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RESEARCH ARTICLE

The Investigation of Anti-Proliferative Effects of [Ag2(sac)2(dap)2] Complex on Different Types of Cancer

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Abstract

Objective: Cytotoxic features of silver and saccharine compounds have taken attention due to the fact that most of them have showed a cytotoxic effect against tumor cells. In this study, it is aimed to investigate anti-proliferative effects of [Ag2(sac)2(dap)2] complex on different types of cancer cell lines.

Methods: HeLa (cervical cancer), PC-3 (prostate cancer), DU-145 (prostate cancer), A549 (non-small cell lung cancer), K562 (chronic myeloid leukemia) and MRC-5 (normal lung fibroblast) cell lines were grown in plates. In order to determine anti-proliferative effects, IC 50 values of [Ag2(sac)2(dap)2] and cisplatin on these cell lines were determined by MTT method**.**

Results: IC 50 value of [Ag2(sac)2(dap)2] complex was 19.53 ± 1.74 µM for HeLa cell line, 17.14 ± 1.41 µM for PC-3 cell line, 18.56 ± 4.04 for DU 145 cell line, 17.93 ± 1.06 µM for A549 cell line, 3.18 ± 0.04 µM for K562 cell line and 7.25 ± 1.00 µM for MRC-5 cell line. Also, IC 50 value of cisplatin was 4.00 ± 0.47 µM for HeLa cell line, 12.29 ± 1.60 µM for PC-3 cell line, 5.05 ± 0.65 for DU 145 cell line, 12.74 ± 1.26 µM for A549 cell line, 5.90 ± 0.59 µM for K562 cell line and 5.91 ± 0.32 µM for MRC-5 cell line. Only in K562 cell line, IC 50 of [Ag2(sac)2(dap)2] complex was lower than IC 50 of cisplatin.

Conclusion: Anti-proliferative activity of [Ag2(sac)2(dap)2] complex is more than cisplatin on chronic myeloid leukemia cells so this complex may be possible to be used as a treatment option especially for chronic myeloid leukemia.

Key words: [Ag2(sac)2(dap)2]; inorganic complex; cancer cell lines; anti-proliferation

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Introduction

Saccharin is an organic compound with a melting point of 225-227 ºC, systematically called 1,2-benzisothiazol-3 (2H) -one 1,1-dioxide or o-sulfobenzimide. Saccharin, which is 500 to 700 times sweeter than sugar, is not metabolized in the body and it is used as a reliable artificial sweetener for diabetics because of this feature. The use of saccharin as an antidote to metal poisoning, its features listed above and its use in the food, beverage, toothpaste, mouthwash and pharmaceutical industries are increasing the importance of studies conducted on saccharin (Baran and Yilmaz, 2006).

In addition, saccharin is a highly preferred ligand in recent years since it has a multi-functional ligand feature. Sodium saccharinate, the sodium salt of saccharin, is highly soluble in water (830 g / L at 20 °C) and is suitable for use both as a ligand and as a sweetener. When sodium saccharinate is used as the ligand, the saccharinate anion recovered in the solution has both negatively charged N atoms and CO and SO2 groups and is coordinated to metals with all these donor parts. The presence of anti-bacterial and anti-carcinogenic effects of metal saccharin complexes synthesized in the literature also increases the importance of studies on these complexes. In recent years, Pt (II) and Pd (II) complexes with mixed ligands containing saccharinates were synthesized and their anticancer effects were determined (Henderson et al., 1999; Yilmaz et al., 2018; Ari et al. 2013)

Since cisplatin complexes, which are the first and most widely used inorganic complex in cancer treatment, have serious side effects, the researchers focused on the synthesis of new metal complexes that are more effective in cancer cells (Florea and Büsselberg, 2011). In our previous study, [Ag2(sac)2(dap)2] complex was successfully synthesized and the chemical structure of it was characterized via spectroscopic techniques. In this study, anti-proliferative effects of [Ag2(sac)2(dap)2] complex on different types of cancer cell lines were investigated.

Methods

This study is designed as experimental in vitro study. HeLa (cervical cancer), PC-3 (prostate cancer), DU-145 (prostate cancer), A549 (non-small cell lung cancer), K562 (chronic myeloid leukemia) and MRC-5 (normal lung fibroblast) cell lines were placed overnight in the incubator after changing the medium of the cell lines. Then, the cells in the centrifuge tube containing a mixture of RPMI-1640, 10% FCS and 1% penicillin + streptomycin were rotated at 1000 rpm and 4 ºC for 5 minutes. The supernatant was discarded and the cells in the bottom were planted in flasks with medium and placed in an incubator containing 5% CO2 and 100% humidity at 37 ºC. The flasks in the incubator were checked by an inverted microscope during the incubation period and the proliferation of the cells was observed, provided that the cells were not displaced for the first two days. Aged media were emptied with the help of a pipette and renewed every 2-3 days with a medium containing 0.1 mL penicillin + streptomycin, 1mL FCS and 8.9 mL RPMI-1640. After the cells covered the flask base by 85-90%, the cells remaining in the flask base were counted with trypan blue staining method, the number of viable cells in 1 ml was determined and the cells seeded in 96-well containers with 1x104 cells per well. In order to determine the effect of the complex on the proliferation of cells and the level of inhibitory concentration 50% (IC50), the cells were treated with concentrations of 0, 250, 500, 750, 1000 and 1250 µM of [Ag2(sac)2(dap)2] complex for 24 and 48 hours. Also, the cells were treated with cisplatin as a control in order to compare anti-proliferative effects of [Ag2(sac)2(dap)2] complex. The primary output measurements of the study were IC50 values of complex on specified cell lines.

MTT method is based on the conversion of the tetrazolium ring of the compound MTT (3- (4,5-Dimethylthiazol-2-Yl) -2,5-Diphenyltetrazolium Bromide) into formazan by a mitochondrial enzyme, succinate dehydrogenase enzyme in the cells (live and mitochondrial functions are intact). Pale yellow MTT in the living cell turns into a dark blue-purple insoluble formazan product as a result of the destruction of the tetrazolium ring. Thus, the cells that live and have intact mitochondrial function are stained in purple, whereas the cells that are dead and have impaired mitochondrial function are not stained. After the cells are dissolved with organic solvent (eg isopropanol, DMSO, etc.), the color intensity of the formazan solution is measured spectrophotometrically at 590 nm. The reduction of MTT occurs only in cells that are metabolically active, and the level of this activity is measured by the viability of the cells. MTT solution was obtained by dissolving 5 mg of MTT in 1 mL of 1 x CMF-PBS. The solution was stored in the dark at + 4ºC. Cultured cells were treated with [Ag2(sac)2(dap)2] complex and cisplatin to each cell line at specified concentrations separately. 20 µL of MTT solution was added to each well of 96-well microplate containing incubated cells. The cells were kept in an orbital shaker outside the incubator at 150 rpm for 5

minutes. The cells were incubated for 3 hours in 5% CO2 incubator at 37°C. Supernatant liquid in the wells was discarded and 100 µL DMSO was added to the wells. Again, the cells were kept in an orbital shaker outside the incubator at 150 rpm for 5 minutes. The intensity of resulting color was measured at 590 nm (against the reference wavelength of 670 nm) on a microplate reader spectrophotometer. The formula specified was used to calculate % of cell viability: [% of cell viability = (total number of cells-total number of dead cells)/total number of cells x 100] (Tam et al., 2011).

SPSS v24 (IBM SPSS Statistics for Windows, Version 24, IBM Corp., Armonk, NY, USA) was used in statistical analysis to evaluate the anti-proliferative effect of [Ag2(sac)2(dap)2] complex on HeLa, PC-3, DU-145, A549, K562 and MRC-5 cell lines. As statistical analysis, Student's t test was used for binary comparisons and one-way ANOVA test was used for more than two comparisons.

Results

Anti-proliferative effects of [Ag2(sac)2(dap)2] complex on HeLa, PC-3, DU-145, A549, K562 and MRC-5 cell lines were investigated.

For this purpose, IC 50 values of [Ag2(sac)2(dap)2] complex on cell lines was determined by MTT method (Table 1). According to this analysis, IC 50 value of [Ag2(sac)2(dap)2] complex was 19.53 ± 1.74 µM for HeLa cell line, 17.14 ± 1.41 µM for PC-3 cell line, 18.56 ± 4.04 for DU 145 cell line, 17.93 ± 1.06 µM for A549 cell line, 3.18 ± 0.04 µM for K562 cell line and 7.25 ± 1.00 µM for MRC-5 cell line. Also, IC 50 values of cisplatin on cell lines was determined in order to compare with [Ag2(sac)2(dap)2] complex. IC 50 value of cisplatin was 4.00 ± 0.47 µM for HeLa cell line, 12.29 ± 1.60 µM for PC-3 cell line, 5.05 ± 0.65 for DU 145 cell line, 12.74 ± 1.26 µM for A549 cell line, 5.90 ± 0.59 µM for K562 cell line and 5.91 ± 0.32 µM for MRC-5 cell line.

Moreover, schematic representation of inhibitory concentrations of [Ag2(sac)2(dap)2] complex on cell lines in comparison with cisplatin was presented in Figure 1.

As can be seen, IC 50 values of [Ag2(sac)2(dap)2] complex on all cell lines were higher when compared to IC 50 value of cisplatin, except K562. Only in K562 cell line, IC 50 of [Ag2(sac)2(dap)2] complex was lower than IC 50 of cisplatin. This means that [Ag2(sac)2(dap)2] complex shows more anti-proliferative effect than cisplatin on chronic myeloid leukemia cells.

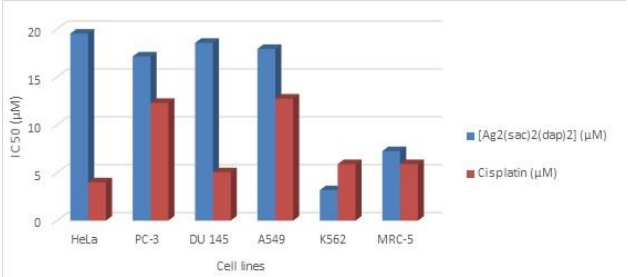

Figure 1. Schematic representation of inhibitory concentrations of [Ag2(sac)2(dap)2] complex on cell lines in comparison with cisplatin

Table 1. Inhibitory concentrations of $[Ag_2(sac)_2(dap)_2]$ complex and cisplatin on cell lines

		IC50 (µM)	
		$[Ag_2(sac)_2(dap)_2]$	Cisplatin
Cell lines	HeLa	19.53 ± 1.74	4.00 ± 0.47
	PC-3	17.14 ± 1.41	12.29 ± 1.60
	DU 145	18.56 ± 4.04	5.05 ± 0.65
	A549	17.93 ± 1.06	12.74 ± 1.26
	K562	3.18 ± 0.04	5.90 ± 0.59
	MRC-5	7.25 ± 1.00	5.91 ± 0.32

Discussion

Mostly, metals are fundamental components of cells chosen by nature. They are commonly placed in the enzyme catalytic region and are included in various biological pathways, from the exchange of electrons to structural and catalysis functions. They are widely utilized in cellular processes. Such metals are silver, gallium, cobalt, zinc, strontium, vanadium, copper and manganese, which are needed in trace quantities to activate catalytic activities (Mourino et al., 2012). Therefore, a homeostasis between cellular requirement and the amount present in the body is crucial for the normal physiological condition. In contrast, metals, including cadmium, nickel, arsenic and chromium, can trigger tumor formation and thus are less useful to the body. These boundaries have encouraged a study for platinum-based complexes showing higher selectivity, lower toxicity and a wider spectrum of function (Benedetti et al., 2011). Platinum (II) complexes like oxaliplatin and carboplatin, also other platinum analogs, are the products of this study. Other metal complexes containing ions like copper, gold and zinc (II) chelating agents have taken significant attention as anti-proliferative molecules. Lately, the chemistry of gold-based and ruthenium complexes has taken intensive examination, owing to renewed attention in giving an alternate to cisplatin and their encouraging

cytotoxic and probable anti-proliferative characteristics (Ndagi et al., 2017).

Newly, cytotoxic characteristics of silver (I) compounds have taken attention thank to the fact that most silver (I) compounds have been observed to show a better cytotoxic effect than cisplatin with comparatively lower toxicity and higher selectivity against tumor cells. In an in vitro research performed to evaluate the cytotoxic features of silver(I) compounds toward tumoral B16 (murine melanoma) and non-tumoral 10T1/2 (murine fibroblast) cell lines, silver compounds containing hydroxymethylene group presented higher cytotoxic activity for B16 (murine melanoma) than cisplatin AgSD and AgNO3. These compounds were detected to show comparatively lower toxicity against non-tumoral 10T1/2. Correspondingly, a research group studying to define the anticancer characteristics of silver (I) and gold (I) N-heterocyclic carbene compounds showed that these complexes displayed similar anticancer effect on H460 lung cancer cell line when compared to cisplatin. Moreover, silver complexes were synthesized from 2,6-disubstituted pyridine ligands. The complexes and the ligands were assessed in vitro in lung adenocarcinoma (A549), hepatocellular carcinoma (HepG2), breast adenocarcinoma (MCF7) and colon carcinoma (HT29) via MMT method by comparing with reference agent doxorubicin. All synthesized compounds displayed greater significant activity than the corresponding ligands and most of synthesized silver compounds showed magnificent cytotoxic activity against cancer cell lines in comparison with doxorubicin (Siciliano et al., 2011; Ali et al., 2013; Kalinowska-Lis et al., 2016). All these features made silver compounds potential metal compounds to be used for chemotherapy in future.

In this study, anti-proliferative effects of [Ag2(sac)2(dap)2] complex on HeLa, PC-3, DU-145, A549, K562 and MRC-5 cell lines were investigated. According to MTT analysis results, IC 50 value of [Ag2(sac)2(dap)2] complex was 19.53 ± 1.74 µM for HeLa cell line, 17.14 ± 1.41 µM for PC-3 cell line, 18.56 ± 4.04 for DU 145 cell line, 17.93 ± 1.06 µM for A549 cell line, 3.18 ± 0.04 µM for K562 cell line and 7.25 ± 1.00 µM for MRC-5 cell line. Also, IC 50 values of cisplatin on cell lines was determined in order to compare with [Ag2(sac)2(dap)2] complex. IC 50 value of cisplatin was 4.00 ± 0.47 µM for HeLa cell line, 12.29 ± 1.60 µM for PC-3 cell line, 5.05 ± 0.65 for DU 145 cell line, 12.74 ± 1.26 µM for A549 cell line, 5.90 ± 0.59 µM for K562 cell line and 5.91 ± 0.32 µM for MRC-5 cell line.

As similar to data of the literature about especially different silver complexes, anti-proliferative activity of [Ag2(sac)2(dap)2] complex on HeLa, PC-3, DU-145, A549, K562 and MRC-5 cell lines was considerably high. Especially, IC 50 of [Ag2(sac)2(dap)2] complex on K562 was lower than IC 50 of cisplatin. This means that [Ag2(sac)2(dap)2] complex shows more anti-proliferative effect than cisplatin on chronic myeloid leukemia cells.

Conclusion

Cytotoxic features of silver compounds have taken attention thank to the fact that most silver compounds have been observed to show a better cytotoxic effect than cisplatin with comparatively lower toxicity and higher selectivity against tumor cells. In our study, as similar to data of the literature about especially different silver complexes, cytotoxic activity of [Ag2(sac)2(dap)2] complex on HeLa, PC-3, DU-145, A549, K562 and MRC-5 cell lines was noticeably high. Especially, IC 50 of [Ag2(sac)2(dap)2] complex on K562 was lower than IC 50 of cisplatin. This means that [Ag2(sac)2(dap)2] complex shows more anti-proliferative effect than cisplatin on chronic myeloid leukemia cells. Even if, anti-proliferative activity of [Ag2(sac)2(dap)2] complex is needed to be confirmed with more complex experimental procedures. If verified, [Ag2(sac)2(dap)2] complex may be possible to be used as a treatment option especially for chronic myeloid leukemia.

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