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Antitumor agents bound to silica nanoparticles: potential technology for the remediation of malignant tumors

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실리카 나노 입자에 결합된 항종양제: 악성종양 치료를 위한 새로운 치료 방법

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Abstract: Commercially widely used antitumor agents such as hydroxy urea, 6-mercaptopurine monohydrate, cytosine arabinoside, cyclophosphamide monohydrate and uracil were reacted with 3-(triethoxysilyl)propyl isocyanate and the product hydrolyzed to give silica nanoparticles bound antitumor agents ranging from 10 nm to micron-sized aggregates. The silyl isocyanate derivative was also reacted neat with water to give hybrid organic-silica nanoparticles containing -CH_2-CH_2-CH_2-NH-COOH or the corresponding decarboxylated propylamine groups depending on solvent and temperature employed. In vitro tests these functionalized silica nanoparticles were effective in the treatment of malignant tumor cells but had little or no effect on normal cells. Malignant human lung, ovarian, melanoma, CNS(Central nervous system) and colon tumor cells were used in this research. The use of silica as a carrier medium in the present research serves as a model material due to its ready functionalization via silation. The proof of concept established by the results suggests that the technique may be applied to other, more biocompatible carrier nanoparticles.

요 약: 현재 상업적으로 널리 사용 되여 지고 있는 항암제인 hydroxy urea, 6-mercaptopurine monohydrate, cytosine arabinoside, cyclophosphamide monohydrate 그리고 uracil를 3-(triethoxysilyl) propyl isocyanate 와 반응시켜 항암제가 붙어있는 3-(triethoxysilyl)propyl amide (compound I)을 합성한후 물과 가수분해 반응시켜 항암제가 결합된 silica 나노입자(10 nm~micronparticles)를 만들 수 있었다. Silyl isocyanate 유도체들은 물과 반응하여 유기물질-silica 나노입자가 포함된 -CH_2-CH_2-CH_2-NH-COOH 그룹이나 혹은 온도와 용매등 반응조건에 따라서 decarboxylated 된 propylamine 그룹이 생성되었다. 생체외 시험에서 항암제가 결합된 silica 나노입자는 종양 세포 제거에 효과적이고 정상세포에 거의 영향을 미치지 않는 것으로 나타났다. 그리고 악성 종양인 폐, 난소, 악성 흑색종,

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중추신경계(CNS)와 결장 종양 세포가 이 연구에 사용되었다. 현재의 연구에서 전달매체 로서 silica는 silation 반응으로 손쉽게 나노입자를 얻을 수 있으므로 본 연구에 쉽게 이용 할 수 있다. 결과로부터 이 기술은 보다 부작용이 적은 생체 의약품에 적합한 carrier nanoparticles에 적용 될 수 있을 것으로 판단된다.

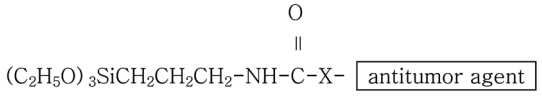
Key words: silica nanoparticle, antitumor agents, 3-(triethoxysilyl)propyl isocyanate

1. Introduction

Commercially available antitumor agents are known to cause serious side effects in patients when administered near. To alleviate this problem many researchers have vigorously investigated alternative methods of delivery, including missile therapy[1] polymer-drug mixtures[2] and anchoring antitumor agents into tumor cells[3] Recently, a nanocapsule system[4] and an agent micellar entrapment method[5] have been reported. Moreover, nanoparticle systems using various materials such as chitosan-acrylic acid,[6] cyclodextrine-polyisobutylcyanoacrylate,[7] oligonucleotides[8] and erythrocyte ghosts[9] have been reported as diluent carriers of antitumor agents.

The results reported in this publication differ from the aforementioned because the selected antitumor agents are covalently bound to the carrier silica nanoparticles either in core-shell fashion or integrated throughout the solid.

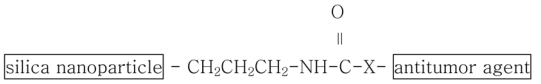
In this work, 3-(triethoxysilyl)propylisocyanate was used as a starting material to react with commercially available antitumor agents to give the compound I.



where X is –O, –S, –NH, and/or –COO

Compound I

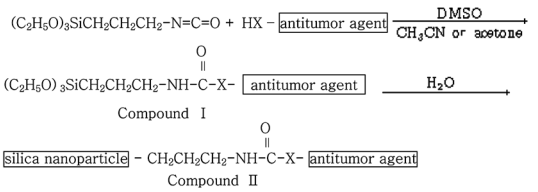
All of the antitumor agents employed have -OH, -NH, -NH_2, -SH, and/or -COOH functional group(s) for reaction with the isocyanate functional group. The triethoxysilyl unit in compound I then reacted with water to give silica nanoparticles containing bound antitumor agents (Compound II).



where X is –O, –S, –NH, and/or –COO

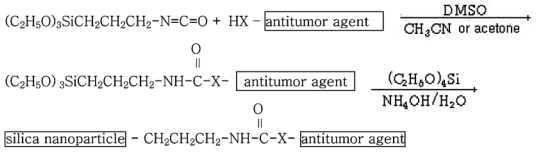
Compound II

Scheme 1. shows the whole reaction scheme.



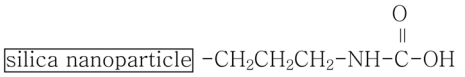
Scheme 1

Alternatively, Scheme 2 also yields the desired silica nanoparticles containing bound antitumor agent but with higher silica: antitumor agent ratio.



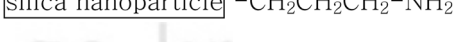
Scheme 2

The reaction of 3-(triethoxysilyl)propylisocyanate with water in the presence of DMSO as a solvent at 38 °C gave compound III which shows very remarkable effect on the various malignant tumors.



Compound III

However, the reaction of 3-(triethoxysilyl)propylisocyanate with water in the presence of CH_3CN as a solvent at 25 °C gave compound IV.



Compound IV

This means that decarboxylation of the initially formed carbamic acid group in compound III depends not only on temperature but solvent.

2. Experimental section

All reagent grade chemicals were used as received unless otherwise noted. Deionized water was used for synthesis and binding experiments. Tetrahydrofuran, 6-mercaptopurine-monohydrate, 2-amino-6-purinthiol, cytosine arabinoside, uracil, cyclophosphamide-monohydrate, tetraethoxysilane, and 3-(triethoxy-silyl)propylisocyanate were obtained from Aldrich Chemical Co. (Milwakee, WI, U.S.A.). Ammonium hydroxide (25%), diethylether, and molecular sieve (4Å) were obtained from Merck Chemical Co.(German). In vitro experiments were conducted by SRB (sulforhodamine B) assay. Shimadzu (Japan) IR-470, and FT-IR-8701, Vision (Korea) refrigerated centrifuge VS-15CF, and Hitach (Japan) S-4700 field-emission scanning electron microscope instruments were used to obtain data, respectively.

2.1. Reaction of 3-(triethoxysilyl)propylisocyanate with hydroxyurea(1).

In a 250 mL round-bottom flask equipped with magnetic stirrer, a thermometer, and 100 mL dropping funnel with calcium chloride drying tube was placed hydroxyurea (1.9 g, 2.5×10^{-2} mole) with dried tetrahydrofuran (70 mL) with stirring at 25 °C. 3-(triethoxysilyl)propylisocyanate (6.1 mL, 2.5×10^{-2} mole) with dried tetrahydrofuran (20 mL) was added dropwise through the dropping funnel with stirring at 25 °C. The solution was stirred for 12 h at 38 °C, and then, the solution was transferred into a 200 mL beaker. The mixture of tetrahydrofuran (25 mL), distilled water (23 mL), and ammonium hydroxide (3.5 mL, 25%) was added to the solution in the beaker with stirring at 38 °C. The solution was stirred at 38 °C for 24 h. The white precipitate formed slowly and was collected by centrifugation at 10,000 rpm, then washed with absolute ethanol (100 mL) twice.

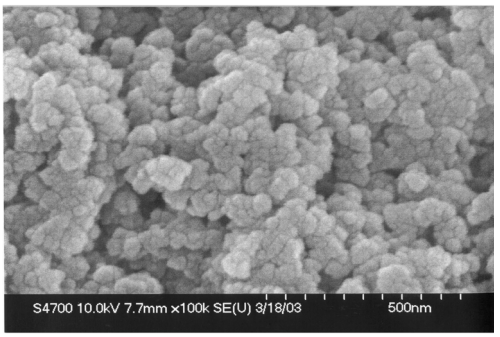


Fig. 1. SEM of silica nanoparticles containing bound cyclophosphamide.

The white precipitate was dried at 90 °C for 24 h. (yield = 70%)

IR (KBr): 3400(-OH, s), 2800~2990 (sat.-CH, m), 1700 and 1660(C=O, s), 1120(C-O, s) cm^{-1}

SEM: 25 nm.

2.2. Reaction of 3-(Triethoxysilyl)propylisocyanate with 6-mercaptopurine·H_2O(2).

In a 250 mL round-bottom flask equipped with magnetic stirrer, a reflux condenser, a thermometer, and a 100 mL dropping funnel with calcium chloride drying tube, was placed 6-mercaptopurine·H_2O (3.4 g, 2.0×10^{-2} mole) with dried acetone (65 mL) with stirring at 25 °C, 3-(triethoxysilyl)propylisocyanate (9.9 mL, 4.0×10^{-2} mole) with dried acetone (35 mL) was added dropwise through the dropping funnel with stirring at 25 °C.

The solution was stirred for 12 h at 100 °C, cooled to 25 °C, then transferred into a beaker (200 mL). The mixture of dried acetone (20 mL), distilled water (18 mL), and ammonium hydroxide (3.0 mL, 25%) was added to the solution in the beaker with stirring at 25 °C. The solution was stirred at 38 °C for 12 h, and a yellow precipitate was formed slowly.

The solids were collected by centrifugation at 10,000 rpm, and washed with dried acetone (100 mL) twice, then dried at 90 °C for 24 h. (yield = 60%)

IR (KBr): 3400(-NH, s), 3100(C=C-H, s), 2900~2990 (sat.-CH, s), 1670(C=O, m), 1620(C=N, s) cm^{-1}

SEM: 50 nm.

2.3. Reaction of 3-(triethoxysilyl)propylisocyanate with cytosine arabinoside(3).

In a 250 mL round-bottom flask equipped with

magnetic stirrer, a reflux condenser, a thermometer, and a 100 mL dropping funnel with calcium chloride drying tube was placed cytosine arabinoside (1.0 g, 3.6×10^{-3} mole) with dried dimethylsulfoxide (30 mL) with stirring at 25 °C, 3-(triethoxysilyl)propylisocyanate (0.9 mL, 3.6×10^{-3} mole) with dried dimethylsulfoxide (20 mL) was added dropwise through the dropping funnel with stirring at 25 °C. The solution was refluxed at 100 °C for 24 h, cooled to 25 °C, then transferred into a beaker (200 mL). The mixture of dimethylsulfoxide (10 mL), distilled water (3.2 mL), and ammonium hydroxide (0.5 mL, 25%) was added to the solution in the beaker with stirring at 25 °C.

The solution was heated at 38 °C for 24 h, but no precipitate was formed. Therefore, tetraethylorthosilicate (0.8 mL) was added to the above solution at 25 °C, and the mixture was stirred for 24 h. A brown precipitate was formed slowly, collected by centrifugation at 10,000 rpm, and washed with absolute ethanol (200 mL) twice, then was dried at 90 °C for 24 h. (yield = 90%)

IR (KBr): 3400 (-OH, s), 2900~2980 (sat.-CH, s), 1690 and 1650 (C=O, s), 1100 (C-O, s) cm^{-1}

SEM: aggregate

2.4. Reaction of 3-(triethoxysilyl)propylisocyanate with cyclophosphamide·H_2O(4)

In a 100 mL round-bottom flask equipped with magnetic stirrer, a reflux condenser, a thermometer, and a 100 mL dropping funnel with calcium chloride drying tube, was placed cyclophosphamide·H_2O(3.0 g, 1.0×10^{-2} mole) with dried dimethylsulfoxide (30 mL) with stirring at 25 °C. 3-(Triethoxysilyl)propylisocyanate (5.0 mL, 2.0×10^{-2} mole) with dried dimethylsulfoxide (20 mL) was added dropwise through the dropping funnel at 80 °C for 24 h.

The solution was cooled at 25 °C, transferred into a beaker (200 mL) of dimethylsulfoxide (10 mL), distilled water (9 mL), and ammonium hydroxide (1.5 mL) were added with stirring at 25 °C. The solution was stirred for 24 h at 25 °C.

The brown precipitate was collected by centrifugation at 10,000 rpm and washed with absolute ethanol (100 mL) twice, then dried at 90 for 24 h (yield = 90%).

IR (KBr): 3400 (-OH, m), 2900~2980 (sat.-CH, m), 1650 (C=O, s), 1120 (C-O, m) cm^{-1}

SEM: 25 nm.

2.5. Reaction of 3-(triethoxysilyl)propylisocyanate with uracil(5)

In a 100 mL round-bottom flask equipped with magnetic stirrer, a reflux condenser, a thermometer, and a 100 mL dropping funnel with calcium chloride drying tube was placed uracil (5.7 g, 5.0×10^{-2} mole) with dried dimethylsulfoxide (30 mL) with stirring at 25 °C. 3-(Triethoxysilyl)propylisocyanate (12.3 mL, 5.0×10^{-2} mole) with dried dimethylsulfoxide (20 mL) was added dropwise through the dropping funnel with stirring at 25 °C. The solution was stirred at 38 °C for 24 h, cooled to at 25 °C, then transferred into a beaker (200 mL).

The mixture of dimethylsulfoxide (200 mL), distilled water (18 mL) and ammonium hydroxide (3 mL, 5%) was added to the solution in the beaker with stirring at 25 °C.

The solution was stirred for 24 h at 25 °C while a white precipitate formed slowly. The white precipitate was collected by centrifugation at 10,000 rpm, washed with absolute ethanol (100 mL) twice, then dried at 90 °C for 24 h. (yield = 30%)

IR (KBr): 3200 (-NH, s), 3100 (C=C-H, s), 2900~2980 (sat.-CH, s), 1700 and 1650 (C=O, s) cm^{-1}

SEM: 25 nm.

2.6. Reaction of 3-(triethoxysilyl)propylisocyanate with water(6).

In the 250 mL beaker was placed 3-(triethoxysilyl) propylisocyanate (12.3 mL, 5.0×10^{-2} mole) with dimethylsulfoxide (30 mL) with stirring, distilled water (18 mL) with dimethylsulfoxide (20 mL) was added slowly with stirring at 25 °C.

The solution was stirred at 38 °C for 24 h. A white precipitate which formed slowly was collected by centrifugation at 10,000 rpm, washed with absolute ethanol (100 mL) twice, and air-dried at 25 °C for 24 h. (yield = 32%)

IR (KBr): 3400 (-NH, s), 2900~2980 (sat.-CH, s),

1650 (C=O, s), 1150 (C-O, s) cm^{-1}

SEM: 25 nm.

2.7. Sample 7

Vial A: hydroxyurea (2.0 g, 2.5×10^{-2} mole) in dimethylsulfoxide (15 mL)

Vial B: 3-(triethoxysilyl)propylisocyanate (6.1 mL, 0.025 mole) in dimethylsulfoxide (15 mL)

Vial A and vial B were mixed together along with water just prior to injection into the tumor cells.

2.8. Sample 8

Vial A: cyclophosphamide·H_2O (1.0 g, 3.3×10^{-3} mole) in acetonitrile (15 mL)

Vial B: 3-(triethoxysilyl)propylisocyanate (1.6 mL, 6.6×10^{-3} mole) in acetonitrile (15 mL)

Vial A and vial B were mixed together along with water just prior to injection into the tumor cells.

2.9. Sample 9

Vial A: uracil (1.1 g, 1.0×10^{-2} mole) in dimethylsulfoxide (15 mL)

Vial B: 3-(triethoxysilyl)propylisocyanate (2.4 mL, 1.0×10^{-2} mole) in dimethylsulfoxide (10 mL)

Vial A and vial B were mixed together along with water just prior to injection into the tumor cells.

3. Results and discussion

The tumor cells used in this experiment were as follows.

A 549: human lung

Table 1. The effect of silica nanoparticles containing bound hydroxyurea agent (1) against various tumor cells

Conc. (μg/mL)	Cells				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
0.1	103.1034	91.8485	105.0623	100.1347	100.8493
0.3	104.4828	87.7596	103.1184	99.1244	97.0276
1	95.4680	90.6166	101.8224	99.6632	99.3631
3	91.6256	86.0822	95.6667	100.5725	99.1507
10	68.6207	81.5477	95.5047	101.6164	101.9108
30	42.8079	70.8276	81.3709	100.4041	65.1805

Table 2. The effect of silica nanoparticles containing bound 6-mercaptopurine(2) against various tumor cells

Conc. (μg/mL)	Cells				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
0.1	68.7533	97.0606	73.9446	72.8690	86.3636
0.3	65.3050	95.1223	43.3254	25.9188	73.5178
1	23.0769	70.4106	41.9147	20.5696	33.7945
3	19.5225	45.2288	40.5041	19.6459	9.2885
10	11.6711	22.9854	36.8945	19.0302	-7.5221
30	8.5411	6.5893	6.4827	10.7177	-39.3229

Table 3. The effect of silica nanoparticles containing bound arabinoside(3) against various tumor cells

Conc. (μg/mL)	Cells				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
0.1	90.9515	102.0575	103.0335	99.8627	98.1567
0.3	87.3134	98.0601	97.1182	89.7339	95.0845
1	84.6549	96.4141	98.1041	100.8240	88.9401
3	76.1194	91.4147	91.2030	94.9871	71.5822
10	34.3284	78.6612	82.1405	91.1073	49.1551
30	8.4422	62.1133	39.7099	57.2189	30.4147

Table 4. The effect of silica nanoparticles containing bound cyclophosphamide (4) against various tumor cells

Conc. (μg/mL)	Cells				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
0.1	91.6135	99.0217	98.7222	98.2755	96.5443
0.3	86.1206	101.3340	99.9201	92.3524	88.5529
1	73.0750	97.7470	93.2515	97.9756	59.3952
3	65.6204	95.4643	93.9303	97.0009	69.1145
10	56.2040	80.0193	80.6329	97.8257	65.8747
30	46.6405	65.7304	54.2777	91.4152	55.9395

Table 5. The effect of silica nanoparticles containing bound uracil agent (5) against various tumor cells

Conc. (μg/mL)	Cells				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
0.1	104.7344	100.4001	99.6358	95.0476	98.5586
0.3	104.1557	99.5713	99.3121	95.2404	96.0360
1	87.8485	101.0288	95.5083	93.3751	92.6126
3	82.8511	93.7987	98.4219	92.7963	81.4414
10	82.3777	88.7976	86.6464	92.5068	76.3964
30	76.2230	79.6528	53.7481	94.0826	77.2973

Table 6. The effect of silica nanoparticles containing bound -$CH_2CH_2CH_2NHCOOH$ agent (6) gainst various tumor cells

Conc. (μg/mL)	Cells				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
$\times10^6$	109.3776	102.7013	104.8950	93.8719	107.2979
$\times10^5$	109.6567	103.8171	102.4475	93.4540	89.0532
$\times10^4$	102.4418	100.6753	95.2373	88.3937	98.7417
$\times10^3$	99.4418	89.2241	86.1750	82.9619	84.5234
$\times10^2$	74.4565	60.0382	18.1082	64.0669	33.8157
×10dilution	-43.4599	-84.0236	-47.9647	-74.5935	-72.8000

Table 7. The effect of hydroxyurea in DMSO/3-(triethoxysilyl)propylisocyanate in DMSO(7) injection against various tumor cells

Conc. (μg/mL)	Cells				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
$\times10^6$	100.7236	96.6462	99.2143	96.8929	104.4378
$\times10^5$	101.9497	102.7859	99.3452	99.6144	103.8421
$\times10^4$	83.5377	96.6191	100.7333	94.4662	99.1958
$\times10^3$	-2.5743	82.9333	89.0002	54.6635	75.4579
$\times10^2$	-11.5512	39.2521	53.3818	5.8343	31.9136
1:10dilution	-98.0198	-99.6277	-92.4017	-98.5262	-85.3445

SK-OV-3: human ovarian
SK-MEL-2: human melanoma
XF 498: human CNS
HCT 15: human colon

The following results (Tables 1~9) show the antitumor activities in vitro (the values are from +100 to -100 where +100 means the most of tumor cells were intact, and -100 means the most of tumor cells were dead).

Tables 1 and 3 show that silica nanoparticles containing bound hydroxyurea (1) and cytosine araboniside (3) have effect on lung tumor only while

Table 8. The effect of cyclophosphamide·H_2O in DMSO/3-(triethoxysilyl)propylisocyanate in DMSO(8) injection against various tumor cells

Conc. (μg/mL)	Cells				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
$\times 10^6$	100.4898	102.1234	96.3624	93.9653	102.0612
$\times 10^5$	96.7755	100.0552	96.2316	86.4731	101.1949
$\times 10^4$	91.3469	97.1596	94.3474	80.6433	100.7968
$\times 10^3$	86.5510	90.7894	85.8685	65.2491	99.9701
$\times 10^2$	58.4490	70.7687	47.5564	50.7885	89.1860
1:10dilution	-77.2937	-87.0427	-59.6061	-94.7924	-94.8228

Table 9. The effect of uracil in DMSO/3-(triethoxysilyl)propylisocyanate in DMSO(9) injection against various tumor cells

Conc. (μg/mL)	Cells				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
$\times 10^6$	99.3594	99.1036	100.0267	100.2129	99.5192
$\times 10^5$	99.5596	102.6893	100.8008	100.8043	99.2080
$\times 10^4$	96.3964	101.8744	102.8295	97.4215	100.8485
$\times 10^3$	98.3584	100.3531	100.6406	93.5419	98.8686
$\times 10^2$	71.6917	67.4024	77.9246	74.0966	19.1911
1:10dilution	-71.4191	-96.8724	-92.9045	-80.1523	-96.3361

In the data (Tables 1~6), the dried silica nanoparticles containing bound antitumor agent powder were suspended in the solvents such as dimethylsulfoxide or acetonitrile and then shaken vigorously before the injection to the malignant tumor cells.

silica nanoparticles containing bound 6-mercaptopurine·H_2O (2) and compound 6 give broad effect on all tumor cells (Table 2 and 6).

It is also possible that 3-(triethoxysilyl)propylisocyanate with DMSO and antitumor agent with DMSO/H_2O mixed together before injection into tumor cells. After the mixed solution together was injection into tumor cells, silica nanoparticles containing bound antitumor was formed in the cells where most of tumor cells were destroyed (see Table 7, 8 and 9).

However, in the data (Tables 7~9), antitumor agent was dissolved in dimethylsulfoxide before it mixed together with 3-(triethoxysilyl)propylisocyanate/DMSO. After two above solutions were mixed together, the solution was shaken very vigorously for 1 h. Then, the sufficient amount of distilled water was added to the above solution with vigorous stirring. It is ready for injection within ten minute when the milky silica nanoparticles could be observed.

By considering Tables 7~9, antitumor effect increased very highly when antitumor agent/water with 3-(triethoxysilyl)propylisocyanate was injected directly to malignant tumor cells since silica nanoparticles containing bound antitumor agent was formed inside of tumor cell directly. Therefore, remediation point of view, direct injection of antitumor agent along with 3-(triethoxysilyl)propylisocyanate was desirable.

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