Marmara Pharmaceutical Journal 19: 73-81, 2015
DOI: 10.12991/mpj.2015198613

ORIGINAL RESEARCH

Synthesis, characterization and antiproliferative activities of novel modified poly (maleic anhydride-co-vinyl acetate)/cytosine β-D-arabinofuranoside hydrochloride conjugate

Gülderen KARAKUŞ, Ayşe ŞAHİN YAĞLIOĞLU, Haci Bayram ZENGİN, Nihat KARAKUŞ

ABSTRACT

Poly (maleic anhydride-*co*-vinyl acetate) (MAVA) copolymer was synthesized by free-radical- polymerization reaction in methyl ethyl ketone (MEK) at 80 ºC using benzoyl-peroxide (BPO) as the radical-initiator. MAVA was then modified with anti-leukemic chemotherapy-agent cytosine β-D-arabinofuranoside hydrochloride (CF). Modification was performed at 70 ºC in dimethylformamide (DMF) containing triethylamine (Et_3N) as the catalyst. Structural characterization of the copolymer and copolymer/drug couple (MAVA/CF) was carried out by Fourier Transform Infrared (FTIR) and Nuclear Magnetic Resonance (^{1}H-NMR). FTIR and ^{1}H-NMR spectra confirmed the modification reaction. UV-Spectrophotometric measurements indicated that MAVA/CF kept its molecular integrity in physiological-body-fluid, PBS, for first four days. Antiproliferative activities of MAVA/CF were also determined by BrdU-cell-proliferation-ELISA assays using C6 and HeLa cell lines (cisplatin and 5-fluorouracil used as positive control). MAVA/CF appeared to have little antiproliferative activity against C6 cell line while samples didn't have antiproliferative activity against HeLa cell line at low concentrations (< 100 µg/ml). Reaction mechanism was also recommended for modification product MAVA/CF.

Keywords: Antiproliferative activity, copolymer modification, cytosine β-D-arabinofuranoside hydrochloride, physiological body fluid PBS, poly(maleic anhydride-*co*-vinyl acetate).

INTRODUCTION

Polymer modification, in order to obtain new materials, based on preparation of nucleophilic compounds attributes to the high reactivity of the anhydride ring of the copolymer toward nucleophilic reagents (1). Maleic anhydride (MA), $C_4H_2O_3$, is a unique electron-acceptor monomer. MA containing copolymers can easily be synthesized by free radical chain polymerization. Furthermore, they can easily be modified by its reactive anhydride group with nucleophilic reagents such as amine and amine derivatives. The highly reactive anhydride ring on repeated unit of MA can be bound to amino or hydroxyl groups of nucleophilic reagents (1,2-4) by the *ring opening reaction*, resulting in either ester/carboxylic acid or amide/carboxylic acid structures.

Drugs carriers for the delivery of biologically active agents are generally classified as either biodegradable or non-biodegradable copolymers. Non-biodegradable

Gülderen Karakuş
Cumhuriyet University Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Sivas, Turkey

Ayşe Şahin Yağlıoğlu
Çankırı Karatekin University Faculty of Science, Department of Chemistry, Çankırı, Turkey

Haci Bayram Zengin3, Nihat Karakuş
Cumhuriyet University Faculty of Science, Department of Chemistry, Sivas, Turkey

Correspondence to:
Gülderen Karakuş
Phone. +90 346 2191010-3910
Fax. +90 346 2191155
E-mail. gulderen@cumhuriyet.edu.tr, gulderenkarakus@gmail.com

polymers have often been used as drug carriers and are expected to retain their structural integrity at least until the completion of the release of their bound drugs (5). The first biological studies have been carried out by using a relatively simple MA containing copolymer, maleic anhydride-divinyl ether (DIVEMA) (6), possessing antiviral, antibacterial, and antifungal activities. It was also used as both an anticoagulant and anti-inflammatory agent (7,8). Most popular copolymer, poly(styrene-*co*-maleic acid/anhydride) (SMA) is another well-known commercialized vinyl base copolymer (1,9). Co-polymeric product obtained by conjugation or modification of SMA and the antitumor protein neocarcinostatin (NCS) (10-14), SMANCS have also been marketed in Japan for the treatment of hepatocellular carcinoma (15).

Many copolymers, synthesized with MA and vinyl base monomers such as styrene, vinyl acetate (Scheme 1) or methyl methacrylate, have often been used as reactive macromolecules observing various biological activities such as direct antitumor inhibitors (6,16). It has been demonstrated that biological activities, for example, in relation with antitumor activity of these copolymers are dependent upon the amount of hydrogen-bonding between carboxyl groups, and the nature of their distribution on the main side chains. In biological systems, it has been implied that even the hydrolyzed copolymers can also show some biological activity especially by modifying proteinaceous drugs (10).

Cytosine β-D-arabinofuranoside hydrochloride (CF), which is commonly known as cytarabine, is a chemotherapy agent used mainly in the treatment of cancers of white blood cells such as acute myeloid leukemia (AML) and non-Hodgkin lymphoma being an efficacious antimetabolite in treatment of leukemia. Synonyms of this drug are cytosine, arabinoside and ara-C (17) and IUPAC name is 4-amino-1-β-D-arabinofuranosylpyrimidin-2(1*H*)-one. The closed formula of CF with white powder appearance is $C_9H_{13}N_3O_5$.HCl, and its molecular weight is 279.68 g mol^{-1}. CF is also known as one of the first serious cancer drugs that altered the sugar component of nucleosides. It has a primary amino group on the pyrimidine ring acting as a functional group in ring opening reactions for maleic anhydride containing copolymers. The presence of amino and phenol groups gives both amphoteric and polar character to the molecule (Scheme 2). Biological roles of CF can be classified as follows: antimetabolite, antiviral, and immunosuppressive agent. Also, it has an application, which is known as antineoplastic agent as well as being antiviral and immunosuppressive agent.

In this study, it was aimed to generate a versatile biologically active substance by modifying a biologically active copolymer, MAVA, through its anhydride group, with an anticancer biomolecule, cytosine β-D-arabinofuranoside hydrochloride (CF). Before its usage, it was demonstrated that MAVA has almost no cytotoxicity on the cultured healthy cell lines (18). In our previous study, the amoebicidal effect of MAVA copolymer was also investigated on *Entamoeba histolytica* trophozoites using different copolymer concentrations and periods of time. The results indicated that MAVA killed all of the trophozoites at 32 mg/mL concentration in 3 h (19). The main aims of the study were two-fold: (1) to use the non-biodegradable and noncytotoxic copolymer as the carrier of a biomolecule, cytosine β-D-arabinofuranoside hydrochloride, and (2) to achieve antiproliferative activity both HeLa and C6 cell lines.

EXPERIMENTAL

Chemistry

All of the organic solvents and reagents were obtained from commercial sources and used without purification. Maleic anhydride (MA), methyl ethyl ketone (MEK), dimethylformamide (DMF), benzoyl peroxide (BPO), petroleum ether and triethylamine (abbreviated as TEA or Et_3N) were obtained from Merck (Germany). Ethyl alcohol was obtained from Carlo-Erba (Rodano). Vinyl acetate (VA) and ethyl acetate were obtained from Sigma-Aldrich (USA). Cytosine β-D-arabinofuranoside hydrochloride (CF) was purchased from Sigma (St. Louis MO, USA).

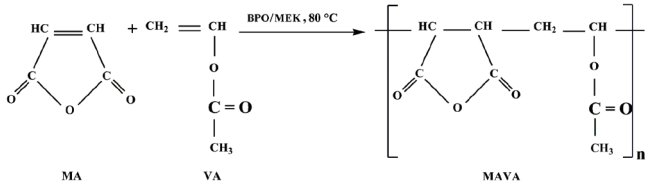
Structural Characterization

MAVA copolymer and modification product MAVA/CF were prepared as KBr pellets (2 mg sample in 100 mg KBr) to be analyzed in a FTIR spectrophotometer (MATTSON 1000 Unicam, USA) at 400-4000 cm^{-1} with 4 cm^{-1} increments. Nuclear Magnetic Resonance, ^{1}H-NMR analysis was performed at 400 MHz (Bruker Avance III, Karlsruhe, Germany) using 6 mg of the copolymer or modified product sample, dissolved in 0.8 mL dimethyl sulfoxide (DMSO). These characterization studies were carried out at Technology Research and Developing Centre, Erciyes University, Kayseri, Turkey.

Synthesis of MAVA copolymer

MAVA copolymer was synthesized by free radical polymerization of maleic anhydride (MA) and vinyl acetate (VA), at a 1:1 molar ratio, in methyl ethyl ketone (MEK) using benzoyl peroxide (BPO) as the initiator for 24 h at 80 °C (Table 1) (16,18). The reaction media was continuously purged and reactions were terminated with ethyl alcohol until the white precipitate was obtained. Un-reacted vinyl acetate

Scheme 1. Copolymerization of the MAVA polymer.



Scheme 2. The modification reaction of the MAVA copolymer with CF.

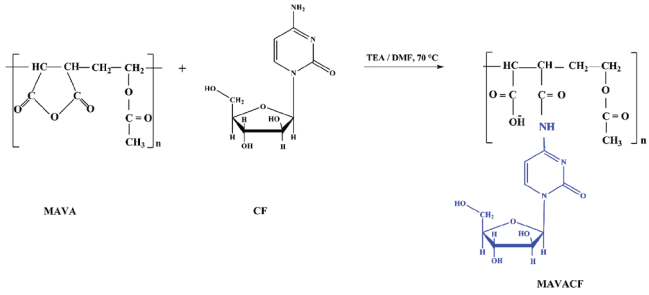


Table 1: Reaction conditions of MAVA copolymer and MAVA/CF conjugate.

SAMPLE	MOL PROPORTIONS	INITIATOR and CATALYST	SOLVENTS	TIME (h)	TEMPERATURE (°C)
MAVA	MA:VA ~ (1:1)	BPO	MEK	24	80
MAVA/CF	MAVA:CF ~ (1:1)	Et_3N	DMF	50	70

BPO: Benzoyl peroxide, Et_3N: Triethylamine, MEK: Methylethyl ketone, DMF: Dimethylformamide.

or homopolymerization products were removed by incubating the precipitate in ethyl acetate for 24 h. MAVA was then precipitated with petroleum ether, filtered under vacuum, and dried in a vacuum incubator for 24 h at 55 °C (20).

Synthesis of MAVA/CF

Poly(maleic anhydride-*co*-vinyl acetate)/Cytosine β-D-arabinofuranoside hydrochloride (MAVA/CF) couple was synthesized by modification of MAVA copolymer with CF, at a 1:1 molar ratio, in dimethylformamide (DMF), using triethylamine (TEA, abbreviated as Et_3N) as the catalyst for 2h (50 °C) and 48h at 70 °C (Table 1) (21). The modification product, MAVA/CF, was precipitated with cold ethyl alcohol, kept at -20 ºC for 1h and dried in a vacuum incubator for 24h at 50 °C (22). The MAVA copolymer powder (0.05 mmol, 9.21 mg) was dissolved in well-stirred DMF (375 µL). TEA (3 µL) was added while under stirring to MAVA-DMF solution. Pharmaceutical active ingredient, CF, (0.05 mmol, 13.98 mg) was prepared in DMF (547.5 µL) and was then added drop-wise into the solution of MAVA at room temperature (21). The final mixture was stirred by shaking for 2h at 50 °C in a shaking-incubator, and the reaction was continued for further 48 h at 70 °C until the obtained dark brown viscous solution. The final reaction sample was then washed repeatedly with excess of cold ethyl alcohol and incubated for 1h at -20 °C to obtain a yellowish-brown precipitate. The light brown precipitate was collected by centrifugation for 10 min at 3,000 rpm. After the removal of liquid phase, the precipitate was crushed into powder, and dried in a vacuum incubator for 24 h at 50 °C.

Behaviour of MAVA and MAVA/CF in Phosphate Buffered Saline

MAVA/CF in dried powder form were incubated after dissolving them in a phosphate buffered saline solution (PBS; biotechnology grade, 137 mM NaCl, 2 mM KCl ve 10 mM phosphate buffer, pH 7.4 ± 0.1) for 45 d at 37 ºC. During the course of incubation, aliquots were periodically taken and the particulate matter was removed by centrifugation. CF release from MAVA/CF was then read within the range from 200 nm to 600 nm with 25 nm increments (Optima®, SP-3000, Japan) by spectrophotometric analysis (4,23).

Antiproliferative activity

Chemicals

All of the antiproliferative chemicals used were in analytical grade and obtained from Sigma–Aldrich, Merck and Roche.

Preparation of sample solutions

Stock solution of the sample, 5-florouracil, cisplatin were solved in DMSO and diluted Dulbecco's modified eagle's medium (DMEM). Final concentration of DMSO is below % 1 in all tests.

Cell culture and cell proliferation assay

Antiproliferative effects of the MAVA/CF, Cisplatin and 5-florouracil (as standard compounds) were investigated on HeLa (human cervix carcinoma) and C6 (rat brain tumor) cell lines using proliferation BrdU ELISA assay (24,25).

HeLa and C6 cells were grown in Dulbecco's modified eagle's medium (DMEM, Sigma), supplemented with 10% (v/v) fetal bovine serum (Sigma, Germany) and PenStrep solution (Sigma, Germany) at 37 °C in a 5% CO_2 humidified

atmosphere. For the proliferation assay, cells were plated in 96-well culture plates (COSTAR, Corning, USA) at a density of 30,000 cells per well. Vehicle (DMSO), 5-Florouracil, cisplatin and several samples in various concentrations were added to each well. The antiproliferative activities were observed at high concentrations, these activities were also investigated at low concentrations (at level of the 100 µg/mL). Concentrations were selected as follows: the first part prepared as 500, 250 and 100 µg/mL, and the second part prepared as 100, 75, 50, 40, 30, 20, 10 and 5 µg/mL. Cells were then incubated for overnight before applying the BrdU Cell Proliferation ELISA assay reagent (Roche, Germany) according to manufacturer's procedure. Briefly, cells were pulsed with BrdU labeling reagent for 4 h followed by fixation in FixDenat solution for 30 min at room temperature. Thereafter, cells were incubated with 1:100 dilution of anti-BrdU-POD for 1.30 h at room temperature. The amount of cell proliferation was assessed by measuring the absorbance of the culture media at 450 nm after addition of the substrate solution by using a microplate reader (Ryto, China). The results were reported as percentage of the inhibition of cell proliferation, where the optical density measured from vehicle-treated cells was considered to be 100% of proliferation. All assays were repeated at least twice against HeLa and C6 cells. Percentages of inhibition of cell proliferation were calculated by using the following equation 1:

$$[1 - (A_{treatments} / A_{vehicle\ control})] \times 100. \quad \text{(equation 1)}$$

Statistical Analysis

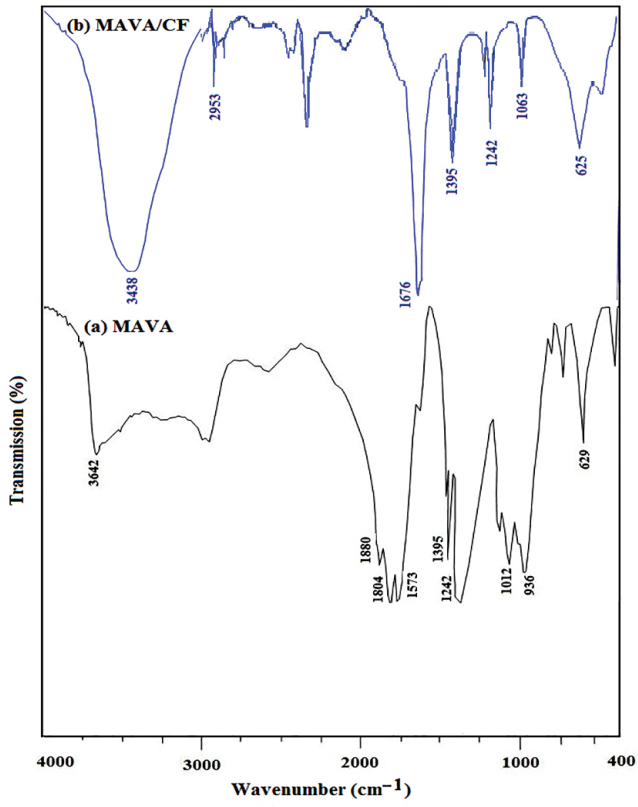
Considering the importance of the pharmaceutical applications on biological media, statistical analysis was implemented for only low concentrations (<100 µg/mL). The results of investigation in vitro were the means ± SEM of six measurements for each cell type. Differences between treatment groups were tested way ANOVA and p values of <0.01 and 0.05 were considered significant.

RESULTS AND DISCUSSION

FTIR analysis

MAVA copolymer (Fig. 1a) had anhydride units at 1880 and 1804 cm^{-1}, indicating symmetric and asymmetric C=O stretching vibrations of MA unit, respectively (22, 26, 27). C-O-C stretching vibrations, from MA ring, were observed at the 936 cm^{-1} (28). Characteristic C–O stretching vibrations at 1242 and 1012 cm^{-1} and a symmetric bending vibration of the CH_3 group of VA at 1395 cm^{-1} were observed (29, 30, 31). CH vibrations in CH-CH anhydride units observed at 629 and 1573 cm^{-1} (22) can be attributed

Figure 1 FTIR spectra of the MAVA copolymer (a) and modification product MAVA/CF (b).



to the CH_2 deformation mode of MAVA copolymer main chain (32). These findings were confirmed by means of the expected MAVA copolymer structure.

Anhydride ring peaks of MAVA/CF were disappeared completely (Fig. 1b). The disappearance of the characteristic anhydride peaks could suggest that almost all of the anhydride rings were opened by CF, via its nucleophilic amino group (33). It has been reported that the ring-opening reaction results in the formation of a carboxylic group and amide or ester structure (33). However, the carbonyl stretching within –COOR at 1500-1600 cm^{-1}, and –NH stretching of NH_2 group at 3000-3700 cm^{-1} were not observed in IR spectra of the modified copolymer. The -NH stretching of –CONHR mono-substituted amide group was observed in the MAVA/CF spectra (Fig. 1b): the intensity of the absorption bands of C=O on the anhydride groups, C-O-C: at 1880 cm^{-1} and C=O at 1804 cm^{-1} completely shifted to 1676 cm^{-1} (-N-C=O) after the modification (22,34,35) due to the amide formation (1,3). It has been observed that this peak is due to an -NH bending and a -CN stretching (C-N-H) (22), indicating the -CONHR mono-substituted amide group (Fig. 1b). These findings clearly demonstrated the formation of amide linkages. The strong peaks at 2953

Figure 2 ^{1}H-NMR spectra of MAVA copolymer (a) and modification product MAVA/CF (b).

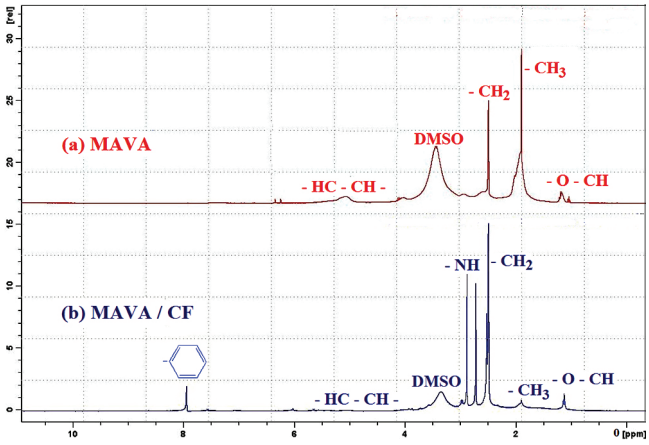
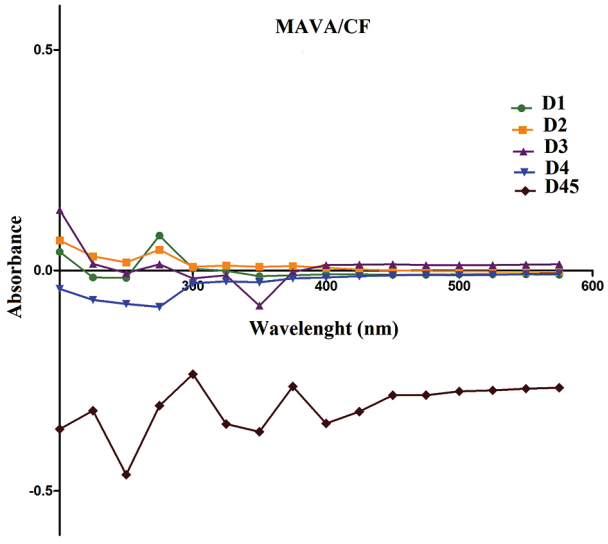


Figure 3 Absorption spectra for the behaviour of MAVA/CF in PBS solution.



and 1242 cm^{-1} were due to backbone stretching and bending, respectively (36). The CH vibrations in CH-CH anhydride units were observed at the 625 cm^{-1} (22). Another strong peak, 3438 cm^{-1}, was assigned to the hydroxyl group stretching vibration and the disappearance of the anhydride peak at both 1880 and 1804 cm^{-1} supported by the fact that the reaction of hydroxylamine (CF) with the anhydride group took place (36). An intense broad peak due to C-O-C stretching was also observed at 1063 cm^{-1} for both CF and VA (Scheme 1) (37,38). The C-H bending of VA unit was also observed at the 1395 cm^{-1} (34). These findings indicated that MAVA was efficiently modified with CF by an amidation reaction. Here, the ring opening reaction of the anhydride unit belong to maleic anhydride was also confirmed by FTIR analysis.

NMR Analysis

^{1}H-NMR Results

^{1}H-NMR results also confirmed those of FTIR for the MAVA copolymer. Characteristic features of the MAVA spectrum were a chemical shift of two protons on MA groups (-CH-CH-) at 5.4 ppm, three methyl (-CH_3) protons at 2 ppm (39,40), -CH_2 protons on VA, at approximately 2 ppm; and a multiplet peak for –CH, bound to oxygen, at approximately 1.1 ppm (16). DMSO deuterated solvent peak at 3.5 ppm were also observed (Fig. 2a).

The amide and carboxylic groups on MAVA/CF was verified by ^{1}H-NMR. The characteristic anhydride peak of MAVA for -CH-CH- protons, methyl ((-CH_3) protons of VA, as the methylene bridges -CH_2 protons on VA and a multiplet peak for –CH (bound to oxygen) were observed at 5.4 ppm, 2 ppm, around 2 ppm and 1.1 ppm (41), respectively (Fig. 2b). 0-5 ppm range of the spectra for both MAVA and MAVA/CF showed approximately similar characteristics. There were noticeable differences at 7,9 ppm for aromatic ring of the CF (42,43) and for the -NH group of the resulting amide structure at 2,5-3,3 ppm (2,42). As can be seen from the Scheme 2, aromatic ring comes from only pharmaceutical active ingredient CF. This peak was the most important evidence for the modification reaction of MAVA copolymer and CF molecule. However, any peak for the –NH_2 group of CF were not observed in ^{1}H-NMR spectra, while a peak is generally observed around 10,55 ppm for the modified copolymer. All of the resulting peak assignments confirmed that CF bound to MAVA by an amidation reaction (44).

Behaviour of MAVA and MAVA/CF in Phosphate Buffered Saline

In our previous study, spectrophotometric measurements indicated that MAVA could retain its molecular integrity in PBS for at least a month (18,21). Generally vinyl-based copolymers have been shown not to be biodegradable in biological media, and it did not undergo any degradation in PBS. For first four days, spectrophotometric measurements indicated that the MAVA/CF kept its molecular integrity in physiological fluid, PBS (physiological pH 7.40 at 37 ºC). In other words, the release of CF from the MAVA/CF appeared to have no started on the incubation times (Day 1, Day 2, Day 3, and Day 4) (Fig. 3). However, after the 45 days of incubation times there was a noticeable behavior difference because MAVA/CF did not kept its molecular integrity in physiological fluid, PBS. UV-spectrophotometric results indicated that the release of CF from the MAVA/CF will need to a detailed research by means of controlled releasing study techniques, for example, dissolution device

Figure 4a Antiproliferation activities of MAVA/CF on C6 at high concentrations.

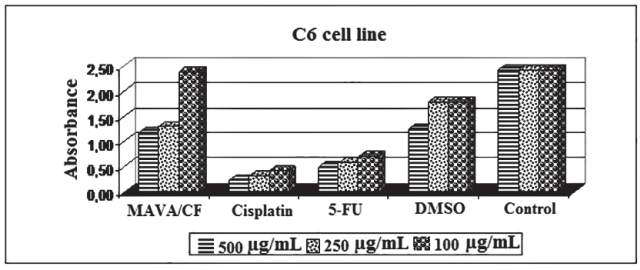


Figure 4a Antiproliferation activities of MAVA/CF on C6 at high concentrations.

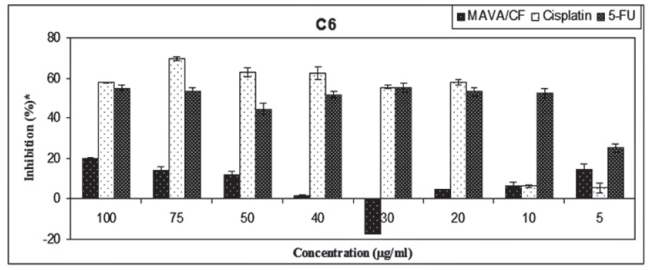


Figure 4b Antiproliferation activities of MAVA/CF on HeLa at high concentrations.

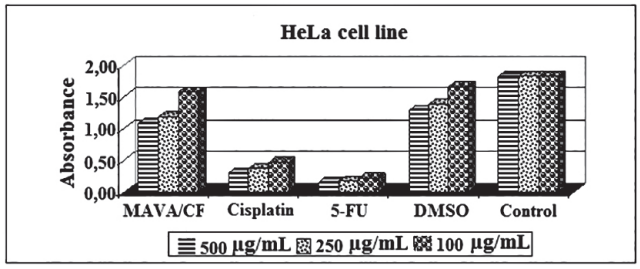
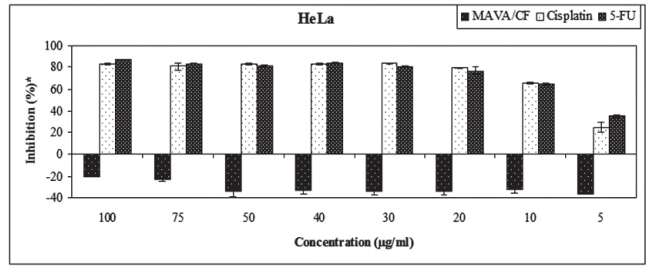


Figure 4b Antiproliferation activities of MAVA/CF on HeLa at high concentrations.



or HPLC (High Performance Liquid Chromatography) for evaluation of the drug release.

The reaction mechanism for the MAVA copolymer and MAVA/NA conjugate

A close inspection of the FTIR, and ^{1}H-NMR results revealed that the reaction mechanism was in agreement with that of a free-radical polymerization of vinyl-based monomers for the MAVA copolymer and that CF was incorporated by a ring opening reaction (Scheme 1 and 2, respectively). Percent yield of the conjugation reaction was approximately calculated as 79,4 %. Repeated units molecular weights of MAVA and MAVA/CF indicated 184 and 428 gmol^{-1}, respectively.

Antiproliferative activity

Antiproliferative activity of MAVA/CF was determined against C6 and HeLa using BrdU cell proliferation ELISA assay. Cisplatin and 5-florouracil were used as standard compounds. The antiproliferative activities were observed at high concentrations such as 500, 250 and 100 µg/mL. By comparing with the control and DMSO, the antiproliferative activities of MAVA/CF at high concentrations (especially 500, 250 µg/mL) were found to be significant (Fig. 4a and Fig. 4b).

Furthermore, this antiproliferative activity of MAVA/CF for both C6 and HeLa cell lines also increased with increasing concentrations. The same activities of samples and standards were deeply investigated for eight low concentrations ranging from 5 to 100 µg/mL (5, 10, 20, 30, 40, 50, 75 and 100 µg/mL). It has appeared that MAVA/CF has very little antiproliferative activity against C6 cell line, while the samples don't have antiproliferative activity against HeLa cell line. The antiproliferative activities of MAVA/CF were not observed to increase depending on increasing concentration against C6 cell line at low concentrations (Fig. 5a). However, MAVA/CF seemed to have exerted much higher antiproliferative activity than cisplatin at 5 µg/mL concentration against C6 cell line. At 10 µg/mL concentration, MAVA/CF had the same activity when compared with cisplatin against C6 cell line (Fig. 5a).

Contrary to having very little antiproliferative activity against C6, MAVA/CF didn't have any antiproliferative activity against HeLa when compared with cisplatin and 5-florouracil, especially under the 100 µg/mL concentrations (Fig. 5b). In addition, the antiproliferative activities of MAVA/CF were not observed to increase depending on increasing concentrations against HeLa. *In vitro* antiproliferative activity assay indicated that the activity of MAVA/CF was almost exclusive to one of the cell lines. It could be argued that the results of this antiproliferative activity of MAVA/CF may be associated with cell or tissue type specificity, having significant pharmaceutical applications/implications. Because of being recently synthesized in our research laboratory, the antiproliferative activities of MAVA/CF have not reported according to our present knowledge against HeLa and C6 cell up till now.

CONCLUSIONS

A biologically active copolymer, MAVA, was chosen as the drug carrier for CF being pharmaceutically active agent. In our previous study, it had been shown that the copolymer had almost no cytotoxicity, at 500 µg/mL, on a cultured mammalian L929 fibroblast cell lines, being least viscous copolymer, and was also kept its molecular integrity in physiological body fluids such as dextrose solution (DX 5%), phosphate-buffer solution (PBS; biotechnology grade, 137 mM NaCl, 2 mM KCl, and 10 mM phosphate buffer, pH 7.40 ± 0.1), and simulated body fluid (SBF; 50 mM tris(hydroxymethyl)aminomethane, 45 mM hydrochloric acid, pH 7.40) at 37 ºC for 5 days (18). In our other previous study, for the first time it was also shown that MAVA could kill *Entamoeba histolytica* trophozoites at 32 mg/mL for short periods of incubation time, or at much lower concentrations using longer incubation periods (19).

Furthermore, as CF has both amino- and phenolic hydroxyl-groups, chemical modifications of MAVA copolymer anhydride unit with this agent was possible by *ring opening reaction*. The results of the FTIR and ^{1}H-NMR spectra have confirmed the modification reaction. The modification product, MAVA/CF appeared to possess the intended below physiological features: (1) UV-Spectrophotometric results showed that the MAVA/CF kept its molecular stability in simulated physiological body fluid PBS (physiological pH 7.40 at 37 ºC) during the first 4 days and the release of CF from the MAVA/CF conjugate appeared to have no started on the incubation times. (2) MAVA/CF showed very little antiproliferative activity against C6 cell line, on the other hand the samples didn't have antiproliferative activity against HeLa cell line (<100 µg/mL). However, when compared with the control and DMSO, the antiproliferative activities of MAVA/CF at high concentrations (> 100 µg/mL) were found to be significant. *In vitro* antiproliferative activity assay indicated that the activity of MAVA/CF was almost exclusive to one of the cell lines, and could be associated with cell or tissue type specificity, having significant pharmaceutical applications/ implications. Both of the animals (C6, rat brain tumor) and human (HeLa, human cervix carcinoma) cell lines were quite important for investigation of the antiproliferative activity of MAVA/CF.

In the lights of the above results, it could be suggested that the modification product deserved further investigation in order to better evaluate its capacity as a biologically active substance by assessing its antitumor (on other cancer cell lines), antimicrobial and antifungal activities. Furthermore, the cumulative or steady release of CF from MAVA/CF conjugate will also be investigated deeply by other spectrophotometric methods.

ACKNOWLEDGEMENTS

This work was supported by the Sciences Research Projects Foundation of Cumhuriyet University (Project No: F258 and F339).

Yeni modifiye Poli(maleik anhidrit-*ko*-vinil asetat)/Sitozin β-D-arabinofuranosid hidroklorid konjugatının sentezi, karakterizasyonu ve antiproliferatif aktivitesi

ÖZET

Poli(maleik anhidrid-*ko*-vinil asetat) (MAVA) kopolimeri, metil etil keton (MEK) içinde 80 ºC'de radikalik başlatıcı olarak benzoil-peroksit (BPO) kullanılarak serbest radikal polimerleşmesi ile sentezlenmiştir. MAVA daha sonra anti-lösemik bir kemoterapötik ajan olan Sitozin β-D-arabinofuranosid hidroklorid (CF) ile modifiye edilmiştir. Modifikasyon tepkimesi 70 ºC'de dimetilformamid içinde trietilamin (Et_3N) katalizörlüğünde yapılmıştır. Kopolimer ve Kopolimer/İlaç (MAVA/CF) ikilisinin yapısal karakterizasyonu Fourier Transform Infrared (FTIR) ve Nükleer Magnetik Rezonans (^{1}H-NMR) ile yapılmıştır. FTIR ve ^{1}H-NMR spektrumları modifikasyon tepkimesini doğrulamaktadır. UV-Spektrofotometrik ölçümleri göstermiştir ki MAVA/CF fizyolojik vücut sıvısı olan PBS (fosfat buffer saline) içinde moleküler bütünlüğünü ilk dört gün için muhafaza etmiştir. MAVA/CF'nin antiproliferatif aktivitesi de BrdU-cell-proliferation-ELISA analiz yöntemi ile C6 ve HeLa hücreleri (cisplatin and 5-florourasil pozitif kontroldür) kullanılarak yapılmıştır. MAVA/CF'nin C6 hücrelerine karşı azda olsa bir antiproliferatif etkiye sahip olduğu, oysa HeLa hücrelerine karşı özellikle düşük derişimlerde (< 100 µg/ml) antiproliferatif etkisinin olmadığı gözlenmiştir. Modifikasyon ürünü MAVA/CF için tepkime mekanizması da önerilmiştir.

Anahtar Kelimeler: Antiproliferatif etki, kopolimer modifikasyon, sitozin β-D-arabinofuranosid hidroklorür, fizyolojik vücut sıvısı PBS, poli(maleik anhidrit-*ko*-vinil asetat).

REFERENCES

1. Saad GR, Morsi RE, Mohammady SZ, Elsabee MZ Dielectric relaxation of monoesters based poly(styrene-co-maleic anhydride) copolymer. J Polym Res 2008; 15:115-23
2. Atıcı OG, Akar A, Rahimian R Modification of poly(maleic anhydride-co- styrene) with hydroxyl containing compounds. Turk J Chem 2001; 25:259-66.
3. Patel H, Raval DA, Madamwar D, Patel SR Polymeric prodrug: Synthesis, release study and antimicrobial property of poly(styrene-co-maleic anhydride)-bound acriflavine. Angew Makromol Chem 1998; 263:25-30.
4. Liu HY, Cao K, Yao Z, Li BG, Hu GH Variations of the glass-transition temperature in the imidization of poly(styrene-co-maleic anhydride). J Appl Polym Sci 2007; 104:2418-22
5. Jain JP, Modi S, Domb AJ, Kumar N. Role of Polyanhydrides as localized Drug Carriers. Control Release 2005; 103:541-63.
6. Breslow DS Biologically active synthetic polymers. Pure & Appl Chem 1976; 46:103-13.
7. Dhal PK, Holmes-Farley SR, Huval CC, Jozefiak TH. Polymers as drugs. Adv Polym Sci 2006;192:9-58.
8. Duncan R. The dawning era of polymer therapeutics. Nat Rev Drug Discov 2003; 2:347-60.
9. Greish K, Sawa T, Fang J, Akaika T, Maeda H SMA-doxorubicin, a new polymeric micellar drug for effective targeting to solid tumors. J Control Release 2004; 97:219-30.
10. Maeda H, Ueda M, Morinaga T, Matsumoto T Conjugation of poly(styrene-*co*-maleic acid) derivatives to the antitumor protein neocarzinostatin: pronounced improvements in pharmacological properties. J Med Chem 1985; 28:455-61.
11. Kobayashi A, Oda T, Maeda H Protein binding of macromolecular anticancer agent SMANCS: Characterization of poly(styrene-*co*-maleic acid) derivatives as an albumin binding ligand. J Bioact Compat Pol 1988; 3:319-33.
12. Ohtsuka N, Konno T, Myauchi Y, Meada H Anticancer effects of arterial administration of the anticancer agent SMANCS with lipiodol on metastatic lymph nodes. Cancer 1987; 59:1560-65.
13. Maeda H, Matsumoto T, Konno T, Iwai K, Ueda M Tailor-making of protein drugs by polymer conjugation for tumor targeting: A brief review on Smancs. J Protein Chem 1984; 3:181-93.
14. Greish K, Fang J, Inutsuka T, Nagamitsu A, Maeda H Macromolecular Therapeutics: Advantages and prospects with special emphasis on solid tumour targeting. Clin Pharmacokinet 2003; 42:1089-105.
15. Maeda H. SMANCS and polymer-conjugated macromolecular drugs: advantages in cancer chemotherapy. Adv Drug Deliv Rev 1991; 6:181-202.
16. Spridon D, Panaitescu L, Ursu D, and Uglea CV. Synthesis and biocompatibility of maleic anhydride copolymers: 1. maleic anhydride-vinyl acetate, maleic anhydride methyl methacrylate and maleic anhydride-styrene. Polym Int 1997; 43:175-81.
17. Vardanyan RS, Hruby VJ. Synthesis of Essential Drugs. Elsevier. Amsterdam. 2006.
18. Karakus G, Zengin HB, Akin Polat Z, Yenidunya AF, Aydin S Cytotoxicity of three maleic anhydride copolymers and common solvents used for polymer solvation. Polym Bull 2013; 70:1591-612.
19. Karakus G, Yenidunya AF, Zengin HB, Malatyali E, Ozcelik S Amoebicidal effect of poly(maleic anhydride-*co*-vinyl acetate) copolymer on entamoeba histolytica trophozoites. Med Chem 2011; 1:1-4.
20. Nguyen V, Yoshida W, Cohen Y. Graft polymerization of vinyl acetate onto silica. J Appl Polym Sci 2003; 87:300-10.
21. Karakus G, Akin Polat Z, Yenidunya AF, Zengin HB, Karakus CB. Synthesis, characterization and cytotoxicity of novel modified poly[(maleic anhydride)-*co*-(vinyl acetate)]/noradrenaline conjugate. Polym Int 2013; 62: 492-500.
22. Kaplan Can H, Doğan AL, Rzaev ZMO, Uner AH, Güner A. Synthesis and antitumor activity of poly(3,4-dihydro-2H-pyran-*co*-maleic anhydride-*co*-vinyl acetate). J Appl Polym Sci 2005; 96:2352-9.
23. Sundell AM, Luttikhedde HJG. Drug release from ionomer cements based on hydrolyzed poly(vinylacetate-maleic anhydride). Pure Appl Chem 1999;A361045-59
24. Demirtas I, Sahin A, Ayhan B, Tekin S, Telci I. Antiproliferative effects of the methanolic extracts of *sideritis libanotica* labill. subsp. *linearis*. Rec Natural Prod 2009; 3:104–9.
25. Demirtas I, Sahin A. Bioactive volatile content of the stem and root of *Centaurea carduiformis* DC. subsp. *carduiformis* var. *carduiformis*. J Chem 2013; 125286: 6 pages.
26. Pal J, Singh H, Ghosh AK. Modification of LLDPE using esterified styrene maleic anhydride copolymer: Study of its properties and environmental degradability. J Appl Polym Sci 2004; 92:102-8.
27. Popa I, Offenberg H, Beldie C, Uglea CV. Benzocaine modified maleic anhydride copolymers-I. Synthesis and characterization of benzocaine modified poly(maleic anhydride-*co*-vinyl acetate), poly(maleic anhydride-*co*-methyl methacrylate) and poly(maleic anhydride-*co*-styrene). Eur Polym J 1997; 33:1511-14.
28. Chitanu GC, Popescu I, Carpov A. Synthesis and characterization of maleic anhydride copolymers and their derivatives. 2. New data on the copolymerization of maleic anhydride with vinyl acetate[1]. Rev Roum Chim 2006; 51: 923-9.
29. Coskun M, Seven P. Synthesis, characterization and investigation of dielectric properties of two-armed graft copolymers prepared with methyl methacrylate and styrene onto PVC using atom transfer radical polymerization. React Funct Polym 2011; 71: 395-401.

30. Qiao Z, Xie Y, Chen M, Xu J, Zhu Y, Qian Y. Synthesis of lead sulfide/(polyvinyl acetate) nanocomposites with controllable morphology. Chem Phys Lett 2000; 321: 504-7.
31. Barakat MF, Abdelkerim FM. Investigation of the polymers formed in reactor irradiated aniline and pyridine. Can J Chem 1973; 51:3860-5.
32. Tripp RA, Dluhy RA, Zhao Y. Novel nanostructures for SERS biosensing. Nanotoday (Review) 2008; 3:31-7.
33. Jeong JH, Byoun YS, Ko SB, Lee YS Chemical modification of poly(styrene-*alt*-maleic anhydride) with antimicrobial 4-aminobenzoic acid and 4-hydroxybenzoic acid. J Ind Eng Chem 2001; **7**:310-5.
34. Ferruti P, Ranucci E, Trotta F, Gianasi E, G. Evagorou E, Wasil M, Wilson G, Duncan R. Synthesis, characterization and antitumour activity of platinum (II) complexes of novel functionalized poly(amido amine)s. Macromol Chem Phys 1999; 200:1644-54.
35. Kim SY, Kim C, Han IS, Lee SC, Kim SH, Lee KS, Choi Y, Byun Y. Inhibition effect of new farnesol derivatives on all-trans-retinoic acid metabolism. Metabolism 2001; 50:1356-60.
36. Kenawy ER, Abdel-Hay F, El-Newehy M, Ottenbrite RM. Effect of pH on the drug release rate from a new polymer-drug conjugate system. Polym Int 2008; 57: 85-91.
37. Balaji R, Nanjundan S. Copolymerization of 3-methoxy-4-methacryloyloxybenzal phenylimine with methyl methacrylate. Eur Polym J 1999; 35:1133-8.
38. Rzayev ZMO. Graft copolymers of maleic anhydride and its isostructurel analogues: High performance engineering materials. Int Rev Chem Eng 2011; 3:153-215.
39. Yoon KJ, Woo JH, Seo YS. Formaldehyde free cross-linking agents based on maleic anhydride copolymers. Fiber Polym 2003; 4:182-7.
40. Nemtoi G, Beldie C, Tircolea C, Popa I, Cretescu I, Humelnicu I, Humelnicu D. Behaviour of the poly(maleic anhydride-co-vinyl acetate) copolymer in aqueous solutions. Eur Polym J 2001; 37:729-35.
41. Ranjbar-Karimi R, Loghmani-Khouzani H. Synthesis of new azines in various reaction conditions and investigation of their cycloaddition reaction. J Iran Chem Soc 2011; 8: 223-30.
42. Sanmathi CS, Prasannakumar S, Sherigara BS. Terpolymerization of 2-ethoxy ethylmethacrylate, styrene and maleic anhydride:determination of the reactivity ratios. Bull Mater Sci 2004; 27:243-9.
43. Bruch M, Mäder D, Bauers F, Loontjens T, Mülhaupt R. Melt modification of poly(styrene-co-maleic anhydride) with alcohols in the presence of 1,3-oxazolines. J Polym Sci 2000; 38:1222-31.
44. Cox CJ, Dutta K, Petri ET, Hwang WC, Lin Y, Pascal SM, Basavappa R. The regions of securing and cyclin B proteins recognized by the ubiquitination machinery are natively unfolded. Febs Lett 2002; 527:303-8.