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Does Vinblastine Retain its Anticancer Effect in the Presence of Cyclodextrin? A Preliminary Study

Péter Keglevich[1*], István Zupkó[2], Lajos Szente[3], István Puskás[3], Áron Szigetvári[4], Szabolcs Mayer[1], László Hazai[1]

[1] Department of Organic Chemistry and Technology, Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics, Műegyetem rkp. 3., H-1111 Budapest, Hungary
[2] Institute of Pharmacodynamics and Biopharmacy, University of Szeged, Eötvös u. 6., H-6720 Szeged, Hungary
[3] Cyclolab Cyclodextrin Research and Development Laboratory Ltd., Illatos út 7., H-1097 Budapest, Hungary
[4] Spectroscopic Research Department, Gedeon Richter Plc., H-1475 Budapest, P. O. B. 27, Hungary
[*] Corresponding author, e-mail: keglevich.peter@vbk.bme.hu

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Abstract

Vinblastine was investigated in the presence of cyclodextrin derivatives on different cancer cells and it was established that the anticancer activity was unchanged. This was the first step of a long procedure resulting in the dosage of vinblastine together with cyclodextrin or its derivatives to improve the adverse effects.

Keywords

cyclodextrin, vinblastine, complex, anticancer effect, NMR titration

1 Introduction

Vinblastine is a well-known anticancer (antineoplastic or cytotoxic) chemotherapy drug [1] (Fig. 1) and is used for treating Hodgkin's and non-Hodgkin's lymphoma, testicular, breast, lung, non-small cell lung cancer, Kaposi's sarcoma, etc. Vinblastine is a very effective agent, however, has some serious side effects e.g., hair loss, fever, several pains, and besides that neurotoxicity [2]. The in vivo disposition of the drug is primarily determined by ABCB1 transporter-mediated efflux and metabolism by cytochrome 3A4, leading to its terminal biological half-life of about one day [3–5]. Vinca alkaloids are typical antimitotic agents eliciting their actions by binding to β-tubulin, disrupting physiological tubulin polymerization, and preventing microtubule assembly. It should be mentioned that vinblastine is a high-charge density cation and thus binds with high affinity to the anionic protein tubulin [6]. The consequences of vinblastin exposure include mitotic arrest, apoptosis, and cell death. In most cases, vinblastine can be administered in a mixture (cocktail) with other anticancer agents or with other compounds decreasing the adverse effects.

Recently, great efforts have been made to produce more effective and less toxic derivatives [7, 8]. Moreover, hybrid molecules, in which two or more pharmacophores are coupled covalently to each other, as a new trend, were synthesized in increasing numbers [9, 10]. In addition, a number of possibilities have been explored where another carrier molecule may be present as a guest in connection with the anticancer host molecule by non-covalent bonds. One of these options is the well-established and widespread cyclic carbohydrate, the cyclodextrin and/or its derivatives [11–17]. Cyclodextrin has been widely utilized to modify anticancer agents' water-solubility and improve their safety profile [18–23]. Another recent example is the study of exciting antiviral agents, coupled with cyclodextrin resulting in several very important outcomes [24, 25]. Moreover, a flavon (galangin) with important activity in the treatment of breast cancer is proved to be more advantageous characteristics using together with β-cyclodextrin [26].

Nevertheless, although the relative strength of interaction between anticancer drugs (including vinblastine) and cyclodextrin derivatives was established [12], a comprehensive and exhaustive study of the effect of cyclodextrins on the anticancer activity and toxicity of vinblastine has not been found. This was the reason why the first step in our research project was to study how cyclodextrins change the antiproliferative effect of vinblastine.

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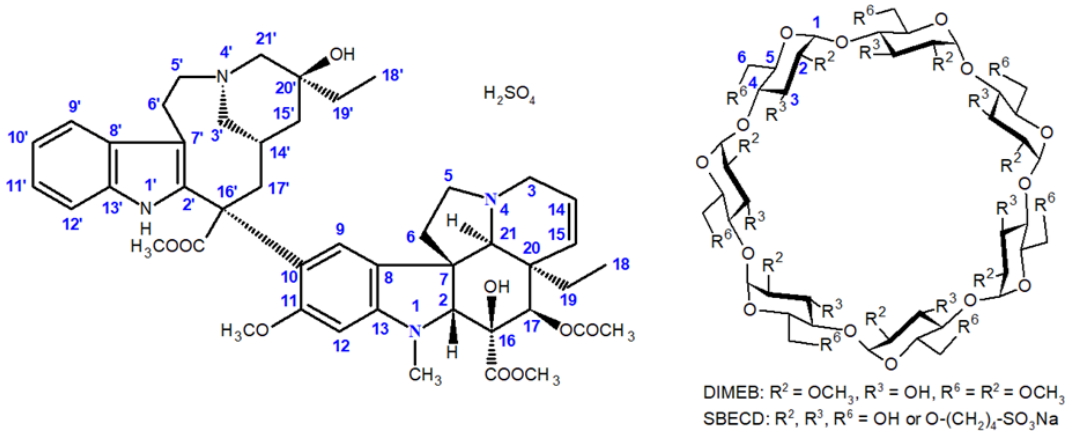

(a) (b)

Fig. 1 (a) The structural formula of vinblastine sulfate, (b) DIMEB and SBECD

2 Results and discussion

2.1 Preparation of complexes

Two types of cyclodextrin-enabled vinblastine (VLB) compositions were prepared, by using an electroneutral heptakis(2,6-di-*O*-methyl)-*β*-cyclodextrin (DIMEB), and a polyanionic, sulfobutylether-beta-cyclodextrin (SBECD). The method of lab scale preparation is described below (see Section 4).

2.2 NMR spectroscopic measurements of the cyclodextrin complexes of vinblastine

First, we have identified the signal sets of vinblastine (Fig. 1 (a)) and the corresponding cyclodextrins (Fig. 1 (b)) in the ^{1}H NMR spectra of the compositions (Fig. 2).

The 800 MHz ^{1}H NMR spectra of vinblastine sulfate/ DIMEB composition and vinblastine sulfate/SBECD composition are shown in Fig. 2 (a) and (b). The molar ratio of vinblastine and either cyclodextrin is estimated by using ^{1}H NMR spectroscopy (Fig. 2) as follows: the peak at δ 7.55 ppm (peak A) corresponds to one hydrogen atom (H-9') in vinblastine; broad signals at $\delta \approx 5$ ppm (peak B) corresponds to seven hydrogen atoms (7 × H-1) in a cyclodextrin (DIMEB: δ 5.10-5.20 ppm; SBECD: δ 4.88–5.20 ppm). Therefore, the ratio of the area of peak A and the 1/7th of the area of peak B gives the molar ratio of the components, which is 1.03:(8.14/7) = 1:1.1 for vinblastine / DIMEB and 0.97:(8.00/7) = 1:1.2 for vinblastine / SBECD.

Then, we performed interproton spatial proximity measurements for the detection of host–guest interactions. The NMR spectroscopic behavior of DIMEB and SBECD differs from each other because DIMEB is a single chemical compound but SBECD is a mixture of randomly substituted cyclodextrin molecules. Therefore, DIMEB gives sharp peaks in the ^{1}H NMR spectrum but SBECD gives broad peaks, which is a characteristic of mixtures of structurally similar compounds. To detect spatial proximity between sharp ^{1}H resonances in vinblastine sulfate/ DIMEB composition, we selected the 2D ROESY experiment (Fig. 3). To detect spatial proximity between a sharp ^{1}H resonance that belongs to vinblastine sulfate and a broad ^{1}H resonance of the SBECD, our choice was the 1D NOESY experiment (Fig. 4). We were able to substantiate the presence of the host–guest interactions by detecting the proximity of H-11' of vinblastine (δ 7.17 ppm) and CH(OR) hydrogen atoms of the cyclodextrins (δ 3.6–3.8 ppm).

Finally, as a further proof of host–guest interactions, we titrated a solution of vinblastine with DIMEB in a carbonate buffer (pH ≈ 10). The concentration of vinblastine base (c_{VLB}) was 36.7 µmol/dm^3 (made by dissolving 0.1 mg vinblastine sulfate in 3 ml buffer). The total concentration of DIMEB (c_{DIMEB}) was varied according to Table 1. To illustrate the strength of complexation, we calculated an apparent association constant by presuming the formation of a 1:1 complex (Eqs. (1) and (2)).

To simplify further calculations, Eq. (3) gives the ratio of vinblastine in complex (VLB·DIMEB, r) and its total concentration, c_{VLB}, where c_{VLB} = [VLB] + [VLB·DIMEB]); the a, b, and c constants are given in Eq. (4). The chemical shift (δ) of any ^{1}H NMR resonance is calculated as the linear combination of the δ of the free VLB and the δ of the VLB in complex (Eq. (5)), given that the association and dissociation kinetics are fast enough for detecting a single δ representing the average state of the system).

Eq. (5) is solved iteratively, by finding the δ of VLB in complex ($\delta^{VLB \cdot DIMEB}$) and the association constant K; δ^{VLB} is known from the measurement of the solution

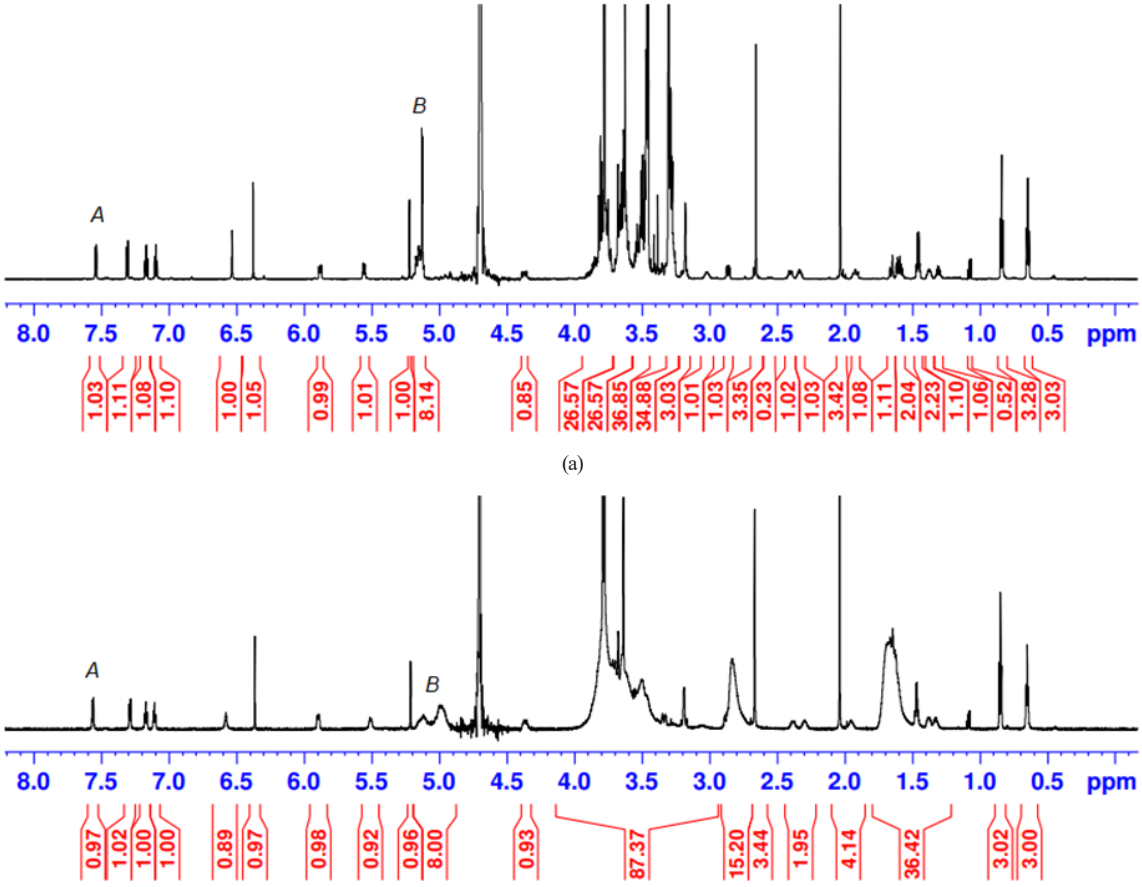

Fig. 2 ^{1}H NMR spectra of (a) vinblastine sulfate/DIMEB composition and (b) vinblastine sulfate/SBECD composition in D_2O. Chemical shift scale (δ, ppm) and area under peaks are shown in blue and red

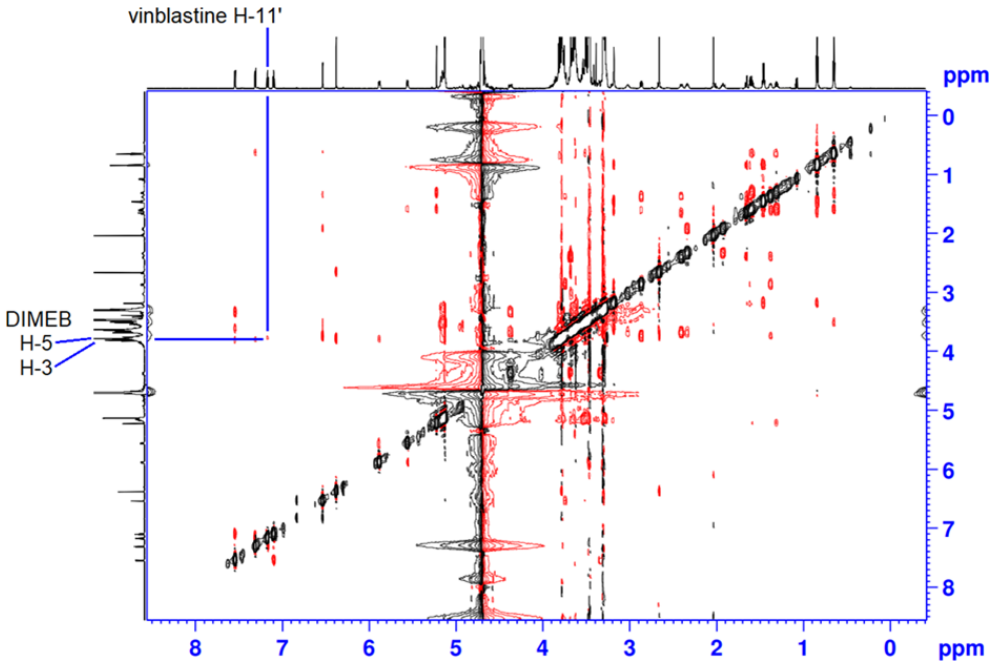

Fig. 3 2D ROESY spectrum of vinblastine/DIMEB composition in D_2O

without any added cyclodextrins (7.1652 ppm, as we used the signal of H-11' as δ^{exp} for monitoring the complexation; Fig. 5). The solution corresponds to the minimum of the sum of the squares of residuals (δ^{fit}–δ^{exp}). We have found

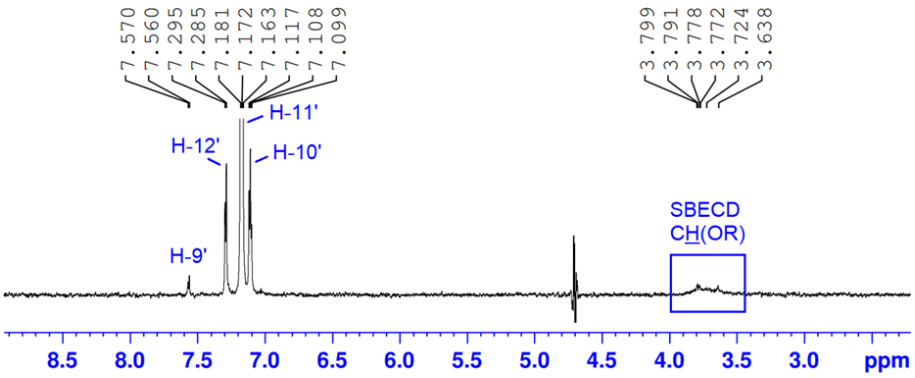

Fig. 4 1D NOESY spectrum of vinblastine/SBECD composition in D_2O

Table 1 ^{1}H NMR titration of vinblastine base with DIMEB in D_2O

c_{DIMEB} (μmol/dm³)	0	13.1	25.0	35.9	45.8	63.5	78.6	127	244	455	943	3300
r (%)	0.0	0.3	0.5	0.8	1.0	1.4	1.7	2.7	5.1	9.1	17.2	42.1
δ^{exp} (ppm)	7.1652	7.1648	7.1642	7.1636	7.1636	7.1633	7.1624	7.1601	7.1565	7.1491	7.1357	7.0911
δ^{fit} (ppm)	7.1652	7.1647	7.1643	7.1639	7.1635	7.1628	7.1623	7.1605	7.1564	7.1495	7.1354	7.0911
$\delta^{fit}-\delta^{exp}$ (ppm)	0.0000	−0.0001	0.0001	0.0003	−0.0001	−0.0005	−0.0001	0.0004	−0.0001	0.0004	−0.0003	0.0000

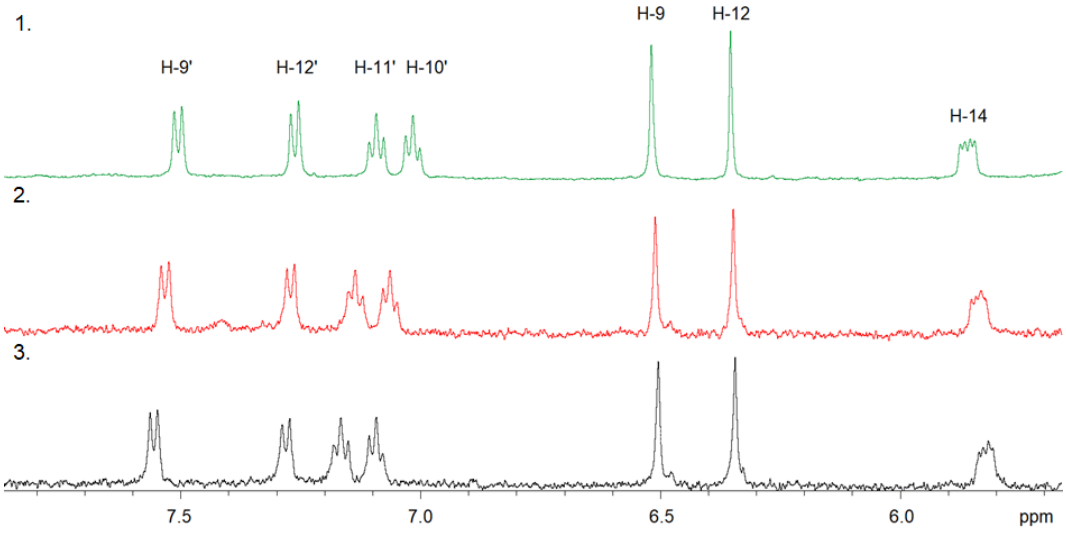

Fig. 5 Selected partial ^{1}H NMR spectra (500 MHz) from the titration series of vinblastine base (c_{VLB} = 36.7 μmol/dm³) with DIMEB. 1. c_{DIMEB} = 3.3 mmol/dm³; 2. c_{DIMEB} = 0.94 mmol/dm³; 3. without any added DIMEB

that $\delta^{VLB\cdot DIMEB}$ = 6.9832 ppm and K = 209 M^{-1} (the error is on order of 10%) gives the best fit (R^2 = 0.9999) to the 1:1 complexation model.

$$\mathrm{VLB} + \mathrm{DIMEB} \rightleftharpoons \mathrm{VLB}\cdot\mathrm{DIMEB} \tag{1}$$

$$K = \frac{[\mathrm{VLB}\cdot\mathrm{DIMEB}]}{[\mathrm{VLB}][\mathrm{DIMEB}]}\left(\mathrm{dm^3\ mol^{-1}}\right) \tag{2}$$

$$r = \frac{[\mathrm{VLB}\cdot\mathrm{DIMEB}]}{c_{\mathrm{VLB}}} = -\frac{b}{2a} + \frac{\sqrt{b^2-4ac}}{2a} \tag{3}$$

$$a = c_{\mathrm{VLB}};\ b = -\left(c_{\mathrm{VLB}} + c_{\mathrm{DIMEB}} + \frac{1}{K}\right);\ c = c_{\mathrm{DIMEB}} \tag{4}$$

$$\delta^{\mathrm{fit}} = r\times\delta^{\mathrm{VLB\cdot DIMEB}} + (1-r)\times\delta^{\mathrm{VLB}} \tag{5}$$

According to our experiments, we have strong evidence for host-guest interactions of DIMEB and SBECD with the aromatic site (H-9' to H-12') of the velbanamine (catharanthine) part of vinblastine; a hypothetical 100% complexation of vinblastine with DIMEB would result in a shift of the ^{1}H NMR signal of H-11' of vinblastine by −0.18 ppm. However, the mapping of (presumably weaker)

competing processes or the possibility of non 1:1 complexation schemes requires further NMR method developments and data analysis.

2.3 Biological investigations

The cell-based assays aimed to characterize the effect of cyclodextrin complex formation on the antiproliferative action of vinblastine. The antiproliferative properties of the prepared complexes and the pure active agent were determined in a wide range of concentrations (1.00 nM – 30 μM). The overlap of the obtained fitted dose-response curves indicates that none of the applied cyclodextrins modified the efficacy of vinblastine (Fig. 6). This finding was confirmed by the narrow ranges of the calculated IC50 values: 3.92–5.39 nM and 1.72–3.13 nM against A2780 and MCF7 cells, respectively.

Though cyclodextrins are generally well-tolerated, they can interfere with the growth of cells at high concentrations. Therefore, the cyclodextrin concentrations corresponding to the three highest vinblastine concentrations were applied to the two types of cancer cells (Fig. 7). None of the two cyclodextrins affected the viability of A2780 cells, even at the highest concentrations corresponding to 30 μM vinblastine. Similarly, no relevant change was detected in the growth of MCF7 cells by SBECD. DIMEB, on the other hand, elicited a moderate but statistically significant inhibition of the viability of the breast cancer cells at the concentrations corresponding to 10 and 30 μM vinblastine. Theoretically, these actions can contribute to the antiproliferative activity of the complex. However, the alkaloid elicits its maximal antiproliferative action at these and even lower concentrations. Based on these findings, it can be concluded that the contribution of DIMEB to the activity of complex against MCF7 cells is negligible.

3 Conclusion

In the course of our work, investigating the synthesis and characteristics of cyclodextrin-vinblastine compositions, derivatives of the electroneutral DIMEB and the polyanionic SBECD containing compounds were successfully prepared. By detailed NMR spectroscopic measurement it could be pointed out that the host–guest interactions presumably occurred between the aromatic ring of the catharanthine part of vinblastine and DIMEB or SBECD.

Based on the results of the in vitro cell-based experiments, it can be concluded that the applied cyclodextrins used for complex formation have no relevant action on the pharmacological activity of vinblastine. Nevertheless, it is clear from these data, that it is worthwhile to carefully examine whether any of the known side effects of vinblastine decrease with administration under these conditions and, if so, to what extent.

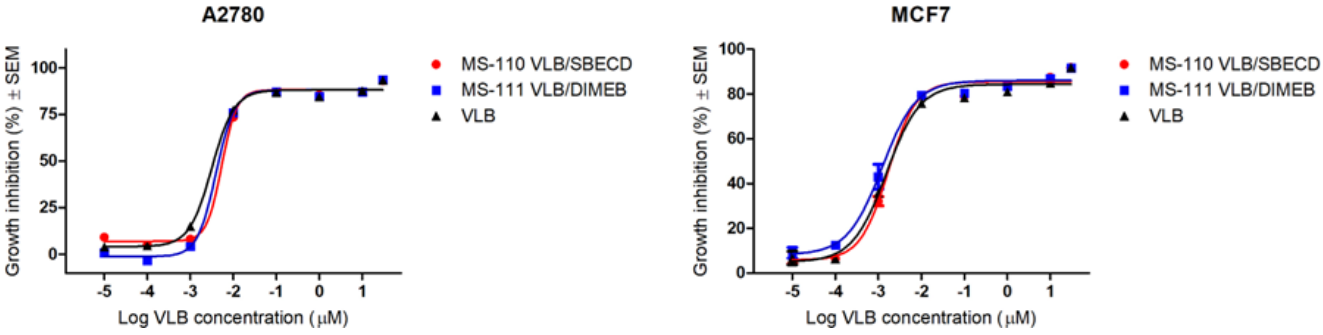

Fig. 6 Antiproliferative actions of vinblastine (VLB) and its cyclodextrin complexes against ovarian (A2780) and breast (MCF7) cancer cells

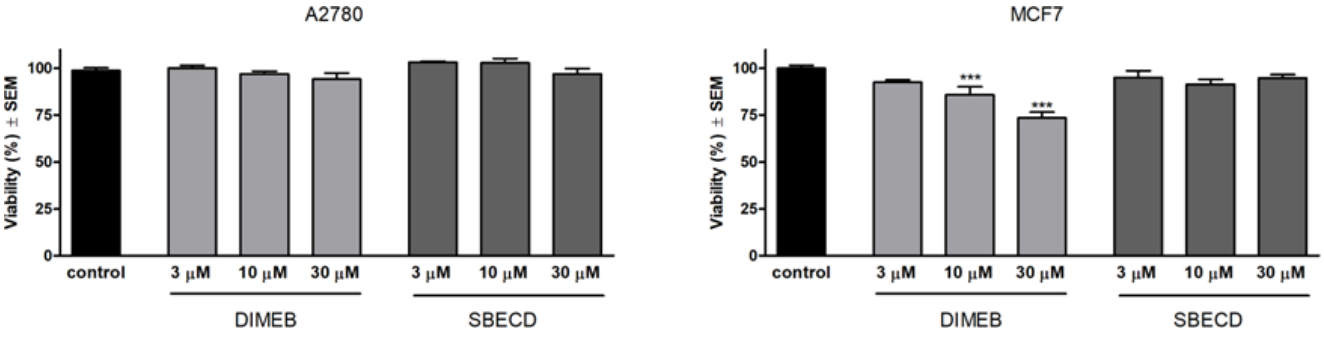

Fig. 7 Actions of DIMEB and SBECD on the viability of A2780 and MCF7 cells. Cyclodextrin concentrations were identical to those applied in complexes at the indicated vinblastine concentrations. *** means $p < 0.001$, as compared to the control samples

4 Experimental methods

4.1 Vinblastine/DIMEB composition

54 mg of heptakis(2,6-di-*O*-methyl)-*β*-cyclodextrin was weighed in an agate mortar and 25 microliter of distilled water was added to the solid powder. The mixture was homogenized at room temperature with an agate pestle, a viscous solution was formed. 46 mg of vinblastine sulfate was then added and the mixture was thoroughly homogenized. The wet binary composition was dried in vacuo under P_2O_5 over 24 hours period at room temperature. The dried substance was pulverized by the pestle.

4.2 Vinblastine/SBECD composition

68 mg of sulfobutylether *β*-cyclodextrin was weighed in an agate mortar and 35 microliter of distilled water was added to the solid powder. The mixture was homogenized with an agate pestle, a viscous solution was formed. 32 mg of vinblastine sulfate was then added and the mixture was thoroughly homogenized. The wet composition was dried in vacuo under P_2O_5 over 24 hours period at room temperature. The dried substance was pulverized by the pestle.

4.3 Cell culture-based experiments

The antiproliferative properties of the prepared complexes and vinblastine were determined through MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay against MCF-7 and A2780 cancer cells isolated from breast and ovarian cancers, respectively [27]. Both cell lines were obtained from the European Collection of Cell Cultures (ECCAC, Salisbury, UK) and maintained in minimal essential medium supplemented with 10% fetal bovine serum, 1% non-essential amino acids, and an antibiotic-antimycotic mixture. All media and supplements were purchased from Lonza Group Ltd., Basel, Switzerland. The assays were performed as described previously [28]. The final concentrations of the tested cyclodextrin complexes always referred to the vinblastine content. When the actions of the cyclodextrins on the cell growth were assayed, the amounts corresponding to the three highest vinblastine concentrations (3, 10, and 30 μM) were applied. All the calculations, including curve fitting of sigmoidal dose-response curves and statistical evaluations (AVOVA followed by Dunnett's posttest), were performed using GraphPad Prism 5.01 software (GraphPad Software Inc., San Diego, CA, USA).

4.4 NMR spectroscopic measurements

^{1}H NMR, 2D ROESY, and selective 1D NOESY spectra were measured on a Bruker Avance III HDX 800 MHz NMR spectrometer equipped with a cryogenically cooled H-F{C,N} triple resonance z-gradient probe and on a Bruker Avance III HDX 500 MHz NMR spectrometer equipped with a cryogenically cooled H{C,N} triple resonance z-gradient probe (Bruker Corporation, Billerica, MA, USA). The spectra were recorded at room temperature (298 K) in water-d_2 (D_2O). ^{1}H NMR chemical shifts are given on the delta (δ) scale with the reference signal of HDO at 4.70 ppm.

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