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EXPERIMENTAL STUDY ON ANTITUMOR EFFECT OF MONOCLONAL ANTI-IDIOTYPIC ANTIBODY-DNR CONJUGATE TO LEUKEMIA*

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In this experiment, the Dextran-T40 (Dex-T40) served as intermidiate carrier, conjugated SM6 monoclonal anti-idiotypic antibody (anti-Id-McAb) against B lymphocytic leukemia cells and chemotherapeutical drug daunorubicin (DNR) to form SM6: Dex: DNR immunoconjugate. The activity of anti-Id-McAb and DNR of the immunoconjugate and their extent of the substitution were measured, and the antitumor activity of SM6: Dex: DNR was assayed *in vitro*. The results demonstrated that using Dex-T40 to prepare immunoconjugate could get a higher extent of substitution of McAb and DNR and maintain the activity of McAb and drug well. The results of cytotoxic assay *in vitro* suggested that the immunoconjugate SM6: Dex: DNR possess a specific cytotoxic effect to target tumor cells.

Key words: Immunoconjugate, Anti-Id-McAb, Cytotoxic assay.

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The immunoconjugate may selectively kill tumor cells without damaging normal tissues and cells.[1,2] The monoclonal anti-idiotypic antibody (anti-Id-McAb) against B lymphocytic leukemia can specifically bind the idiotype of B lymphoma cells.[3] In this experiment, 25% oxidized dextran T40 was used as intermediate carrier for conjugating drug daunorubicin (DNR) and SM6 anti-Id-McAb against SmIgM of B lymphocytic leukemia to form immunoconjugates, SM6: Dex: DNR, and its specific antitumor effects on lymphoma cell lines *in vitro* were studied.

MATERIALS AND METHODS

Reagents

Anti-Id-McAb (SM6) was purified mice McAb to IgM of B lymphocytic leukemia and preparated by our laboratory.[4] Daunorubicin (DNR) was produced by Farmitalia Co Italy) Dextran T40 (Dex-T40, molecular weight 40000) was purchased from Sigma.

Cell Line

Both Raji and Daudi cells are lymphoma cell lines, and target cell of SM6. K562 is a human ery-throid leukemic cell line and non-target cell of SM6.

Preparation and Purification of SM6: Dex: DNR Conjugate

25% oxidazed Dex-T40 served as intermidiate carrier to form immunoconjugate SM6: Dex: DNR. The conjugate was separated and purified from the unbound drugs and antibodies and/or others on sepha-dex G100. The first peak was then collected.[5]

The Extent of Drug Substitution in Purified Conjugates

The ratio of the number of moles of the drug and antibody in the conjugate represents the extent of drug substitution. The methods of measuring concentration of DNR and antibody were according to the references.[5, 6]

Determination of Antibody Activity in the Conjugates

The reactivity of target cell Raji and different concentration of immunoconjugate was detected by indirect immunofluorence. The activity of McAb was judged by the percentage of above 95% cell stained with fluorescence.

Determination of Pharmacological Activity of the Conjugates (24 h Cytotoxicity)

Target cell Raji and non-target cell K562 in concentration of 1×10^5/ml, mixed with conjugate and free DNR in different concentration separatively, were cultured at 37°C, 5% CO_2 for 24 h and the cytotoxicity was judged by MTT colorimetry.[7] Briefly, the suspension of 10^5/ml cell containing 15% fetal cow serums (FCS)-RPMI1640 were poured into the 96 well costar plates (100 μl/well), and 50 μl different concentration of free and conjugate DNR wee added to every well respectively, then incubated at 37°C, 5% CO_2 for 24 h. After the supernatant was replaced by 50 μl concentrating 500 μg/ml MTT 1640 solution, the cell were cultured for 4 h and treated by 50 μl/well acidified isopropylalchol, then distracted by agitation. The OD values of the supernatant were detected by ELISA-detector at 570 nm or 630 nm. The percentage of cytotoxicity was calculated according to following formal.

$$\text{Cytotoxicity}(\%)=\frac{\text{OD - experiment (570 nm or 630 nm)}}{\text{OD - control (570 nm or 630 nm)}}\times100\%$$

The Specific Cytotoxicity Assay of the Conjugate (30 min Cytotoxicity)

The specific cytotoxicity assay was similar to 24 h cytotoxicity assay. Raji, Daudi and K562 were mixed with free and conjugated DNR for 30 min at 37°C. After washed twice with RPMI1640 by centrifugation, the every well was added 100 μl RPMI1640 containing 15% FCS and cultured at 37°C 5% CO_2 for 24 h. Other process were as same as 24 h cytotoxicity assay.

RESULTS

The Extent of Drug Substitution in the Immunoconjugate

The content of SM6 was 2.0×10^{-5} m mol/l, and DNR was 1.39×10^{-3} m mol/l. The ratio of McAb and DNR in conjugate was 1:63 (number of moles:number of moles).

The Activities of McAb and DNR in the Conjugate

The activity of McAb and DNR in the conjugate was declined in different degree. The activity of McAb in the conjugate was less one titer than that of free McAb, and the activity of DNR in the conjugate was

also less one to two titer than that of free DNR when compared in LD50 (Table 1, 2).

Table 1. The activity of anti-Id-McAb (SM6) of the conjugate and free anti-Id-McAb (SM6)

SM6 content (μg/ml)	SM6: Dex: DNR		SM6	
	Raji	K562	Raji	K562
523	+	-	+	-
264	+	-	+	-
132	+	-	+	-
66	-	-	+	-
33	-	-	-	-

Specific Cytotoxicity of the Conjugate

The reaction of the conjugate and target cell in short time not only showed specific target effect of McAb, but also escaped direct cytotoxicity of DNR in the conjugate. Therefore, we determinant specific cytotoxicity of the conjugate using 30 min cytotoxic assay. The result showed that the conjugate possessed a specific cytotoxicity on target cell Daudi and Raji, but not non-target cell K562 (Figure 1). The cytotoxic effects of SM6 plus DNR and free DNR on Raji were markedly less than that of the conjugate (Figure 2).

DISCUSSION

Idiotypic determinants on the surface of tumor cell can serve as the specific marks of tumor cell.[3] The res-

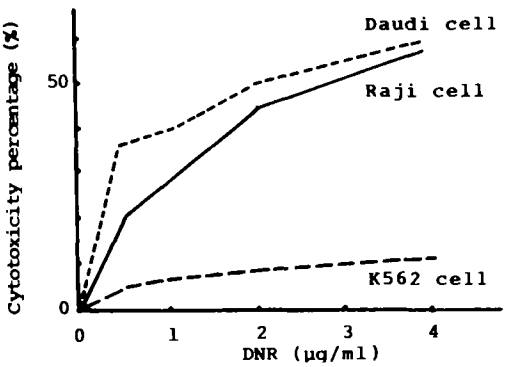

Fig. 1. The curves of cytotoxicity of the conjugate toward Daudi, Raji and K562 cells (30 min cytotoxic assay).

Table 2. The cytotoxitic activity of DNR of the conjugate and free DNR (24 h cytotoxitic assay)

DNR content (μg/ml)	Cytotoxicity (%)			
	Raji		K562	
	Conjugate	Free DNR	Conjugate	Free DNR
0.25	10.00	30.00	19.82	40.02
0.50	34.12	50.20	40.19	60.00
1.00	44.87	59.80	50.15	70.01
2.00	60.01	72.85	65.00	80.09
4.00	69.80	94.12	77.64	94.20
8.00	75.00	100.00	83.00	100.00
lgLD50± SD	3.24± 0.7[a]	2.70± 0.53[b]	3.00± 0.80[c]	2.65± 0.6[d]

a compared with b: $P<0.01$ c compared with d: $P<0.01$

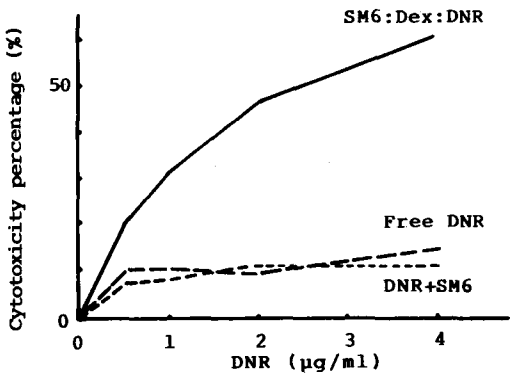

Fig. 2. The curves of cytotoxicity of conjuagte, free DNR and DNR+SM6 toward Raji cell (30 min cytotoxic assay).

ults of our experiment indicated that immunoconjugate prepared with antitumor Id-McAb can bind and kill target tumor cells Daudi and Raji specificity, but didn't kill non-target cell K562 (Figure 2). This finding suggested that the specific cytotoxicity of the conjugate toward tumor cells Raji and Daudi targeted by antitumor Id-McAb. Therefore antitumor Id-McAb could be used to prepared immunoconjugate that killed tumor cells specificity.

The results also showed that cytotoxicity of the conjugate on Daudi was greater than that on Raji at low concentration of drug DNR in the conjugate (0.5 μg DNR/ml, $P<0.05$). This different was gradually less as increase in concentration of the conjugate. This phenomenon may be related to the expressing degree of the idiotype on the surface of tumor cell beside the different sensibility of different tumor cell lines to DNR. In our previously study was found that the expression of the idiotype corresponding to SM6 on Daudi was greater than that on Raji cells, and the biological modulating effects of SM6 on Daudi and Raji cells were different either.[8] It is necessary for further investigation.

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