財團法人明日醫學基金會專題研究計畫申請書

一、基本資料： 申請條碼：

本申請案所需經費(單選)	■A 類(研究主持費及執行計畫所需經費) ☐B 類(研究主持費，限人文處計畫，不須填寫表 C002 及 C004 至 C009)				
計畫類別(單選)	■一般型研究計畫 ☐特約研究計畫 ☐新進人員研究計畫 ☐其他				
研究型別	■個別型計畫 ☐整合型計畫				
申請機構/系所(單位)	中國醫藥大學 醫學系				
本計畫主持人姓名	賴志河	職稱	副教授	身分證號碼	
本計畫名稱	中文	應用細菌基因毒素於胃癌之治療			
	英文	Application of bacterial genotoxin in gastric cancer therapy			
整合型總計畫名稱					
整合型總計畫主持人				身分證號碼	
全程執行期限	自民國 102 年 1 月 1 日起至民國 102 年 12 月 31 日				
研究學門(請參考本申請書所附之學門專長分類表填寫)	學門代碼	名稱(如為其他類，請自行填寫學門)			
	BI				
研究性質	☐純基礎研究 ☐導向性基礎研究 ■應用研究 ☐技術發展				
本計畫是否為國際合作計畫	■否； ☐是，合作國家：_____，請加填表 I001～I003				
本計畫是否申請海洋研究船	■否； ☐是，請務必填寫表 C014。				
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二、申請補助經費：

金額單位：新台幣元

執行年次 / 補助項目		第一年（101 年 01 月～101 年 12 月)	第二年（___年___月～___年___月）	第三年（___年___月～___年___月）	第四年（___年___月～___年___月）	第五年（___年___月～___年___月）
業務費		300,000				
研究人力費						
耗材、物品及雜項費用						
國際合作研究計畫國外學者來臺費用						
研究設備費						
國外差旅費						
國外或大陸地區差旅費						
出席國際學術會議差旅費						
國際合作研究計畫出國差旅費						
管理費						
合計		300,000				
貴重儀器中心使用額度						
博士後研究	國內、外地區	共_____名	共_____名	共_____名	共_____名	共_____名
	大陸地區	共_____名	共_____名	共_____名	共_____名	共_____名

申請機構或其他單位（含產業界）提供之配合項目（無配合補助項目者免填）

配合單位名稱	配合補助項目	配合補助金額	配合年次	證明文件

三、主要研究人力：

（一）請依照「主持人」、「共同主持人」、「協同研究人員」及「博士後研究」等類別之順序分別填寫。

類 別	姓名	服務機構/系所	職稱	在本研究計畫內擔任之具體工作性質、項目及範圍	*每週平均投入工作時數比率(%)
主持人	賴志河	中國醫藥大學/醫學系	副教授	統籌及推動本研究計畫、參與主要實驗工作、整理文獻背景及撰寫研究成果	80%

※註：每週平均投入工作時數比率係填寫每人每週平均投入本計畫工作時數佔其每週全部工作時間之比率，以百分比表示（例如：50%即表示該研究人員每週投入本計畫研究工作之時數佔其每週全部工時之百分五十）。

（二）如申請博士後研究，請另填表 CIF2101 及 CIF2102(若已有人選者，請務必填註人選姓名，並將其個人資料表併同本計畫書送本會)。

五、耗材、物品及雜項費用：

（一） 凡執行研究計畫所需之耗材、物品及雜項費用，均可填入本表內。

（二） 說明欄請就該項目之規格、用途等相關資料詳細填寫，以利審查。

（三） 若申請單位有配合款，請於備註欄註明。

（四） 請分年列述。

金額單位：新台幣元

項 目 名 稱	說明	單位	數量	單價	金額	備註
消耗性器材	細胞培養耗材：胎牛血清、無菌培養皿、無菌吸管、培養基、液態氮、CO_2 氣體及離心管等	年	1	52,000	52,000	
消耗性器材	微生物培養耗材：綿羊血、無菌吸管、培養基、培養皿、洋菜膠、棉棒等	年	1	75,000	75,000	
消耗性器材	蛋白質電泳試劑與耗材：電泳配製試劑、protein marker、PVDF membrane 及濾紙等	年	1	30,000	30,000	
消耗性器材	Reporter assay kit，ELISA assay kit 分析 IL-8，NF-κB 及 AP-1 promoter activity	年	1	40,000	40,000	
消耗性器材	分子生物學實驗藥品與耗材：限制酶、聚合酶、電泳膠、緩衝液及 DNA marker 等	批	1	46,000	46,000	
消耗性器材	塑膠瓶、吸管、玻璃	年	1	10,000	10,000	

	瓶、及血清瓶等耗材					
消耗性器材	清潔劑、酒精、有機溶劑及化學試劑等	批	1	10,000	10,000	
資訊設備費	碳粉夾、紙張、光碟片等資訊耗材	年	1	5,000	5,000	
論文投稿費	期刊雜誌投稿	篇	2	16,000	32,000	
合　　計					300,000	

Abstract

Campylobacter spp. are the most common zoonosis disease and causes of diarrhea worldwide. Human disease likely results from improper handling or incomplete cooking of the contaminated poultry. *Campylobacter*-associated enterocolitis is typically associated with a local acute inflammatory response and involves intestinal tissue damage in both animal and human. Cytolethal distending toxin (Cdt), produced by *Campylobacter*, is a putative virulence factor that induces cell-cycle arrest and apoptosis in eukaryotic cells. Our preliminary study has been demonstrated that cell intoxication by CdtB delivery into the nucleus through the association of CdtA/CdtC and membrane cholesterol-rich microdomains. Translocation of CdtB into nucleus induced cell cycle arrested at G2/M phase. In this proposal, we will develop novel pH-responsive CdtB/chitosan/heparin nanoparticles in the gastric cancer therapy. The delivery efficiency of CdtB-loaded nanoparticles for tumor therapy will be further evaluated. Studies from this proposal, we anticipate inspire the future inauguration for application of agricultural microbe in biotech industries.

Keywords: *Campylobacter*, cytolethal distending toxin, nanoparticle, gastric cancer

A. BACKGROUND AND SIGNIFICANCE

1. *Campylobacter jejuni* infection and cytolethal distending toxin

Watery diarrhea is commonly seen in children infected with *Campylobacters* (2). Infection by the pathogen usually occurs in humans through consumption of contaminated poultry products (6). However, the virulence factors responsible for induction of host diarrhea remain to elucidate. Several reports have been revealed that *C. jejuni* adheres to and invades into host cells (10,30). Infection of cells with *C. jejuni* stimulates nuclear factor (NF)-κB, which mediates the production of several proinflammatory cytokines (1). In addition, *C. jejuni* induces the release of interleukin (IL)-8 from infected epithelial cells (29). Moreover, a bacterial membrane-associated protein–cytolethal distending toxin (Cdt)–has subsequently been identified as one of the virulence factors required for the induction of IL-8 (32).

Cdt holotoxin functions as an AB_2 toxin in which CdtA and CdtC serve as the binding (B) unit and CdtB is the active (A) unit (14). Previous study has been revealed that both of CdtA and CdtC can interact with cell membrane and enable translocation of the holotoxin across the cell membrane (20). The nuclear-translocated CdtB subunit exhibits type I deoxyribonuclease (DNase) activity, which causes DNA damage and in turn leads to cell-cycle arrest at the G2/M phase (13). A study of Cdt from *A. actinomycetemcomitans* also revealed that both CdtA and CdtC not only bind to the cell surface but are associated with membrane lipid rafts (3). Lipid rafts are microdomains that contain large fractions of cholesterol, phospholipids, and glycosylphosphatidylinositol-anchored proteins (8). Studies *in vitro* showed that the structure of lipid rafts is stabilized in the cold non-ionic detergents such as Triton X-100 , but it can be disrupted by the cholesterol depletion agent methyl-β-cyclodextrin (MβCD) (26). A recent study of *A. actinomycetemcomitans* Cdt revealed that the CdtC subunit contains a cholesterol recognition/interaction amino acid consensus (CRAC) region, which is required for CdtC binding to lipid membrane microdomains (4). This finding indicates that cholesterol provides an essential ligand for Cdt binding to the cell membrane and also serves as a portal for CdtB delivery into host cells for the induction of cell intoxication.

2. Gastric cancer

Around seventy thousand residents in Taiwan (24) and 1.5 million residents in America are diagnosed with cancer annually (9). Among this, gastric cancer is the second most common cause of cancer-related death in the world. The correlation of *Helicobacter pylori* (*H. pylori*) etiology and gastric cancer was virtually certain. Numerous studies of epidemiology and animal model have subsequently confirmed this association [review in (11)]. In a very recent report by Hatakeyama's group demonstrated that cytotoxin-associated gene A (CagA) as a bacterial oncoprotein which can act in mammals and enable CagA to deregulate SHP-2, that contributed in the development of *H. pylori*-associated neoplasms (21). These types of cancers were thought to have a high potential for organ metastasis and thus increased their mortality rates in patients. Therefore, it is critical to develop a new strategy to suppress proliferation or migration of gastric cancer as well as prostate cancer cells.

3. Rational and significance

Very few studies reported the association of *C. jejuni* or its toxin with cell receptors. Our recent study revealed that the association of the *C. jejuni* Cdt subunits with the host membrane is mediated through a cholesterol-dependent manner (18). Binding of Cdt subunits (CdtA and CdtC) to the cell membrane, delivery of CdtB into the nucleus, and G2/M arrest were all reduced when cellular cholesterol was depleted. Our preliminary results provide evidence that membrane cholesterol plays an essential role in the binding of *C. jejuni* Cdt subunits to membrane rafts, which enhances cell cycle arrest and cell death in host cells. Further investigation regarding the specific cholesterol-recognition sites on CdtA and/or CdtC in detail will be executed in the fist year proposal of this project. Despite understanding the epidemiology and molecular mechanism of the *Campylobacter* Cdt, there are not any reports on the establishment of Cdt delivery system applied to induce tumor cell death *in vitro* and *in vivo*. Thus, the major mission of this project in the second year and the third year is to "*establish a model system for application of bacterial toxin—Campylobacter CdtB in tumor therapy"*. With the development of bacterial toxin for tumor therapy, it becomes evident that an effective and versatile delivery system is the key to successful application of this novel treatment modality for possible clinical use (31).

B. RESEARCH DESIGN AND METHODS

The cholesterol recognition/interaction amino acid consensus (CRAC) region contains a conserved pattern $L/V(X)_{1-5}Y(X)_{1-5}R/K$, which is present in all proteins that associate with cholesterol (15). A recent report showed that the *A. actinomycetemcomitans* CdtC subunit contains a CRAC region, which contributes to the interaction between CdtC and cholesterol (4). Our present data indicated that both CdtA and CdtC were mainly localized in the cholesterol-rich microdomains. Thus, these evidences indicated that *C. jejuni* CdtA and CdtC may contain a hypothetical CRAC region, which contribute to cholesterol-binding activity.Analysis of the motif within the *C. jejuni* CdtC subunit also revealed a CRAC-like region (^{77}LPFGYVQFTNPK^{88}) (Fig. 1). This analysis suggests that the CRAC region, essential for the association of CdtC with cholesterol, is commonly preserved in these bacterial species. Moreover, our analysis of the *C. jejuni* CdtA amino acid sequence also revealed a CRAC-like region (^{17}LYACSSK^{23}) (Fig. 1). These sequence analyses indicate that although CdtA and CdtC have diverged, both have a CRAC-like region that may contribute to cholesterol-binding activity.

Recently, particles of nanometer scales are increasingly used as antigen carriers, and it is generally accepted that nanoparticles have adjuvant activity (27). Chitosan [b(1-4)2-amino-2-deoxy-D-glucose] is a biodegradable polysaccharide and mucoadhesive copolymer of N-acetyl-D- glucosamine and D-glucosamine (22), which has been proven to be safe. It allows a prolonged interaction between the delivered drug and the membrane epithelia, facilitating more efficient drug diffusion into the mucus/epithelium layer (28). Thus, it has potential to be used as a cationic polymeric condensing carrier (23), and since then chitosan has been shown

to have high transfection efficiency (25) and successfully used as a non-viral gene delivery system both *in vitro* (16) and *in vivo* (12). Therefore, the major project of the second year is to develop *in vitro* and *in vivo* systems for delivery of recombinant CdtB for tumor therapy using pH-responsive chitosan/heparin nanoparticles.

Figure 1. Identification of CRAC-like region in CdtA and CdtC. Deduced amino acid sequences of CdtA (upper panel) and CdtC (lower panel) are shown. The predicted amino acid motifs containing the putative CRAC-like region are boxed. Numbers indicate the positions of the amino acid residues at each end of the motif. Residues in red represented conserved pattern in CRAC-like region.

Specific aim 1. To develop an *in vitro* system for delivery of recombinant CdtB for tumor therapy using pH-responsive chitosan/heparin nanoparticles. Our previous study collaborated with Dr. Yu-Hsin Lin (Department of Biotechnology, China Medical University) was demonstrated that chitosan/heparin nanoparticles could internalize into human gastric adenocarcinoma cell cytoplasm and cell nuclei (19). We have been developed a novel nanoparticle-drug carrier with a heparin shell. The prepared nanoparticles were further found to be significantly increased the suppressive effect of several drugs on *H. pylori* growth and efficiently reducing cytotoxic effects in *H. pylori*-infected cells (5). Based on above description, the development of "*CdtB-loaded nanoparticles*" will be more feasible and accurate for assessing the biological effect for chemotherapeutic agents of cancer cells.

***Approach 1*. Preparing of chitosan/heparin nanoparticles.** We first prepared CdtB-loaded nanoparticles for test their biological activity on cancer cells. Two compositions of nanoparticles (protein/heparin and protein/heparin/chitosan) were prepared by a simple ionic gelation method with magnetic stirring at room temperature as described previously (5). In brief, aqueous heparin (1.0 mg/mL, 2 mL, pH 7.4) will add by flush mixing with a pipette tip into aqueous chitosan at various concentrations (0.3, 0.6, 0.9, 1.2 or 1.5 mg/mL, 10 mL, pH 6.0). The production of nanoparticles will be collected by ultracentrifugation at 15,000 rpm for 50 min. The supernatant will be discarded and the nanoparticles will be resuspended in deionized water for further studies. The

size distribution and zeta potential of the particles at pH 6.0 and pH 7.4 (10 mM PBS), will then be determined with a Zetasizer (Malvern Instruments Ltd., Worcestershire, UK) (17). The morphology of the prepared nanoparticles will be examined by transmission electron microscopy examination at different pHs. The nanoparticle suspension will be placed onto a 400 mesh copper grid coated with carbon. About 2 min after deposition, the grid will be tapped with a filter paper to remove surface water and positively stained with an alkaline bismuth solution.

Approach 2. Preparation of CdtB-loaded nanoparticles. To study the loading efficiency of CdtB from test samples (chitosan/heparin nanoparticles), the CdtB–loaded nanoparticle system will be prepared. Briefly, 0.1 ml of protein at various known concentrations (24.0, 12.0, 6.0, and 3.0 by mg/ml) was premixed with a heparin solution (2.0 mg/ml 0.1 ml) and added to a chitosan solution (1.2 mg/ml, 1.0 ml) under magnetic stirring as described before (n=5). To determine the loading efficiency, the CdtB-loaded nanoparticles will be collected by ultracentrifugation at 15,000 rpm, 4 °C for 50 min, and the concentration of free protein in the supernatant will be determined by ELISA analysis. The CdtB loading efficiency of nanoparticles will be determined as described in the literature and calculated from the following equation (7):

$$\text{Loading efficiency} = \frac{\text{total amount of CdtB} - \text{free CdtB}}{\text{total amount of CdtB}} \times 100\%$$

Table 1. Particle sizes and zeta potentials of nanoparticles prepared with different chitosan and heparin concentrations in deionized water (n = 5).

Chitosan Concentration (mg/ml)	Heparin Concentration (mg/ml)	Mean Particle Size (nm)	Zeta Potential (mV)
0.3	1.0	●	●
0.6	1.0	208.4 ± 10.6	30.4 ± 1.5
0.9	1.0	248.5 ± 8.7	33.6 ± 0.8
1.2	1.0	273.1 ± 10.6	37.4 ± 1.9
1.5	1.0	298.1 ± 9.6	39.4 ± 21.4

● Precipitation of aggregates was observed.

As shown in Table 1, chitosan:heparin in distinct chitosan concentration (0.3, 0.6, 0.9, 1.2, 1.5 mg/ml; 12.0 ml) and heparin (1.0 mg/ml; 2.0 ml) formed complexes on the nanometer scale. The mean particle sizes of the prepared nanoparticles were in the range 200–300 nm, with positive zeta potentials, depending on the relative concentrations of chitosan and heparin used. The amount of positively charged chitosan significantly exceeded that of negatively charged heparin, because some of the excessive chitosan molecules were entangled on the surfaces of the nanoparticles produced. A

chitosan concentration was 1.2 mg/ml, it was appeared to produce the particle size (273.1 ± 10.6 nm) with a significantly zeta potential of 37.4 ± 1.9 mV (Table 1). Therefore, the nanoparticles prepared with this specific composition were used for the rest of the study.

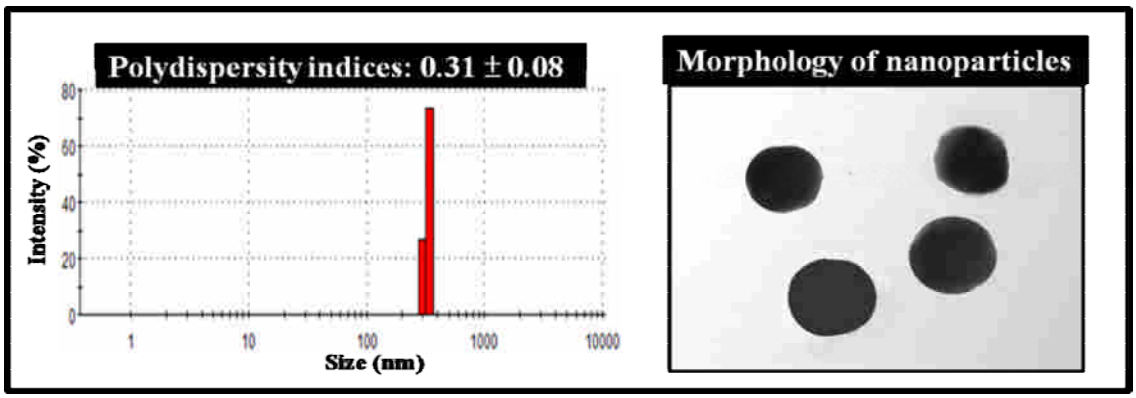

Figure 2. The polydispersity index (left panel) and transmission electron microscopy examination (right panel) of CdtB-loaded chitosan/heparin nanoparticles.

Approach 3*. *Characterization of nanoparticles. The size distributions and zeta potential values of the prepared nanoparticles at DI water were measured using a dynamic light scattering analyzer (Zetasizer ZS90, Malvern Instruments). FTIR spectra of the prepared nanoparticles were recorded with a fourier transformed infrared spectroscopy (Shimadzu Scientific Instruments). TEM was employed to examine the morphology of the heparin/chitosan nanoparticles as prepared previously (19). As shown in Table 2, the chitosan (1.2 mg/ml, 1.0 ml) and the protein:heparin in distinct compositions (12.0:1.0, 6.0:1.0, 3.0:1.0, and 1.5:1.0 by mg/ml, 0.2 ml) had a mean size range of 2800–300 nm, with different positive zeta potentials, depending on the relative concentrations of protein used. The polydispersity index of nanoparticles measured by a dynamic light scattering analyzer revealed a narrower distribution (polydispersity indices: 0.31 ± 0.08), when the protein:heparin composition at 1.5:1.0 (mg/ml) was used (Fig. 2). Additionally, the result obtained by the transmission electron microscopy examination showed that the morphology of the prepared CdtB-loaded in chitosan/heparin nanoparticles remained spherical and smooth shaped (Fig. 2).

Table 2. Particle sizes and zeta potentials of the prepared CdtB-loaded chitosan/heparin nanoparticles in deionized water (n = 5).

Protein:Heparin Concentration (mg/ml)	Chitosan Concentration (mg/ml)	Mean Particle Size (nm)	Zeta Potential (mV)
12.0:1.0	1.2	2788.1 ± 300.9	14.8 ± 5.9
6.0:1.0	1.2	983.5 ± 163.9	20.8 ± 3.1
3.0:1.0	1.2	414.3 ± 8.3	29.9 ± 1.7
1.5:1.0	1.2	312.4 ± 15.7	32.3 ± 0.7

Approach 4*. *Assessment of in vitro CdtB release. The release profiles of CdtB from test nanoparticles will be investigated in simulated dissolution medium (pH 1.2 for 120 min, pH 6.0 for 120 min, and pH 7.0 for 240 min, then simulating gastric acid and gastric mucosa) at 37°C. At set time intervals samples will be removed and centrifuged and the supernatants subjected to HPLC. The percentage cumulative amount of CdtB released will be determined using a standard calibration curve. The stability of CdtB will determine by analyzing the conformation of the CdtB released from nanoparticles using electrospray ionization–mass spectrometry (ESI–MS) and comparing the spectrum with that of standard CdtB. For ESI–MS measurements CdtB released from nanoparticles and standard CdtB were separately dissolved in alcohol at a concentration of 10 p.p.m., then directly injected into the electrospray ionization interface of the mass spectrometer. The ionization is in positive mode with a spray voltage of 4.25 kV

Approach 5*. *Viability of AGS cells treated with CdtB-loaded nanoparticles. To evaluate the cytotoxic activity of CdtB/heparin/chitosan nanoparticles, the AGS cell line (ATCC CRL 1739) will be used in this study. The cells were initially grown in 25 cm^2 tissue culture flasks in RPMI 1640 medium containing 10% FBS, penicillin (100 U/ml) and streptomycin (100 μg/ml) in an incubator at 37 °C, 95% humidity and 5% CO2. The cells will be harvested for subculture every 3 days with 0.25% trypsin plus 0.05% EDTA solution and used for the cytotoxicity experiments. The cytotoxicity of the test samples will be evaluated *in vitro* by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay.

***Possible problems and alternatives*.** The MTT assay is based on mitochondrial dehydrogenase activity as an indicator of cell viability. To further determine whether the cytotoxic effect is mediated through apoptosis pathway, flow cytometry for analysis of sub-G1 population of AGS cells will be used. Further quantitative assessment of apoptotic cells was detected by using PI/AnnexinV assay (Calbiochem) and TUNEL assay (Roche Diagnostics) according to manufacturer's instructions. The stained cells were then analyzed using a FACScan and the Cellquest program (Becton Dickinson).

Anticipated results. Studies from this specific aim would develop the most potent CdtB/heparin/chitosan nanoparticles and establish the basis for further characterizations of the signal pathway in the induction of cancer cell apoptosis.

Specific aim 2. To investigate the apoptotic pathway in the tumor cells upon treatment of cells with CdtB/heparin/chitosan nanoparticles. To investigate potential cell death pathway of CdtB-loaded nanoparticles in gastric cancer cells, we will investigate the cell viability and signaling pathway through an apoptotic mechanism.

***Approach 1*. Determination of the mitochondrial membrane potential.** To explore whether CdtB/heparin/chitosan nanoparticles alter mitochondrial membrane potential, the mitochondrial membrane potential (ΔΨm) will be assessed using a fluorometric probe JC-1 (Calbiochem), with a positive charge of a mitochondrial-specific fluorophore, indicated by a fluorescence emission shift

from green (525 nm) to red (610 nm). AGS cells were plated in 6-well culture dishes and treated with vehicle or gentamicin. After incubation, cells were stained with JC-1 (5 μg/ml) for 15 min at 37^{o}C. Samples were analyzed by FACScan using an argon laser (488 nm). Mitochondrial depolarization is specifically indicated by a decrease in the red to green fluorescence intensity ratio and analyzed by a FACScan and the Cellquest program (Becton Dickinson).

***Approach 2*. Measurements of reactive oxygen species (ROS).** To detect the level of ROS production, CdtB-loaded nanoparticles treated cells will be loaded with 10 μM dihydrorhodamine 123 (DHR 123) for 15 min. The fluorescence intensities were obtained by recording the FITC fluorescence. AGS cells were collected and analyzed by a FACScan and the Cellquest program (Becton Dickinson).

***Approach 3*. Detection of Ca^{2+} concentrations.** We will further assess the effect of CdtB/heparin/chitosan nanoparticles on mobilization of Ca^{2+} in HEI-OC1cells. CdtB-loaded nanoparticles treated AGS cells (5×10^5 cells/well) were cultured in 12-well plates for the indicated time intervals to detect changes in Ca^{2+} levels. Cells were harvested and washed twice, and re-suspension in FURA-PE3/AM (3 μM) at 37 °C for 30 min and analyzed by a FACScan and the Cellquest program (Becton Dickinson).

***Approach 4*. Western blot analysis of molecules involved in apoptotic pathway.** To ascertain CdtB/heparin/chitosan nanoparticle-induced AGS cell apoptosis by triggering the mitochondrial apoptotic pathway or ER-stress, we will measure change in expression of Bcl-2 family proteins (including Bcl-2, Bax and Bak), calpain, caspase-9 and caspase-3. Proteins of the cellular lysates were resolved on SDS-PAGE and transferred to Immobilon polyvinyldifluoride membranes (Millipore). The blots were blocked and then probed with antibodies against Bcl-2, Bcl-xl, Bax, Bak, caspase 3, caspase 9, PARP, calpain-1 and calpain-2 (Cell Signaling) for 1 h at room temperature. After three washes, the blots were subsequently incubated with a donkey anti-rabbit peroxidase conjugated secondary antibody (Santa Cruz) for 1 h at room temperature. The blots were visualized by enhanced chemiluminescence using Kodak X-OMAT LS film (Eastman Kodak).

Anticipated results. We will examine the effects of injection of CdtB/heparin/chitosan nanoparticles into nude mice on tumor growth *in vivo*. We anticipate at the end of the experimental period (48 days after initiation of CdtB-loaded nanoparticle therapy), all tumors in the mice had decreased in size or disappeared. In addition, we anticipate that a large number of TUNEL-positive or caspase 3/9-positive cells were detected in tissue sections from mice treated by CdtB/heparin/chitosan nanoparticles. The results from this proposal will suggest possible application of focused CdtB-loaded nanoparticles for chemotherapy as treatment for gastric cancer cells.

C. CONCLUDING REMARKS

The main focus of this grant proposal is to reveal molecular basis of *Campylobacter Cdt* in the interaction of cholesterol-enriched microdomains and induced pathogenesis of host. Here, we will develop novel technology to apply the bacterial toxin in the cancer therapy. We plan to sustain and

develop a program of international quality sciences, integrating experimental and theoretical means to cross-field science research in the three-year road map. We will address bacterial toxin-cell interactions at the subcellular level. *In vitro* experiments by these newly developed techniques will be also performed for the application of cancer therapy. The efficiency of novel drug—CdtB for tumor therapy will then be identified in an *in vivo* mouse model system. Based on our previous results in this peculiar microbe as well as understanding of Cdt biological functions, we are confident in the execution of this proposal.

This project will emphasize both research and novel approaches. We anticipate inspire the future inauguration for molecule-based biotech industries in tumor clinical therapy. The cross-field of advanced methodology may be also developed. The benefits in executing this proposal are:

(1) The scientific issues related to the molecular mechanisms of *Campylobacter* Cdt pathogenesis will be addressed.

(2) The development of novel bacterial toxin delivery systems will be carried out.

(3) Promote infection-related sciences as well as cross-fields with the established comprehensive supports to the biology community, including nanotechnology, and cancer biology.

(4) Overall, the strength of the proposal is that our team and collaborations have the experience in this field and the subject of study could be important and potentially have impact on the application of bacterial toxin in cancer therapy.

References

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