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Evaluation of antiproliferative and antimicrobial activity of polyanhydride based poly[(maleic anhydride)-*co*-(vinyl acetate)]/noradrenaline conjugate

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Abstract

Aim: Personalized medicine has increased the interest in polymer-drug conjugates. In recent years, many polymer-drug conjugates have been developed to increase the specificity and selectivity of drugs for diseases. Noradrenaline (NA) is involved in the pathophysiology of many different neurological, psychiatric, and peripheral conditions. Moreover, NA has been reported to play a role in angiogenesis and tumor development. The MAVA/NA conjugate, a maleic anhydride-vinyl acetate (MAVA) copolymer modification product, and the noradrenaline biomolecule were examined for their antiproliferative and antimicrobial properties.

Materials and Methods: MAVA-NA conjugate was synthesized as the modification product between maleic anhydride-vinyl acetate (MAVA) copolymer and noradrenaline (NA) biomolecule. The conjugate was previously characterized in terms of chemical structure by Fourier Transform Infrared (FTIR) and Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C-NMR) spectroscopic methods, and its topographic properties were characterized by atomic force microscopy (AFM). The minimum inhibitory concentration (MIC) method evaluated the antimicrobial properties against gram-positive and gram-negative bacteria and fungi. Antiproliferative activity was determined by XTT assay on lung (A549), brain (C6), bone (MG-63), and breast (MCF-7) cancer cells and healthy fibroblast (WI-38) cell lines.

Results: The MAVA-NA conjugate showed no toxicity within the 3.125-100 µg/mL dosage range in WI-38 cells. Compared to normal fibroblast cells, MAVA-NA exhibited selective toxicity against A549, C6, MG-63, and MCF-7 cancer cells. MAVA-NA showed high antibacterial activity on *S. aureus* and MRSA bacteria compared to standard antibiotics (*S. aureus* MIC=50 µg/mL, MRSA MIC=25 µg/mL).

Conclusion: These results indicate that the newly synthesized and characterized MAVA-NA conjugate has antiproliferative and antimicrobial effects and may have a promising role in developing new anticancer drugs.

Introduction

Recent advances in designing different types of polymer-based drug conjugates, such as dendrimers, polymer-drug conjugates with protein or small molecules attached, polymer-based man-sized particles, and functional systems, are attracting attention. Polymer-drug conjugates represent a significant part of recently developed polymer-based macro therapeutics [1]. The physicochemical and biological properties of conjugates as a delivery system are closely related to the route of administration but also seriously affect the ability of drug molecules to reach the target site and the therapeutic index [2]. Such systems have desirable properties and consist of three parts: a solubilizer, a targeted, and attached pharmaceutical therapeutics. In the model first proposed by Helmut Ringsdorf in 1975 (Figure 1), the units are attached to the polymer backbone by covalent bonds [3,4]. Three different methods are proposed for the production of polymer-drug conjugates. These methods can be listed as either incorporating the drug into a synthesized polymeric carrier, a monomer before polymerization, or as monomers or initiators during polymerization [5]. Based on this model, the first biological studies used maleic anhydride-divinyl ether [6], a simple copolymer with antiviral, antibacterial, and antifungal [7, 8] activities.

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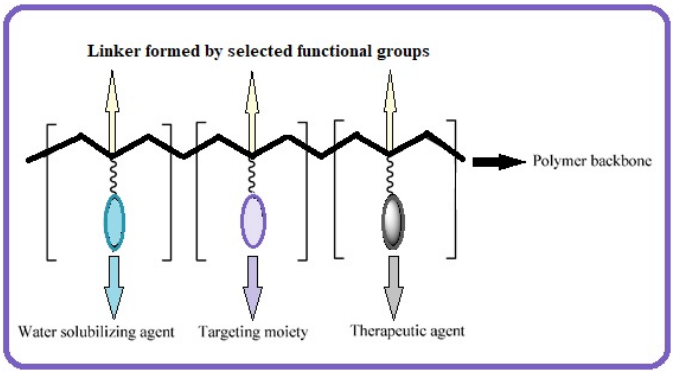

Figure 1. General schematic diagram of polymer-drug conjugates according to the Ringsdorf model [3].

Modification products of regularly used copolymers containing anhydride groups along the main chain, such as maleic anhydride (MA), are considered promising versatile materials in many industries [9]. Being water soluble is very advantageous in biological environments. For example, copolymers containing carboxyl functional groups that can dissolve in aqueous media are essential in many pharmaceutical applications [10]. The highly reactive anhydride ring on copolymers with MA groups, also known as polyanhydrides, binds to amine (-NH2) or hydroxyl (-OH) groups of reactive compounds with nucleophile character via a ring-opening reaction, both allow the reformation of ester or amide structures in addition to the carboxylic acid [11-14].

Noradrenaline (NA), or norepinephrine, is an organic chemical compound with a catecholamine structure naturally synthesized in nerve endings in the body. Although NA is used as an effective vasoconstrictor drug, it is mainly applied in emergencies such as bleeding, burns, traumatic shock, and fainting resulting from poisoning [18, 19]. Noradrenaline has also been reported to stimulate angiogenesis via VEGF signaling and increase tumor growth [20]. An increasing amount of research indicates that medications that inhibit NA signaling slow the development and spread of different cancers [21,22].

Polymer-based drug delivery systems are employed in biomedical applications to administer therapeutic chemicals to the intended biological environment. They have a variety of characteristics, including reduced drug toxicity, enhanced compliance among patients, increased solubility of the drug, increased drug bioavailability, biocompatibility, and biodegradability. They also regulate the drug release mechanism, guard against deactivation, and sustain drug activity while it is in circulation [23]. Numerous polymer-based drug delivery technologies, including polymer capsules, polymeric nanoparticles, dendrimers, micelles, hydrogels, nanogels, in situ gels, polymer-drug conjugates, and nanoliposomes, have been developed to enhance the therapeutic results of anticancer medications [24].

According to estimates, there were 18.1 million new instances of cancer worldwide in 2018 and 9.6 million cancer-related deaths. While there are many different kinds of cancer, the most prevalent ones are colorectal, lung, and breast cancers. The brain and bones are where these malignancies most frequently metastasize [25, 26].

In this study, maleic anhydride-vinyl acetate copolymer was proposed as the carrier of the pharmaceutically active substance, and NA was chosen as the conjugation agent in the development of the polymer-based conjugate structure containing the amide/carboxylic acid main backbone. This study aimed to investigate the antimicrobial effect of MAVA/NA conjugation on gram-positive and gram-negative bacteria and fungi and its antiproliferative properties on A549, C6, MG-63, and MCF-7 cancer cells.

Materials and Methods

Maleic anhydride (MA) monomer, benzoyl peroxide (BPO), methyl ethyl ketone (MEK), triethylamine (TEA), dimethylformamide (DMF), and were purchased from Merck (Germany). Absolute ethanol was purchased from Smyras (Teknik, Turkey). Vinyl acetate (VA) monomer and ethyl acetate were purchased from Sigma-Aldrich (USA). Noradrenaline (NA), used as the conjugation agent, was purchased from Sigma (St Louis, MO, USA). All chemicals were of analytical grade and were used without the need for additional purification.

Synthesis of the MAVA and MAVA-NA

The MAVA carrier and MAVA-NA conjugate used in this study were previously synthesized in our polymer research laboratory according to the free radical polymerization and the Ringsdorf model, respectively (Figure 1) [3]. Our previously published article provides detailed data on the synthesis, characterization, and toxicity of the copolymer and conjugate [15,16]. Briefly, the MAVA copolymer was synthesized by the free radical polymerization method of MA and VA in a 1:1 molar ratio in MEK accompanied by a BPO initiator at 80 °C for 24 h. The reaction medium was kept under constant control at 80 °C for 24 h, and the reactions were terminated by treatment with excess ethyl alcohol until a white precipitate was obtained. Unreacted VA or homopolymerization product was carefully removed by ethyl acetate incubating for 24 h. MAVA was then precipitated with light petroleum, filtered under vacuum, and dried at 55 °C for 24 h in a vacuum incubator. MAVA/NA was synthesized by chemical modification of MAVA copolymer with NA at a molar ratio of 1:119.25 in DMF medium in the presence of TEA catalyst at 70 °C for 24 hours. TEA (20 µL) was added to the solution prepared by dissolving MAVA copolymer powder (0.5 mmol) in DMF (2.5 mL). NA (0.5 mmol) was prepared in DMF/water (1.25 mL/250 µL) and then added dropwise to the previously prepared MAVA solution at room temperature. The final mixture was mixed while shaking in an incubator at 50 °C for 2 h, and the reaction was terminated at 70 °C after 48 h. The precipitate from the reaction medium was washed with cold ethyl alcohol. After removing the liquid phase, the precipitate was dried in the open air, converted to powder, and dried in a vacuum incubator at 50 °C for 24 h.

Structural characterization of the MAVA and MAVA-NA

Structural identification of the MAVA copolymer and the MAVA-NA was performed by Fourier Transform Infrared

(FTIR) and Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C-NMR) spectroscopic techniques. It has been demonstrated that the NA small molecule is successfully covalently attached to the MAVA copolymer chain. In addition, surface morphology is imaged with atomic force microscopy [14,15].

Antimicrobial activity

The antimicrobial activity of MAVA, NA, and MAVA-NA polymeric materials against gram-positive and gram-negative bacteria and yeast fungi was determined using the Minimum inhibitory concentration (MIC) test. For this test, standard strains of *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212), *Streptococcus mutans* (ATCC 25175), Methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), *Candida albicans* (ATCC 10231) and *Candida tropicalis* (ATCC 4563) were used. The polymeric materials were first dissolved in 40% Dimethyl sulfoxide (DMSO). Rows 11 and 12 of a 96% U-bottom microplate were used as negative and positive controls. Polymeric materials were added to the first well, and serial dilutions were made to 10 concentrations. Microorganisms cultured on general-use solid media were transferred to Mueller Hinton Broth (Accumix® AM1072) for bacteria and Saboraud Dextrose Broth (Himedia ME033) for yeasts and adjusted to a density of 0.5 McFarland. 10 µL of microorganism culture was added to all wells. Bacterial microplates were incubated at 37°C, and yeast microplates were incubated at 35°C for 24 hours. The first well in which microorganism growth disappeared was considered the MIC value. The analysis was performed in 3 replicates [27, 28].

Antiproliferative activity

Using human lung cancer cells (A549, ATCC-CCL-185), human breast cancer cells (MCF-7, ATCC-HTB-22), mouse glioma cells (C6, ATCC-CCL-107), human bone cancer cells (MG-63, ATCC-CRL-1427), and human normal lung fibroblast cell (WI-38, ATCC-CCL-75), the antiproliferative effects of MAVA, NA, and MAVA-NA polymeric materials were assessed using the XTT assay method.

First, the cells were passaged and developed until they were at the proper density for the right kind of experiment. 10^4 cells were then placed in each well of microplates. Eight concentrations of the polymeric materials were applied to the cells. The positive control in this experiment was antineoplastic drugs, while the negative control was DMSO. Microplates were incubated in a 5% CO_2 atmosphere at 37°C for a whole day. Following the end of the incubation, each well contained 100 µL of an XTT solution that the experimental protocol had made. The medium was then withdrawn. Using a microplate reader, optical density values were obtained at 495 nm following a 4h incubation period [29]. The selectivity index, which represents the ratio of the IC_{50} values of MAVA, NA, and MAVA-NA polymeric materials in normal cells to the IC_{50} values in all cancer cells, was calculated according to the relevant formula [30], indicating that this compound affects cancer cells many times more specifically than normal cells: fibroblast IC_{50}/cancer cell IC_{50}.

Statistical analysis

Each experiment was studied in triplicate, and data were presented as the mean ± standard deviation. Kolmogorov–Smirnov test was used to compatibility with normal distribution. One-way analysis of variance (ANOVA) was performed. Intergroup comparisons followed by the post-hoc Tukey's test. The statistically significant difference was set at $p<0.05$.

Results

Structural characterization of MAVA copolymer and MAVA-NA conjugate

The structural characterization of MAVA and MAVA-NA was analyzed on an FTIR spectrophotometer (Mattson 1000 Unicam, USA) at 400-4000 cm-1 in 4 cm-1 increments. A more detailed functional group analysis of the samples was carried out with ^{1}H-NMR and ^{13}C-NMR analyses using a nuclear magnetic resonance instrument (Bruker Avance III, Karlsruhe, Germany) at 400 MHz. The surface morphology of the samples was also visualized by atomic force microscopy (AFM) (NanoScope Veeco, Digital Instruments, Mannheim, Germany).

According to the data obtained in our previous study, from a functional group perspective, since NA has both amino and phenolic hydroxyl groups, it was possible to modify the poly-anhydride-based MAVA copolymer with this agent chemically. Taking into account the spectroscopic measurements, it was observed that the characteristic symmetrical and asymmetrical peaks of the anhydride ring, which are regularly arranged in the main copolymer backbone, were replaced by newly formed carboxyl (-COOH) and amide (-CO-NH-) groups as a result of conjugation with the NA agent via the amide mechanism. In particular, the formation of (-N-C-O), (CO-N-H) amide, and

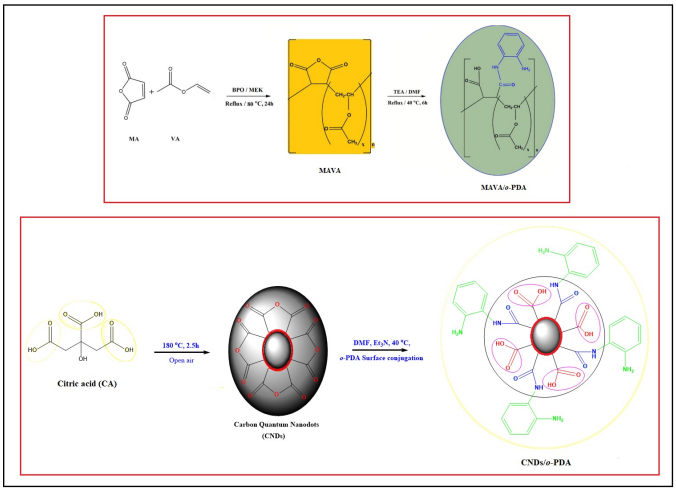

Figure 2. Conjugation mechanism of MAVA copolymer with NA biomolecule with identification of functional groups [16].

Table 1. MIC results of MAVA, NA, and MAVA-NA (µg/mL).

Microorganisms (Bacteria and Yeasts)	MIC (µg/mL) MAVA	MIC (µg/mL) NA	MIC (µg/mL) MAVA-NA	MIC (µg/mL) Antibiotics	Antibiotics used
Escherichia coli	100	>200	100	8	Amoxicillin
Klebsiella pneumoniae	100	>200	100	16	Piperacillin / Tazobactam
Pseudomonas aeruginosa	25	100	25	16	TMP-STX
Staphylococcus aureus	50	100	50	64	Chloramphenicol
Methicillin-resistant Staphylococcus aureus (MRSA)	25	50	25	64	Ciprofloxacin
Enterococcus faecalis	100	>200	50	8	Chloramphenicol
Streptococcus mutans	50	>200	25	2	Imipenem
Candida albicans	>200	>200	>200	0.25	Fluconazole
Candida tropicalis	>200	>200	>200	0.25	Fluconazole

MAVA: poly[(maleic anhydride)-co-(vinyl acetate)] copolymer, NA: Noradrenaline MAVA-NA: polyanhydride-based Poly[(maleic anhydride)-co-(vinyl acetate)]/Noradrenaline conjugate.

Table 2. IC_{50} values of MAVA, NA, MAVA-NA.

Samples	IC_{50}(µg/ml) A549	C6	MG-63	MCF-7	WI-38
MAVA	2.58±0.11*	3.17±0.15*	6.09±0.53*	3.37±0.22*	46.92±0.34
NA	3.17±0.17*	2.42±0.17*	7.85±0.62*	3.76±0.31*	47.44±0.32
MAVA-NA	4.35±0.19*	3.56±0.34*	4.15±0.75*	6.09±0.19*	51.76±0.26

*Represents significant results (p<.05) compared to WI-38 group. MAVA: poly[(maleic anhydride)-*co*-(vinyl acetate)] copolymer, NA: Noradrenaline MAVA-NA: polyanhydride-based Poly[(maleic anhydride)-co-(vinyl acetate)]/Noradrenaline conjugate.

free acid as a result of the conjugation proves the existence of the monosubstituted amide group. As a result, FTIR, ^{1}H-NMR, and ^{13}C-NMR spectra confirm the successful covalent attachment of NA to the MAVA copolymer backbone. The roughness analysis of the samples was evaluated in terms of surface morphology by visualization with an atomic force microscope. The results indicate that the surface irregularity of the modified product has increased [16].

Reaction mechanism of (Maleic anhydride-co-Vinyl acetate)/Noradrenaline conjugate and functional group-biological activity relationship

Polyanhydride-based conjugates can become more functional due to the carboxyl (-COOH) and amide (–C(=O)N=) or ester (R-CO-OR) groups formed on their surfaces during conjugation. The MAVA/NA conjugate used in this study has regularly distributed carboxyl and amide functional groups on its surface (Figure 2). After the conjugate form is formed, the hydrophilic groups arranged regularly in the macromolecule chain increase the polarity, thus increasing the water solubility and biological activity.

Antimicrobial activity

The results were compared with reference sources and MIC values of standard antibiotics (Amoxicillin, Piperacillin / Tazobactam, TMP-STX, Chloramphenicol, Ciprofloxacin, Chloramphenicol, Imipenem, Fluconazole, Fluconazole). Reference sources given as [Effective (MIC $<$ 100 µg/mL), Moderate (100 $<$ MIC $\leq$ 625 µg/mL), and Weak (MIC $>$ 625 µg/mL)] were used [31,32]. The MIC values of the polymeric materials and antibiotics are given in the table below (Table 1).

NA molecule was found to be moderately effective against *P. aeruginosa*, *S. aureus* and MRSA according to reference sources. MAVA was effective against all bacteria except fungi. Compared to standard antibiotics, MAVA was effective against *S. aureus* (MIC=50 ug/mL) and MRSA (MIC=25 ug/mL), while NA was effective only against MRSA (MIC=50 ug/mL). The antibacterial activity of MAVA-NA conjugate against *E. faecalis* and *S. mutans* was increased compared to its components. In other bacteria, the combination of NA did not change the antibacterial activity of MAVA.

Among the synthesized substances, the antibacterial activity of MAVA-NA conjugate was found to be effective and antifungal activity was found to be weak (ineffective) according to the reference source [high effective (MIC $\leq$ 100 µg/mL), moderate (100 $<$ MIC $\leq$ 625 µg/mL) and weak (MIC $>$ 625 µg/mL)] [31,32]. The MAVA-NA conjugate was found to have moderate antibacterial activity against *P. aeruginosa* and *S. mutans*, but highly effective against *S. aureus* and MRSA compared to standard antibiotics' MIC values [28].

Antiproliferative activity of MAVA, NA, and MAVA-NA

The results of the study on cells belonging to the most common cancer types (A549, MG-63, MCF-7, and C6) in the world are shown in Table 2. The most effective concentrations for MAVA in A549 cells were found to be 2.58±0.11 µg/ml, for NA in C6 cells 2.42±0.17 µg/ml, and for MAVA-NA conjugate in C6 cells 3.56±0.34 µg/ml.

The effects of MAVA-NA conjugate, MAVA, and NA on

Table 3. Selectivity Index (SI) values of MAVA, NA and MAVA-NA

Samples	Selectivity Index (SI)			
	A549	C6	MG-63	MCF-7
MAVA	18.19	14.80	7.70	13.92
NA	14.95	19.60	6.04	12.62
MAVA-NA	11.90	8.50	12.47	14.54

MAVA: poly[(maleic anhydride)-co-(vinyl acetate)] copolymer, NA: Noradrenaline, MAVA-NA: polyanhydride-based Poly[(maleic anhydride)-*co*-(vinyl acetate)]/Noradrenaline conjugate.

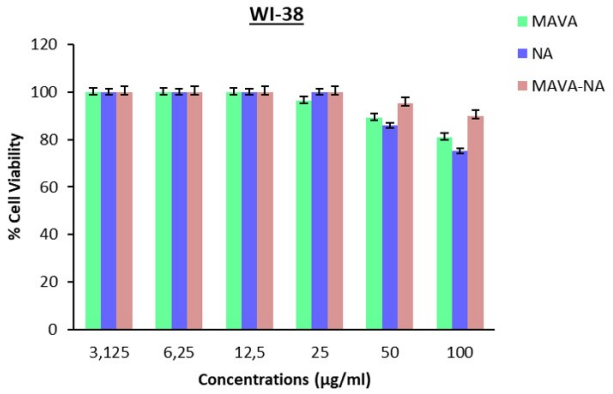

Figure 3. Cell viability of MAVA, NA and MAVA-NA conjugate on WI-38 cells.

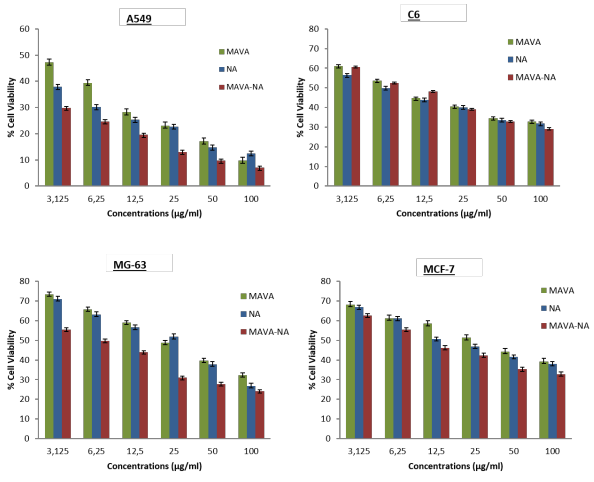

Figure 4. Cell viability of MAVA, NA, and MAVA-NA conjugate on A549 (a), C6 (b), MG-63 (c) and MCF-7 (d) cell lines.

cytotoxicity results in WI-38 are shown in Figure 3. Compared to its constituents MAVA and NA, the MAVA-NA conjugate exhibited almost no toxic effects. This finding demonstrates that MAVA-NA can be employed in cancer treatment and is not harmful to healthy cells in size or physical characteristics.

MAVA-NA conjugate significantly reduced cell viability dose-dependently in all cancer cells ($p<0.05$) (Figure 4). MAVA-NA conjugate was more effective than MAVA and NA alone in MCF-7, MG-63, and A549 cancer cells. A significant difference was observed in 25-100 µg/ml concentrations in C6 cells, and the conjugate was more effective.

The selectivity indexes of MAVA, NA, and MAVA-NA are given in Table 3.

According to the antiproliferative activity results, it was determined that the conjugate was highly influential in all cancer cell lines used with low IC_{50} values and had high selectivity.

Discussion

New approaches are required for improved diagnosis and treatment of severe illnesses and incapacitating disease states that lower quality of life, such as cardiovascular disease, infectious diseases, multiple cancers, etc. [33]. The field of polymer-drug/protein conjugates has emerged significantly in recent years, with an increasing number of PDC-based therapeutics entering clinical trials [26]. Maleic anhydride and its derivatives are actively employed in the pharmaceutical and medical industries as copolymers, controlled delivery systems, and drug-polymer systems (conjugates) in medication formulations [34].

This study investigated the antimicrobial and antiproliferative properties of MAVA/NA conjugation formed as a maleic anhydride derivative. Maleic anhydride-co-Vinyl acetate)/Noradrenaline conjugate has carboxyl and amide functional groups (Figure 2).

Functional groups formed regularly on the MAVA-NA conjugate synthesized as a candidate molecule are very important for the desired chemical functionality and biological activity. Carboxyl and amide groups are polar and, therefore, can form hydrogen bonds, meaning the molecules can dissolve in a polar solvent such as water. Formation of both functional groups of the conjugate resulted in increased water solubility. Water solubility allows for increased or improved activity for most drugs and drug-like molecules. It is thought that the non-toxicity and the antiproliferative and antimicrobial activity of the conjugate are due to the functional groups regularly distributed on the surface [16].

Maleic anhydride polymers exhibit activity against pathogenic bacteria, fungi, and drug-resistant bacteria [35]. According to MIC data, the MAVA-NA conjugate's antibacterial activity was shown to be higher than that of both MAVA and NA. According to reference sources, MAVA-NA reached highly effective MIC values on bacteria (MIC= 25-100 µg/mL) but not effective on fungi (MIC= >200 µg/mL) [31,32]. Compared with standard antibiotics, it was highly effective against *S. aureus* and MRSA, while it showed moderate antimicrobial activity against *P. aeruginosa* and *S. mutans* [28]. Upon reviewing similar research in the literature, Ye et al. synthesized a polymer-drug conjugate and reported that they synthesized an antimicrobial conjugate that can significantly enhance the transport of antibiotics into bacteria and bypass the efflux pump [36]. Gakiya-Teruya et al. demonstrated increased antibacterial activity against microorganisms by conjugating silver nanoparticles with synthetic peptides

[37]. The antibacterial activity of poly(4-styrenesulfonic acid-co-maleic acid) polymer-coated silver nanoparticles was studied by Tamiyakul et al. Their findings indicated a more significant effect against gram-negative bacteria [38]. With their new functional groups, the conjugated compounds were found to have significant antimicrobial activity.

Biological challenges for polymer-drug conjugates include toxicity, degradation pathways, and accumulation of non-biodegradable components [39]. The MAVA-NA conjugate, produced by binding the NA molecule to the MAVA molecule, has a cell survival of % 92,95 at 100 µg/ml in WI-38 (Figure 3). Recent studies have indicated that polymer-drug conjugates have cytotoxic effects and antiproliferative properties [26,40].

The literature review revealed that MAVA conjugate showed a cytotoxic effect on different cancer cells. Karakuş et al. showed that MAVA-doxorubicin conjugate tested its anticancer effect, and found a higher anticancer effect on HeLa and C6 cells than MAVA copolymer [17]. Can et al. reported P(MA-co-VA-co-AA) cytotoxicity and P(MA-sub-AA) on Raji cells. It was stated that P(MA-sub-AA) had a more effective cytotoxic effect on tumor cells than P(MA-co-AA-co-VA) [10]. In another study, the cytotoxic and apoptotic effects of MAVA on MDA-MB-231 and MCF-7 breast cancer cells were demonstrated [41]. Our study indicated that MAVA-NA conjugate reduced the cell viability dose-dependent (3,125–100 mg/mL) by XTT assay. The IC50 values of MAVA, NA, and MAVA-NA are shown in Table 2.

A sample's cytotoxicity against non-cancerous cell lines should be ascertained to compute the SI value, which will be needed to assess anti-cancer efficacy. The medication should ideally be able to destroy cancer cells while not affecting healthy cells. If the sample has a low SI value (< 1), it may be harmful and should not be utilized as medicine. If the computed SI value falls between 1 and 10, additional assessments utilizing additional biosystem(s) for validation are advised. A lower SI value (≥ 3) was proposed by Weerapreeyakul et al. [42] for the classification of a prospective anti-cancer sample. A549 for MAVA, C6 for NA, and MG-63 for MAVA-NA have significant selectivity indexes.

Although several PDCs have shown promising results in preclinical studies, many processes need to be addressed, especially for the findings to be clinically applicable. First, it is essential to determine the stability, solubility, and interaction of the synthesized compounds with the active compounds. Also, challenges include reduced drug conjugation, reduced bioactivity, poor sanitary chemistry, and polymeric stress [39].

This study has a few limitations. In this study, some experiments have been used for anti-proliferative and antimicrobial effects. These experiments should be performed in different cell types and organisms. More studies are needed for the mechanism of MAVA-NA and its potential targets in cancer development. We believe that it can be a potential drug conjugate for the treatment of cancer patients and that animal studies should be conducted on this topic. It is obvious that the polymer conjugation strategy will provide novel and cutting-edge treatment approaches in the evolving therapeutic environment given its particular tactics and advantages.

Conclusion

In conclusion, this study represents the first report on the antiproliferative activity of MAVA-NA in the MCF-7, MG-63, C6, and A549 cells. Additional studies in animal models and clinical trials are now required to fully evaluate the potential of MAVA-NA in preventing and treating cancer. Also, MAVA-NA conjugate has antibacterial activity.

Ethical approval

Ethics committee permission is not required for this study.

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