by step-wise gradient elution.

2.2.1. Isolation of Exosome from Whole Blood

Serum was obtained from a freshly procured liver by squeezing the liver to release blood. The blood was subjected to ultracentrifugation at 3000xg over night at 4°C to obtain serum containing exosomes [6].

2.2.2. Preparation of Exosome-Loaded Plant Extract (CE)

Exosome was dissolved in DEPC (diethylpyrocarbonate)-treated water to a final concentration of 0.88 mg/mL (stock solution). Purified plant extract from the construction reaction was diluted to 2 μm in DEPC treated water (pH 4.6). The Isolated extract solution was added to exosome solution to obtain the weight ratios of 4, 8, 16, and 32. The mixture was gently pipetted and vortexed for 3–5 s to initiate EX/PE complex formation and left for 30 min at room temperature. Complex formation was confirmed by gel electrophoresis [6].

2.2.3. Morphological Studies of Exosome-Loaded Plant Extract

The morphology of exosome-plant complex was determined by an atomic force microscope (AFM) using tapping-mode AFM in air and scanning electron microscope [7-8].

2.2.4. Complex Formation

Exosome was dissolved in DEPC (diethylpyrocarbonate) - treated water to a final concentration of 0.88 mg/mL (stock solution). Purified plant extract from the construction reaction was diluted to 2 μm in DEPC treated water (pH 4.6). The solution was added to exosome solution to obtain the weight ratios of 4, 8, 16, and 32. The mixture is gently pipetted and vortexed for 3–5 s to initiate complex formation and left for 30 min at room temperature. The complex formation was confirmed by electrophoresis.

2.2.5. Size and Zeta Potential Measurement

The particle sizes and surface charge of the complexes was measured by photon correlation spectroscopy (PCS) using the Zeta sizer at room temperature. The complexes was diluted with phosphate buffer saline (PBS) pH 7.2 which was passed through 0.22 μm membrane filter prior used. All samples was measured in triplicate.

2.2.6. Morphology Study of Exosome

The morphology of exosome was determined by an atomic force microscope (AFM) using tapping-mode AFM in air. The complexes was diluted with distilled water which was passed through 0.22 μm membrane filter prior used. These samples was dropped immediately onto freshly cleaved mica and air-dried. Ovarian cancer cell line OV1063 was obtained. The cell line was maintained in suitable medium supplemented with 10% fetal bovine serum and antibiotics (100 units / mL penicillin and 100 mg / mL streptomycin), then incubated at 37℃ in a 5% CO_2 incubator. At 24 hours before transfection, the cells were added to a 96 well plate at a concentration of 2×10^5 cells / well, then transfected with the complexes. Fresh medium was changed after transfection for 24 h and continued to culture for another 48 h.

2.2.7. Cytotoxicity

Evaluation of exosome complexes and standard anticancer drug doxorubicin cytotoxicity were performed by MTT assay, and using tadpoles**.** Tadpoles were scooped from ponds at Daniya Bali Local Government Area of Taraba State and was properly identified by Mr. Ukwubile Cletus Anes of Biology Unit. Ten tadpoles of similar sizes were selected with the aid of a broken Pasteur pipette into different beakers containing 30 mL of the natural water from the habitat of tadpoles. This was made up to 49 mL with distilled water. The mixture was made up to 50 mL with 20, 40, 100, 200 and 400 μg/mL of the leaf extract in 5% DMSO. The assay was performed in triplicate and a control assay was performed using 50 mL containing 1mL of 5% DMSO in distilled water. This procedure was repeated for three times [8].

2.2.8. Determination of Antiproliferative Effects on *Sorghum Bicolor* (Guinea corn) Radicle Length

Seeds of *S. bicolor* (Guinea corn) was purchased from Bali market in Taraba State. A simple viability test carried out by placing the seeds in distilled water. The viable seeds sank because of their denser embryonic tissues, unlike the non-viable seeds which floated and were therefore discarded. The floating seeds were decanted and separated from the viable ones. The viable seeds were washed with 95% ethanol for sterilization for 1 minute and were finally rinsed with distilled water. Ten (10) mL different concentrations of the leaf methanol extract (1-30 mg/mL) containing 5% DMSO was poured into the Petri-dish of about 9 cm wide containing filter (Whatman No. 1) underlay with cotton wool, after which twenty (20) of the sterile Petri-dishes was incubated in a dark cupboard at room temperature and the lengths of the radicle emerging from the seeds was measured at 24, 48, 72 and 96 hours. The control seeds was treated with 10 mL distilled water containing 5% DMSO. The experiment was carried out in triplicates for all concentrations and controls while the radicle lengths was measured to the nearest millimeter. The procedure was carried out for both the leaf extract and the complexes.

2.2.9. Statistical Analysis

The data obtained were expressed as mean ± SEM. Significant difference between means were determine at $p \leq 0.05$ (one-way ANOVA).

3. Results and Discussion

In Table 1, exosome-complex formulation showed percentage entrapment efficiency in dose dependent manner. This showed that the higher the percentage of entrapment efficiency of exosome, the better the therapeutic measures in the cell. In Table 2, the various kinetic equations used in order to understand the kinetic and mechanism of drug release from the in vitro studies showed that exosome-loaded drug exhibited a slow drug release at 0.8-1.0 and entrapment between 45.88 ± 0.1^a and 62.93 ± 0.01^b in concentration dependent fashion. This revealed that exosome as a nano-carrier for anticancer drugs does not show exponential release of its content thereby exposing potent drugs to enzymatic actions [9-10]. It then means that exosome offers distinct advantages that uniquely positioned them as high effective drug carriers composed of cellular membranes with multiple adhesive proteins on their surface, specialized in cell-to-cell communication and provide an exclusive approach for the delivery of various therapeutic agents to target cells. In table 3, exosome-loaded plant extract showed strong inhibitory efficacy on ovarian cancer cell line in concentration dependent manner, with CE1 having the least IC_{50} value of 322.50 µg/mL while CE V showed the highest inhibitory concentration of 550.12 µg/mL on the growth of ovarian cancer cell. It is possible therefore, that exosome-loaded *C. sinensis* leaf methanol extract inhibited the growth of ovarian granulosa cells by correcting defective gene like FOXL2 and promoting apoptosis of the cell (Table 3). Therefore, the cell is devoid of metastasis of any kind [11]; showing the potential of exosome as a strong drug delivery system in cancer therapy. This result was comparable to the inhibitory effect showed by a first line anti-cancer drug doxorubicin at $p \leq 0.05$ (one-way ANOVA) that was delivered ordinarily (i.e. not in exosome).

Table 1. *Formulation of exosome-loaded extract complexes (CEC).*

Batch code	Drug: Carrier ratio (mL)	Experimental Weight (g)	% EE
CE I	1:1	161. 5	45.88 ± 0.1^a
CE II	1:2	191. 5	54.40 ± 0.1^a
CE III	1:3	201.5	57.24 ± 0.01^b
CE IV	1:4	211.5	60.1 ± 0.1^a
CE V	1:5	221.5	62.93 ± 0.01^b

Results are mean ± SEM, n = 3, numbers followed by the same alphabet are statistically significant at $p \leq 0.05$, CE (*C. sinensis*- exosome complex), EE (entrapment efficiency), % EE = (Experiment drug weight/ Theoretical drug weight) X 100.

Table 2. *Correlation coefficients according to different kinetic equations.*

Formulation	Cumulative drug Released (%)	Zero order	First order
FM1	84.31	0.8	1.0
FM2	82.70	0.9	1.0
FM3	75.30	0.8	1.0
FM4	73.01	0.8	1.0
FM5	70.02	0.8	1.0

FM1-5 = formulations, zero order (cum. drug released Vs time), first order (log % drug remaining Vs time)

Table 3. *Growth inhibition assay of human ovarian cancer cell line OV1063.*

Sample code	Concentration (µg/mL						IC_{50} (µg/mL)
	6.25	12.5	25.0	50.0	100.0	200.0	
CE I	2.11	2.51	4.12	17.20	24.20	30.11	322.50
CE II	3.13	5.00	6.17	22.00	30.11	42.10	400.10
CE III	4.22	8.12	10.00	25.00	32.01	45.02	405.12
CE IV	6.12	12.02	10.50	28.12	38.14	51.04	500.45
CE V	8.10	14.45	12.88	32.00	41.12	57.10	550.12
Do.RuB	1.22	1.88	2.11	16.00	20.12	22.00	108.04

n= 3, Do.Rub (doxorubicin anticancer drug), CE I-V (*C. sinensis*-exosome formulations)

4. Conclusion

Cancer nanotechnology is on its way to accelerating early detection of ovarian cancer and improving therapy of resistant and recurrent cases. In order to enhance this further, training of bio-researchers is a must, and this will create additional interdisciplinary collaborative opportunities with their non-bio-researchers or engineering colleagues to advance the field further into a direction of research that is disease oriented, and thus accelerate specific disease needed solutions.

The study therefore, showed that exosome nanoparticles serve as effective and safe therapeutics in cancer therapy as drug carrier, and this research aspect should be sustained for cancer treatment.

Acknowledgements

The authors are grateful to Prof. Alexander Nyarko and Mr. Clement of the University of Ghana School of Pharmacy, College of Health Sciences, Accra, Ghana.

References

[1] Li, E, Zeringer, T., Barta, J., Schageman, A., Cheng, and Vlassov, A. V. (2016). Analysis of the RNA content of the exosomes derived from blood serum and urine and its potential as bio-maker. *Philosophical transactions of the Royal Society Biological Science*: 3-33.

[2] Basu, J., B Ludlow, J. W. (2016). Exosomes for repair, Regeneration and Rejuvenation *Expert opinion on Biologic therapy*; 3: 34-40.

[3] Leiserowitz, G. S., Lebrilla, C., Miyamoto, S., An, H. J., Duong, H., Kirmiz, C., *et al. (*2008). Glycomics analysis of serum: a potential new biomarker for ovarian cancer? *International Journal of Gynecologic Cancer*; 18: 470-475.

[4] Park, J. E., Tan, H. S., Datta, A., Lai, R. C., Zhang, H., Meng, W., Lim, S. K., Size, S. K. (2010). Hypoxic Tumor cell Modulates its micro environment to enhance Angiogenic and metastatic potentials by secretion of proteins and exosomes. *Molecular and cellular proteomic*s; 9 (6): 56-67.

[5] Qin, J., Q. xu. Function and application of exosomes. *Acta Pol Phar*. 2014: 71.

[6] Shabbir, A., Cox, A., Rodriguez-menocal L., Salgodom. And Badiavas E. V. (2015). Mesenchymal stem cell exosomes induce proliferation and migration of normal and chronic wound fibroblast and enhance development; 24 (14): 1635-1647.

[7] Alvarez-Erviti, L., Seow, Y., Yin, H., Betts, C., Lakhal, S., and Wood, M. S. (2011). Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat. Biotechnology*; 29 (4): 341-5.

[8] Vanderpole, E., Boing, A. N., Harrison P., Sturk A., and Nieuwland, R. (2012). Classification, function and clinical relevance of extracellular, vesicles. *Journal of Pharmacology*; 64 (3): 676-705.

[9] Yeo, R., Lim, W. Y., and Lim, S. K. (2010). Exosomes and their therapeutic application inadvances in pharmaceutical cell therapy: principle of cell-based; *Biopharmceutic*; 3 (5): 477-501.

[10] Kahlert, C., Kalluri, R. (2012). Exosomes in tumor microenvironment influence cancer progression and metastasis. *Journal of Molecular Medicine* (Berl); 91: 431-437.

[11] Batrakova, E. V., Kim, M. S. (2015). Using exosomes, naturally-equipped nano-carriers for drug delivery. *Journal of controlled release*; 219: 396-405.