Journal of Korean Medical Science
Vol 7, No. 2, 128-135 June 1992

Thermo-stability and Antitumor Activity on Colon Cancer Cell Lines of Monoclonal Anti-CEA Antibody-Saporin Immunotoxin[1]

Jiradej Manosroi, Dr.rer.nat.*, Sabine von Kleist, M.D., Ph.D**,
Aranya Manosroi, Ph.D* and Fritz Grunert, Dr.rer.nat**

*Department of Pharmaceutical Technology, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50002, Thailand
**Institut für Immunbiologie Albert-Ludwigs-Universität, D-7800 Freiburg, Germany

Eight saporin peaks were obtained from the purification of seed extracts of Saponaria officinalis *L. Saporin peak No. 6 (SAP-6) showed the highest activity in the inhibition of protein synthesis (98%) in an in vitro translation study. An immunotoxin (IT) was prepared from SAP-6 conjugated to a monoclonal anti-CEA antibody 26/5/1 (mab B) using N-succinimidyl pyridyl dithiopropionate (SPDP) and 2-iminothiolane as a cross linker. Under thermal stability study by a DSC (differential scanning calorimetry), the IT showed a denature temperature of 75°C. In in vitro translation studies, the purified IT showed the same activity as SAP-6 at 10^{-7} M and 10^{-9} M protein concentration at 0, 30 and 60-min incubation effects with mab B and SAP-6 not conjugated at 24-hr incubation periods on human promyelocytic cell line HL 60 and on human colon adenocarcinoma cell lines which were SW 403, LoVo and LS 174 T. SAP-6, mab B and IT had no cytotoxic effect on HL-60. The IT showed a higher cytotoxic effect than SAP-6 in CEA-positive cell lines. The IT demonstrated the highest cytotoxic effect of 51% inhibition of control at 10^{-7} M on the LS 174 T.*

Key Words: *Saporin, Monoclonal anti-CEA antibody, Immunotoxin, DSC, Colon cancer*

INTRODUCTION

Chemotherapeutic agents are most widely used in cancer treatment. None of the cytotoxic agents in current use act specifically and they also cause several side effects. Carcinomas are often unresponsive to or eventually acquire resistance to chemotherapy. Therefore, numerous studies for an alternative and effective cancer treatment have been performed.

Several drug-monoclonal antibody conjugates have been extensively studied for their cytotoxicity (Casson et al, 1987; Corvalan and Smith, 1987; Corvalan et al, 1987b; Konno, 1986). Since these drugs act stoichiometrically, the number of drug molecules delivered to each cell is rather small and might not be sufficient to result in cytotoxicity. There are more potent substances such as toxins which act catalytically so that even one single toxin molecule delivered to the cytoplasm can kill a cell (Eiklid and Olsnes, 1980; Eiklid et al, 1980; Yama Izumi et al, 1987). These toxins have been substituted for those drugs. The study of toxins is currently focused on preparing immunotoxin (IT), a conjugate of toxins to an antibody (Pirker et al, 1987; Shih et al, 1987). Currently, saporin is one of the most widely used toxins for IT preparation since it is a single chain (type I) basic proteins with a MW of about 30,000 and has advantages over ricin, a type II toxic protein. Type I toxins are extremely stable proteins and do not bind to cells spontaneously (Stirpe et al, 1983; Stirpe et al, 1980) and therefore, are non-toxic to intact cells, while being safe to purify and handle in the laboratory (Lappi et al, 1985; Olsnes and Phil, 1982).

Monoclonal anti-CEA antibody has been conjugated with many toxins such as ricin (Griffin et al, 1984; Masuho et al, 1982), PAP (Shih et al, 1987), and with many drugs (Konno, 1986). However, some of these

Address for correspondence: *A. Manosroi, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand*

[1]This study was supported by the DAAD (Deutsche Akademische Austauschen Dienst) and The AvH (Alexander von Humboldt Stiftung) in Bonn, Germany, (1987-1989).

conjugated products appeared not to give promising results and have problems (Watanabe et al, 1984). For saporin, the IT obtained from saporin conjugated with a monoclonal anti-Thy 1.1 antibody showed potent antitumor effects both in vitro and in vivo (Thorpe et al, 1985). The study of saporin conjugated with monoclonal anti-CEA antibody on colon cancer cell lines would be interesting but has never been performed.

Therefore, the objective of the present study was to investigate the thermostability and the antitumor activity in colon cancer cell lines of an IT composed of monoclonal anti-CEA antibody and saporin, a ribosome inactivating toxic protein from the seed of Saponaria officinalis, in an attempt to evaluate the potential of this IT for future colon cancer treatment.

MATERIALS AND METHODS

Purification of Saporin

Saporin was extracted and purified from the seeds of *Saponaria officinalis* as described elsewhere (Stirpe et al, 1983) with modifications. Briefly, a batch of 25 g of seeds was homogenized with 200 ml of 0.14 M NaCl in 5 mM sodium phosphate buffer, pH 7.2. The homogenate was stirred over night at 4°C, strained through a cotton gauze and centrifuged at 28,000 g for 30 min. The crude extract solution was dialyzed against 5 mM sodium phosphate buffer, pH 6.5. The dialysate was passed through a CM-Sepharose CL-6B column (Pharmacia Fine Chemicals, Uppsala, Sweden) equilibrated with the same buffer used in the dialysis procedure. The bound fractions were eluted with the 0 to 0.3 M NaCl gradient. The amounts of protein were determined by the Lowry method (Protein Assay Kit, Sigma Diagnostics, St. Louis, MO, USA).

Purification of Monoclonal Anti-CEA Antibodies

Ascitic fluid from mice inoculated with hybridoma cells was thawed and filtered through a filter paper pre-wetted with starting buffer. Saturated ammonium sulfate solution was slowly added drop by drop with gentle stirring until precipitation completely occurred in a cold room. After standing for 30 min, the suspension was centrifuged at 7,700 g for 30 min. The resulting precipitate was dissolved in starting buffer and dialyzed against buffer over night. The dialysate was passed through a Protein A-Sepharose CL-4B column (1.6 cm × 20 cm, Pharmacia, Uppsala, Sweden). The column was eluted by 0.1 M glycine buffer, pH 3. The fraction was dialyzed against phosphate buffered saline (PBS) overnight. The resulting purified antibody was stored in aliquots at −70°C.

The monoclonal anti-CEA antibody used in the preparation of the IT was 26/5/1 (mab B) which belongs to the IgG2a subclass and has already been described and characterized elsewhere (Hammarstrom et al, 1989).

Preparation of IT

Purified mab B (1 mg/ml) was first derivatized by treating with SPDP solution (10 mg/ml) in 100 mM sodium phosphate buffer pH 7.0 containing 0.5 mM ethylenediamine tetraacetate (EDTA, Sigma, Mo, USA). The reaction mixture was incubated at 30°C for 30 min and terminated by dialysis with 1,000 times volume of 100 mM sodium phosphate buffer, pH 7.0 at 4°C.

SAP-6 powder was dissolved in 60 mM triethanolamine (Sigma, Mo, USA) and 1 mM EDTA in PBS, pH 7.4, degassed and derivatized by treating with a freshly prepared 0.5 M 2-iminothiolane (Sigma, Mo, USA) in 1 M triethanolamine/HCl buffer, pH 8.0 solution under nitrogen gas at 0°C for 10 min. The excess reagents were then removed by dialysis against 5 mM bis-tris/acetate buffer (Sigma, Mo, USA), pH 5.8 containing NaCl (50 mM) and EDTA (1 mM) at 4°C for 24 hr.

In preparing the IT, the derivatized mab B (0.5-1 mg/ml) in 100 mM sodium phosphate buffer, pH 7.0 and EDTA (0.5 mM) solution was mixed with an equal weight (about a 5-fold molar excess) of modified SAP-6 (0.5-1 mg/ml) in 5 mM bis-tris acetate buffer, pH 5.8 containing NaCl (50 mM) and EDTA (1 mM). The pH of the mixture was raised to 7.0 by adjusting with 0.5 M triethanolamine/HCl buffer, pH 8.0. The mixture was held under nitrogen gas at 4°C for 20 hr. A 0.5 M iodoacetamide (Sigma, Mo, USA) solution was added to the mixture and further incubated at 25°C for 1 hr.

Purification of IT

The above mixture was passed through a DEAE-Cellulose CL-6B (1.6 cm × 20 cm column) equilibrated with 20 mM tris-HCl buffer, pH 7.5. The column was eluted by the same buffer containing 1 M NaCl. The eluted fraction was dialyzed against 5 mM PBS, pH 7.0 containing 20 mM NaCl and 0.4 mM NaN_3. The dialysate was passed through a CM-Sepharose CL-6B (1.6 cm × 20 cm column) equilibrated with the above buffer at pH 7.0. The purified IT was eluted by the same buffer containing 1M NaCl. The eluted purified IT fraction was then submitted to a gel filtration on a Sephacryl S-300 column (1.0 cm × 30 cm) equilibrated with 10 mM potassium phosphate buffer, pH 7.2 containing 145 mM NaCl. The fraction was finally sterilized by filtration through a 0.22 μm membrane filter and stored at

−40°C.

SDS-PAGE Analysis

Purified SAP-6, mab B and IT were analyzed for purity by 5-20% linear gradient sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions as previously described (16).

Differential Scanning Calorimetry Study (DSC)

Calorimetry measurements can be used to gain insight into protein thermodynamic properties such as T_m's (denature temperatures) and H_{cal}'s (enthalpy changes of protein denaturation). Thermal techniques, in particular, DSC have become standard methods.

In our study, T_m's of SAP-6, mab B and IT were determined using a scanning calorimeter (DASM-1, Mashpriborintorg, Moscow, USSR) at a heating rate of 0.75°C/min. The T_m's were obtained from the temperature at the highest point of the peaks. All of the experiments were performed in triplicate.

In Vitro Translation Study

This study was based on the method previously described (30). A 25μl (25μ Ci) of (^{3}H)-leucine (Amersham Buchler GmbH, Braunschweig, FRG) was lyophilized. A 35μl of rabbit reticulocyte lysate (Promega, WI, USA) together with 4μl of bidistilled water, 8μl of saporin or IT, 1μl of 1 mM amino acid mixture (no leucine, Promega, WI, USA), 2 μl of RNA substrate in water (Promega, WI, USA) at 0.5 μg/ml were added at 0°C.

The reaction mixtures were incubated at 30°C for 60 min. Samples of 2μl were taken at intervals. Each sample was added to 1.0 ml of 1 N NaOH, 1.5% of H_2O_2 and incubated for 10 min at 37°C. An amount of 4 ml of ice-cold 25% trichloroacetic acid/ 2% casamino acid was added and incubated in ice for 30 min. The precipitates were collected through a Whatman GF/C filter (Whatman, England) with suction. The filter was washed with 10 ml of ice-cold 8% TCA and then with a few ml of acetone under vacuum. The vacuum was pulled until the filter was dried. Radiolabelled protein was finally quantified by a B-counter (Beckman LS-330) using 4 ml Quickszint 501 (Zinsser, FRG) as scintillant.

For the saporin purification study, the optimal final concentration of saporin from all peaks in the test systems were all equal to 20 ng/ml. The samples were lyophilized saporin powder from all peaks which were diluted to 0.125μg/ml with 10 mM potassium phosphate buffer, pH 7.4 containing sodium chloride 20 mM, BSA 0.2 mg/ml, IT and SAP-6 at 10^{-7} M and 10^{-9} M.

Determination of CEA Production on Supernate and Cell Lysate in Different Cell Lines

An amount of 5×10^4 cells in 0.2 ml RPMI 1640 medium (Flow Laboratories, USA) of each cell line was plated into 96-well (flat bottom) tissue culture plates (Falcon, USA). The plates were incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 24 hours. The plates were centrifuged at 500 g at 18°C for 10 minutes. The supernate was collected and the total number of cells was determined. An amount of 0.2 ml of lysis buffer was added to the cell sediment. The plates were then incubated at 4°C for 45 minutes. After incubation, the plate was centrifuged at 500 g at 18°C for 10 minutes. The supernate which was a cell lysate portion was collected. Both supernate and the cell lysate portion were quantified for CEA by an enzyme immunoassay (EIA) using the Abbott CEA-EIA test kit (Abbott Laboratories, Diagnostic Product GmbH, Delkenheim, FRG).

Cytotoxicity Testing

The purified IT was compared for its cytotoxic effects with mab B and SAP-6 at 10^{-7} M to 10^{-14} M and, at 24-hr incubation periods on 4 different cell lines, which were the human promyelocytic cell line HL-60, and the human colon adenocarcinoma cell lines SW 403, LoVo and LS 174T. An amount of 5×10^4 cells in 0.1 ml RPMI 1640 medium (Flow Laboratories, USA) was plated into 96-well tissue culture plates (Falcon, USA). Equal volumes of medium containing serial dilution of the protein samples were added to each well. The plates were then incubated at 37°C in a humidified atmosphere containing 5% CO_2. After incubation, the cells were pulsed for 2 hr with 0.8 μCi/well of (^{3}H)-thymidine (Amersham, England). The cells were then harvested onto a glass fiber filter disc (Skatron AS, Norway). The radioactivity retained on the filters was measured by a B-counter using 4 ml Quickszint 501 as scintillant. All assays were done in triplicate and each experiment was repeated 3 times. Mean and SE percentages of inhibition relative to control were calculated and evaluated.

RESULTS

Purification of Saporin

After passing a crude extract from seeds of *Saponaria officinalis* through a CM-Sepharose CL-6B column, a chromatogram of 8 peaks of saporin was obtained by elution with a 0 to 0.30 M NaCl gradient (Figure

1). SAP-6 was eluted at 0.21 M NaCl and gave a maximum yield of 10.98% as protein contents of the crude extract or about 0.12% of seeds (dry weight). All saporin peaks from the purification procedure were tested for protein synthesis inhibition as measured by the inhibition of (^{3}H)-leucine incorporation in order to determine the peaks with maximum activity. All samples, except saporin peak No. 1, showed less cpm than the control at all incubation periods indicating inhibition of protein synthesis. The cpm values were found to decrease with time and almost stay stable after 60 min. SAP-6, which gave the highest yield of protein contents, showed the highest percentages of protein synthesis inhibition at 97.88.

Purification, Protein Synthesis Inhibition Activity and Thermal Stability of IT

SAP-6, IT and mab B were compared by a SDS-PAGE under reducing conditions (Figure 2). SAP-6

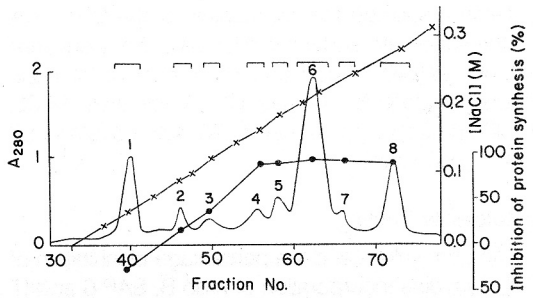

Fig. 1. Purification of *Saponaria officinalis* seed extract. The dialysed extracts were chromatographed on a column (20 × 1.6 cm) of CM-Sepharose CL-6B, eluted with 0.5-litre gradient of 0 to 0.3 M NaCl (X ———— X) and 4 ml fractions were collected. Inhibitory activity on protein synthesis (• ———— •) was assayed in the *in vitro* translation system at one hour.

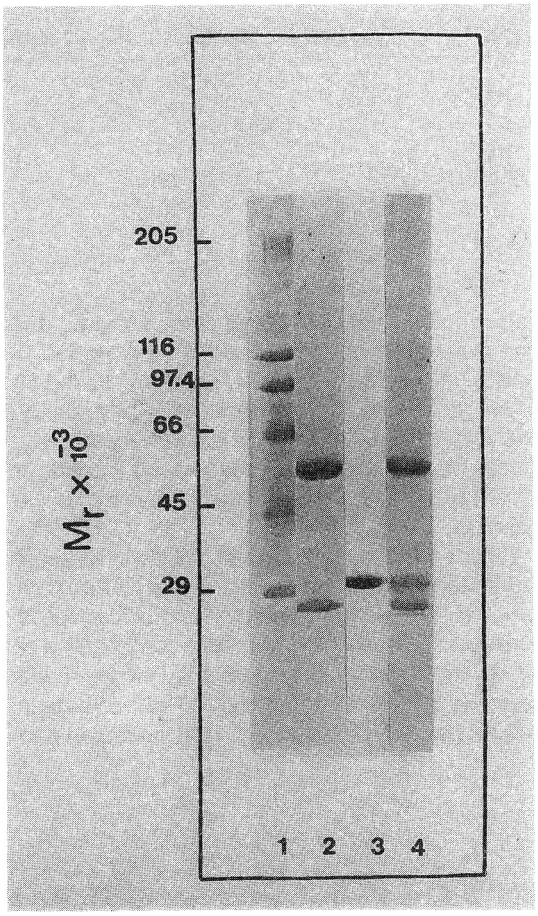

Fig. 2. Polyacrylamide/dodecyl sulfate gel (5-20%) analysis of purified IT under reducing conditions.
Lane 1, standard
Lane 2, monoclonal anti-CEA antibody (mab B)
Lane 3, SAP-6
Lane 4, purified IT
At approx. at, 30 and 50 kd are light chain of mab B, SAP-6 and heavy chain of mab B respectively.

Table 1. Inhibition of protein synthesis by saporin (10^{-7} and 10^{-9}M) and monoclonal anti-CEA-saporin IT (10^{-7}M and 10^{-9}M) at 30, 60 and 90 minutes incubation

Time (min)	Experimental system[a]								
	Control	Saporin 10^{-7}M		Saporin 10^{-9}M		IT 10^{-7}M		IT 10^{-9}M	
	cpm	cpm	%[b]	cpm	%[b]	cpm	%[b]	cpm	%[b]
30	58,900 ± 4,875	2,803 ± 217	4.75 ± 0.41	5,950 ± 499	10.09 ± 1.00	1,903 ± 208	3.22 ± 0.35	2,513 ± 199	4.26 ± 0.33
60	95,238 ± 9,764	3,083 ± 548	3.20 ± 0.23	3,969 ± 714	4.23 ± 1.16	2,163 ± 125	2.44 ± 0.26	3,367 ± 243	3.56 ± 0.41
90	124,903 ± 3,534	3,174 ± 79	2.54 ± 0.14	3,399 ± 6.00	2.72 ± 0.08	1,718 ± 257	1.39 ± 0.23	3,173 ± 402	2.53 ± 0.26

a) All numbers are mean ± SE of triplicate assay of three experiments.
b) Percentage of control

Table 2. Comparison of CEA level on the supernate and cell lysate of the cell lines at 24 hr-incubation period

Cell line	CEA in supernate ($ng/10^4$ cells)	CEA in cell lysate ($ng/10^4$ cells)	Total CEA ($ng/10^4$ cells)
HL-60	0.00	0.00	0.00
SW-403	0.17	14.19	14.36
LoVo	0.38	17.95	18.33
LS 174 T	0.00	21.46	21.46

had one distinct band at 30 kd M.W. indicating that pure saporin was obtained (lane 3). The purified IT lane 4) gave 3 distinct bands, at about 25, 30 and 50 kd MW corresponding to a light chain of the antibody, SAP-6 and a heavy chain of the antibody respectively, hereas the monoclonal antibody showed 2 bands of the light and heavy chain in lane 2. Lane 1 is the standard.

Table 1 showed the comparison of the inhibition of protein synthesis by the purified IT and SAP-6, cpm values of (^{3}H)-leucine incorporation and percentage of control of 10^{-7} and 10^{-9} M protein concentration at 30, 60, and 90-min incubation periods. Both the IT and saporin at 10^{-7} and 10^{-9} M showed no difference in the inhibition of protein synthesis at all incubation periods except at 10^{-9} M and 30 min of incubation, where saporin showed higher activity than the IT.

From the DSC study, figure 3 showed a cvalorigram of mab B, SAP-6 and IT. A single distinct peak was observed in the temperature ranges studied. Different reference solutions were used since different solvents were used in the samples. The mab B, SAP-6 and IT gave an average T_m of 68, 67 and 75°C respectively.

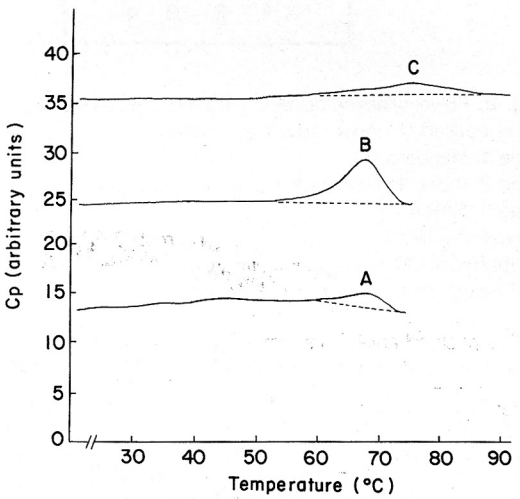

Fig. 3. Differential scanning calorigram by a DASM-1 calorimeter of:
A: monoclonal anti-CEA antibody B (mab B) (3.1×10^{-6}M)
B: SAP-6 (1.4×10^{-5}M)
C: IT (1.8×10^{-7}M)
Distilled water was used as a reference in B and C. PBS is a reference in A.

Determination of CEA Production on Supernate and Cell Lysate in Different Cell Lines

Table 2 showed the comparison of the CEA level on the supernate and the cell lysate of the 4 cell lines (HL-60, SW 403, LoVo and LS 174 T) at 24 hour-incubation periods. The total CEA levels were 14.36, 18.33 and 21.46 $ng/10^4$ cell in SW 403, Lovo and LS 174 T respectively.

Cytotoxicity Testing

The cpm values and the percentage of inhibition of (^{3}H) thymidine incorporation of mab B, SAP-6 and IT on the 4 cell lines at 10^{-7} to 10^{-14} M protein concentration at 24-hr incubation periods were investigated. The maximum cytotoxic effect was observed at 10^{-7} M protein concentration.

Figure 4 shows the plot of the (^{3}H)-thymidine incorporation in a percentage of the controls versus incubation periods of mab B, SAP-6 and IT 10^{-7} M protein concentration and 24 hr incubation period on the 4 cell lines. The mab B did not appear to have a cytotoxic effect on the 4 cell lines. The IT demonstrated a maximum cytotoxic effect on LS 174 T.

Both IT and SAP-6 had no cytotoxic effect on HL-60 (CEA-negative cell). In comparing the cytotoxic effect of IT and SAP-6 on CEA-positive cells, IT showed a higher percentage of inhibition of 12% and 49% in SW 403 and LS 174 T more than SAP-6 and at about the same effect on LoVo.

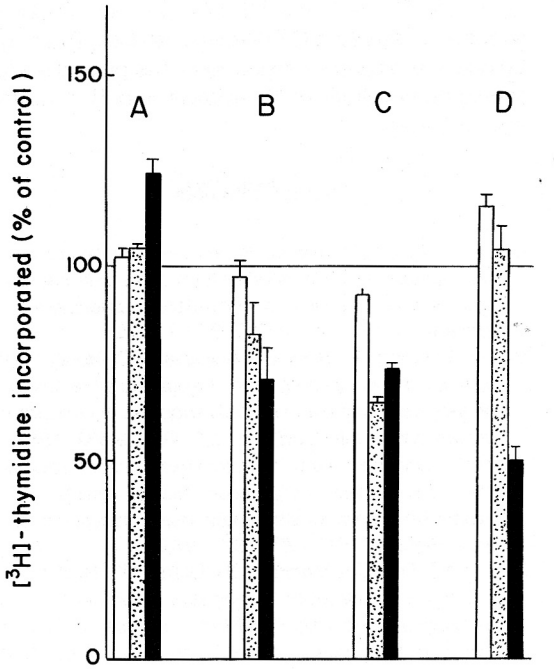

Fig. 4. The plot of the (^{3}H)-thymidine incorporation in percentages of the controls versus incubation periods of mab B (□), SAP-6 (▨), and IT (■) at 10^{-7}M protein concentration and 24 hr incubation periods on the cell lines HL-60 (panel A), SW 403 (panel B), LoVo (panel C) and LS 174 T (panel D). Bars, SE.

DISCUSSION

Toxins have an advantage over drugs in the inhibition mechanisms of protein synthesis. Toxins can kill both dividing and non-dividing cancer cells and can act catalytically so cancer cells can hardly escape from their action. However, toxins cannot be used alone since they lack specificity. Various toxins have been used for preparing IT with the purpose of increasing their selectivity. Among them saporin appeared to be more advantageous than any other toxins for the reasons already mentioned (Siena et al, 1988; Stirpe et a, 1983; Thorpe et al, 1985; Trowbridge and Domingo, 1981).

In our study, the 8 peaks of saporin obtained from the purification of seeds extract of *Saponaria officinalis*. Peak No. 6 (SAP-6) was used to prepare IT. It contained the highest amount of high purity and more importantly, it showed the greates activity in the inhibition of protein synthesis of 98% as compared to the control in the *in vitro* translation studies.

In preparing purified IT, it is important to separate the unconjugated mab B and SAP-6 from the IT, since they may interfere with the IT activity in the cytotoxicity testing. It has been previously demonstrated that certain monoclonal antibodies alone can already have a cytotoxic effect (Bumol et al, 1983; Masuho et al, 1982).

In SDS-PAGE analysis (under reducing conditions), IT gave three distinct bands. Disulfide bondings in both IT and antibody were broken by 2-mercaptoethanol into SAP-6 and light and heavy chains of the antibody. Since only one distinct peak was obtained by chromatography, this together with the result from SDS-PAGE analysis confirms the formation and purity of our IT. This also proved that mab E, which was prepared in our laboratory can form an IT with saporin. Not all antibodies can be used to form IT (24, 38). In *in vitro* translation studies, the IT showed no different activity than saporin alone at all concentrations and incubation periods except at a 10^{-9} M protein concentration and after 30-min of incubation. It appeared that even when saporin had been conjugated with antibody, it still kept the same activity as unconjugated saporin.

In DSC study, IT gave a higher T_m more than mab B and SAP-6. This confirmed that a covalent association between SAP-6 and mab B of a more thermo stable conjugated product which had stable linkage was obtained.

There are many factors which can affect the cytotoxicity of IT, e.g. the isotype of the antibodies used, the types of toxin used, the stability of the linkage of the IT, the number of toxin chains bound to the antibodies and the cross linker used.

The evidence of covalent conjugation as well as the average number of toxins per antibody of many monoclonal antibodies using the same cross linker has been previously discussed (Carlson et al, 1978; Krolick et al, 1983). It has been shown that the prepared ITs consisted of about 2-4 toxin molecules per antibody molecule (Carlson et al, 1987).

Different cell lines can have a different density of cell surface antigens and hence the binding efficiency with toxin-coupled antibodies may vary. These differences may consequently affect the rate of endocytosis and translocation of IT (Pastan et al, 1986; Pirker et al, 1987; Vitetta and Uhr, 1985). The most active IT has been achieved only, if the conjugate could be endocytosed and released to the cytosol after binding to the cell membrane antigen. It has been suggested that the CEA molecule is capable of directing productive internalization of IT (23). The radioimmunoassay binding studies of the ITs with monoclonal anti-CEA antibody showed that the antibody portions of the

molecules retained the ability to form a complex with CEA after conjugation to toxins (Levin et al, 1987; Siena et al, 1988).

As concerns the properties of the cell lines from our experiment, the HL-60 cell line expressed no CEA. The other cell lines, i.e. SW 403, LoVo and LS 174 T have been found to have CEA at different amounts, of 14.36, 18.33, and 21.46ng/10^4 cells after 24 hours incubation, respectively.

IT, mab B and saporin appeared to show different cytotoxic effects on different cell lines and protein concentrations. The maximum cytotoxic effect was observed at 10^{-7} M which was the maximum protein concentration used and the maximum cytotoxicity was observed. For mab B,there was a slight cytotoxic effect not more than 15% inhibition. This agrees with the previous work reported by Thorpe and Ross (Thorpe and Ross, 1982). The mab B itself could also bind to cells and was possibly endocytosed when the incubation time was longer. By flow cytometry analysis of monoclonal antibody alone and a ricin A-chain IT, the same binding and endocytosis on target LoVo cells has already been demonstrated (Levin et al, 1986).

Although the IT showed slightly cytotoxic effects on both the SW 403 the LoVo cell line, the IT showed a slightly higher cytotoxicty effect on the SW 403 than the LoVo. This may be due to more CEA of LoVo being present in the supernatant as shown in the experiment which may neutralize the IT and thus reduce its cytotoxic effect. Thus, a correlation was not always found in the sense that cells having high contents of antigen will always exhibit a high cytotoxic effect by IT (Pirker et al, 1987; Siena et al, 1988).

It has been demonstrated that when saporin conjugated with antibodies, it is more toxic than antibody-coupled ricin and geloin, as previously reorted in in vivo studies (Blakey et al, 1988). The mechanism of cytotoxicity of saporin is known to be due to a protein synthesis inhibition by the inactivation of 60 S ribosomes rendering the ribosome unable to react with EF2 which leads to cell death (Barbieri et al, 1982; Olsnes and Pihl 1986; Stirpe et al, 1983). However, the mechanism of toxins penetrating into the cell is not clearly understood.

The present study demonstrated that an IT prepared from SAP-6 coupled to mab B by covalent bonding is purified, thermo-stable and effective in protein synthesis inhibition and cytotoxicity as compared to unconjugated saporin at 24 hr incubation period. The IT showed specificity since it did not react with CEA-negative cells (HL-60). The IT gave a higher efficacy in cytotoxicity activity than the saporin not conjugated under the SW 403 and LS 174 cell lines. Saporin showed cytotoxicity to CEA-bearing cell lines. This may be due to spontaneous endocytosis. This present study should be beneficial for the application of IT in future cancer therapy.

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