行政院國家科學委員會專題研究計畫成果報告

計畫編號：NSC 90-2314-B-002-200
執行期限：90 年 8 月 1 日至 91 年 7 月 31 日

主持人：魏淑?　執行機構及單位名稱: 台灣大學醫學院附設醫院內科部
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一、摘要

The “Vogelgram” proposed by Vogelstein and his colleagues, described the adenoma-adenocarcinoma sequence, had mentioned the adenomatous polyposis coli (APC) gene mutation in the early stage of colorectal carcinogenesis. Mutation of the APC gene leads to abnormal epithelial proliferation and differentiation. β-catenin can bind members of the Tcf/Lef family of transcription factors and activate gene transcription. C-MYC, cyclin D1 and matrix metalloproteinase-7 (MMP-7) were found to be the downstream targets of this β-catenin/Tcf-regulated transcription. It appears that c-MYC activation is a direct consequence of APC loss and thus a primary cause of tumor cell proliferation. Aspirin and/or nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to reduce the adenoma size and numbers in FAP patients. Disease prevention is receiving more attention in the developed country today. The risk-benefit ratio for chemoprevention of colorectal cancer would likely improve if high-risk groups could be identified and less side effect drugs could be used. COX-2 selective inhibitors and curcumin have less side effects than sulindac but their effects on colorectal cancer remained to be clarified. This study was designed to first realize the serial gene expression changes after sulindac treatment, especially focusing on APC, β-catenin, TCF, cyclin D1, MMP-7, axin-2 and c-MYC; and to second compare the anti-proliferation and apoptosis-inducing ability of sulindac, curcumin and COX-2 inhibitor. The results of this study revealed that Sulindac still remained the most constant effective (anticancer) agents among these three test compounds which has the effects of inducing cancer cells apoptosis and cell cycle paused at G1 phase effects. Curcumin has the effects of inducing cancer cells apoptosis and cell cycle paused at G2M phase. Lonine (Etolodac), a COX-2 inhibitor, has less apoptosis-inducing effects but can pause the cell cycle in G1 phase. All these effects were time and dose dependent. The Northern blot results showed that only c-myc expression decreased in HCT116 after sulindac treated for 48 hours but no significant changes of other genes. Further study is indicated for confirming the results.

Keywords: Sulindac, COX-2 inhibitor, Curcumin, Colorectal carcinogenesis

二、緣由與目的

A. SPECIFIC AIMS:

(1). To define the serial gene expression changes after sulindac usage, especially focusing on APC, β-catenin, c-MYC, TCF, cyclin D1, axin-2 and MMP-7.

(2). To compare the effects of sulindac, curcumin and COX-2 inhibitors on:

<1>. the inhibition of cell growth

<2>. cell cycle progression

<3>. apoptosis

<4>. the expression of APC, β-catenin, c-MYC, TCF, cyclin D1, axin-2 and MMP-7 genes

B. BACKGROUND

Colorectal cancer is a major cause of death in Western civilizations. The incidence of colorectal cancer in Taiwan has increased recently, which might be due to the westernizing of lifestyle and food in recent years. The “Vogelgram” proposed by Vogelstein and his colleagues, described the adenoma-adenocarcinoma sequence, has mentioned the adenomatous polyposis coli (APC) gene mutation in the early stage of colorectal carcinogenesis (1). The APC gene is a tumor suppressor gene located on the long arm of chromosome 5. Most of the familial adenomatous polyposis (FAP), a hereditary type colorectal cancer, is thought to be due the mutation in APC gene (2-5). Mutation of the APC gene leads to abnormal epithelial proliferation and differentiation. APC mutations are present

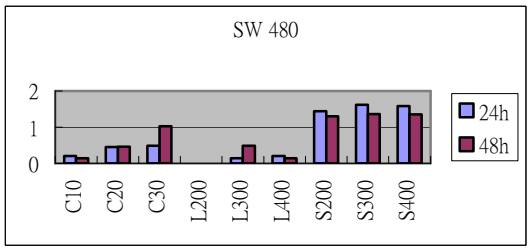
in more than 85% of sporadic colorectal cancer (6). Therefore, understanding the function of APC is crucial to studies of carcinogenesis and tumor prevention. It is now known that the APC is a cytoplasmic protein (7), which acts coordinately with the GSK-3β (glycogen synthase kinase-3β) and axin/conductin (protein with unknown function yet) to control the β-catenin in cytoplasm (8). β-catenin can bind members of the T-cell factor (TCF)/lymphocyte effective factor (LEF) family of transcription factors and activate gene transcription (9,10). Accordingly, human colorectal tumors with APC or β-catenin mutations exhibit increased β -catenin/TCF-mediated transcription (11,12). C-MYC oncogene was found to be one of the downstream targets of this β -catenin/TCF-regulated transcription (13). It appears that c-MYC activation is a direct consequence of APC loss and thus a primary cause of tumor cell proliferation. Recently, there are two more components reported to be involved in this APC-β -catenin /TCF (LEF) pathway. The cyclin D1 gene is a direct target for transactivation by the β-catenin /TCF (LEF) pathway through a LEF-1 binding site in the cyclin D1 promoter. Cyclin D1 protein levels were induced by β-catenin overexpression and reduced in cells overexpressing the cadherin cytoplasmic domain. Increased β -catenin levels may thus promote neoplastic conversion by triggering cyclin D1 gene expression and, consequently. Uncontrolled progression into the cell cycle (14, 15). Matrix metalloproteinase MMP-7 was found to be another target gene of β -catenin /TCF (LEF). MMP-7 is overexpressed in 80% of human colorectal cancers and known to be an important factor for early tumor growth, with a potential function also for later progression steps, like invasion and metastasis (16).

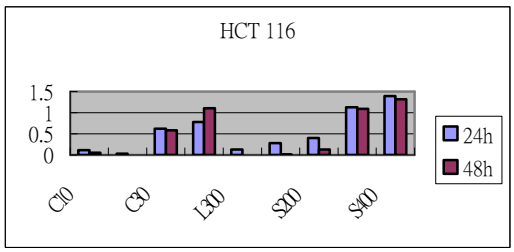
Waddell and Loughry (17) first reported that sulindac led to regression of rectal adenomas in four patients with FAP. Aspirin and/or nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to reduce the adenoma size and numbers in FAP patients in the later studies (18,19). However, the actual mechanism underlined the chemoprevention of colorectal cancer by aspirin/sulindac still remains to be resolved. The basic mechanisms for the decrease pre-cancer lesion (adenoma) size and numbers, also for the cancer chemoprevention, include inhibiting proliferation and/ or inducing apoptosis of tumor cells. Actually, aspirin and NSAIDs had been reported to have the effects of inhibiting cancer cells proliferation, inducing apoptosis and normalizing enterocytes migration both in the in vitro and in vivo studies (20-25). However, the exact component and the detail pathway that the drugs intervening the carcinogenesis remain unclear (26). Some claimed that the effect was prostaglandin-independent (24,25). But others thought it as prostaglandin-dependent and even pointed to the cyclooxygenase-2 (COX-2)-related events (24, 29-31). Anyhow, there is no report about the serial gene changes in the Vogelgram after NSAIDs usage till now. Disease prevention is receiving more attention in the developed country today. Initially, it was hoped that early detection would reduce the high prevalence of colorectal cancer. However, despite recent evidence that early detection by fecal occult blood tests and flexible sigmoidoscopy can decrease the risk of colorectal cancer by 20-30%, most people do not undergo appropriate screening. The risk-benefit ratio for chemoprevention of colorectal cancer would likely improve if high-risk groups could be identified (26). Additionally, if agents were available that lack platelet effects and far less gastrointestinal gastrointestinal toxicity (such as, potentially, COX-2 inhibitors (32)), the risk to benefit ratio might be favorably affected. Curcumin, a phenolic compound that has been identified as the major pigment in tumeric, possesses both anti-inflammatory (33) and antioxidant properties (34). It had been shown that 2000 ppm of curcumin in the diet significantly suppressed the azotymethane-induced colonic aberrant

crypt foci formation which are early preneoplastic
lesions, colon tumor incidence and tumor multiplicity in male F344 rats (35,36). It also had been suggested that the chemoprevention action of curcumin might be mediated through the inhibition of formation of COX metabolites as NSAIDs do (37). Moreover, after centuries of use as a natural product, curcumin has no known toxicity and side effects yet (37). As a chemopreventive agent, curcumin seems to have advantage than the NSAIDs for long-term use in that it is in large qualities as a natural product and no side effects and toxicity. Therefore, the chemoprevention effect of selective COX-2 inhibitors or curcumin is worth of testing.

三、結果與討論

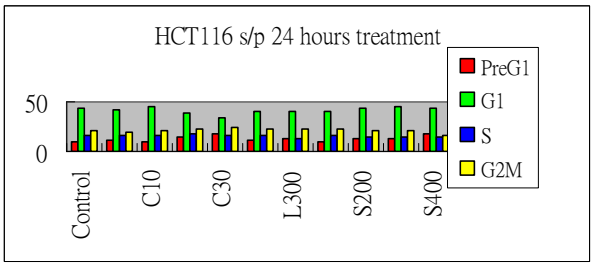
(1). MTS (viable cell counts assay): shown by Inhibition rate

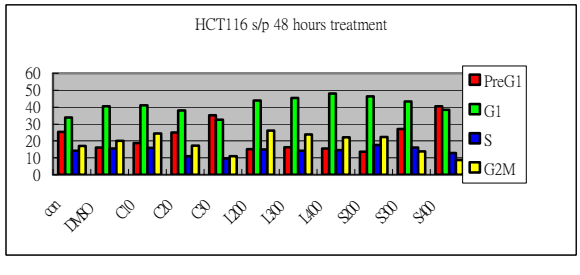


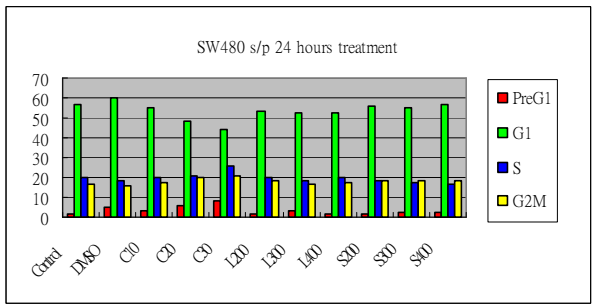


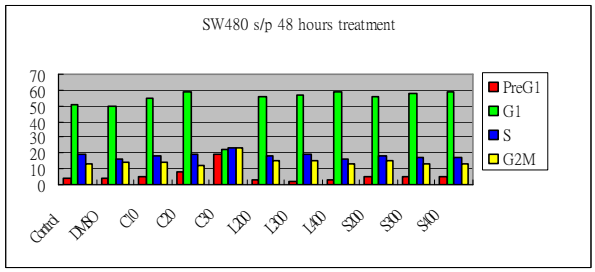
(C: Curcumin; L: Lonine <Etolodac>;
S: Sulindac; concentration: μM in uint)

(2). Cell cycle changes: by PI staining, FACS analysis

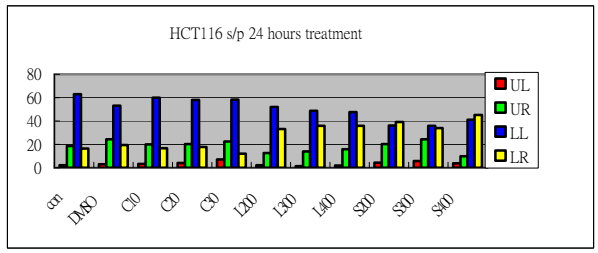


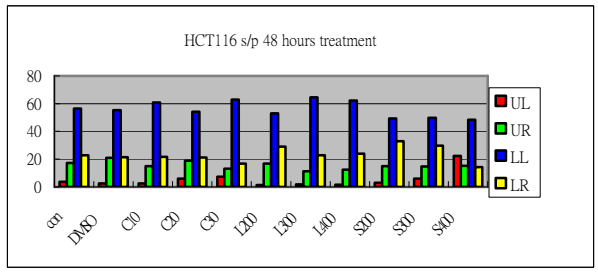


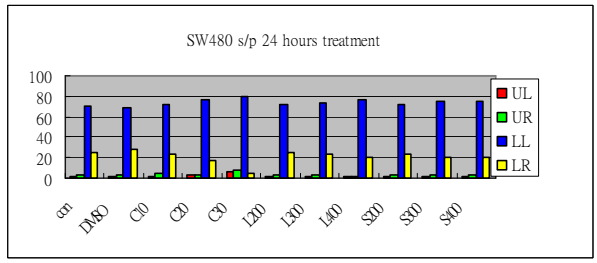


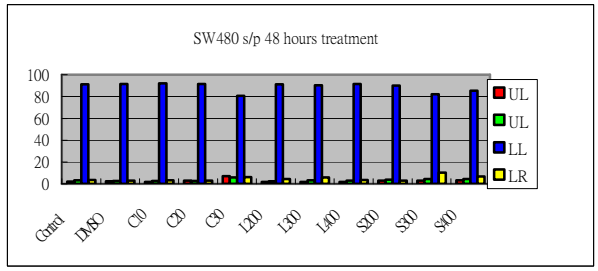


(3). Apoptosis analysis: by PI/Annexin V staining, FACS analysis



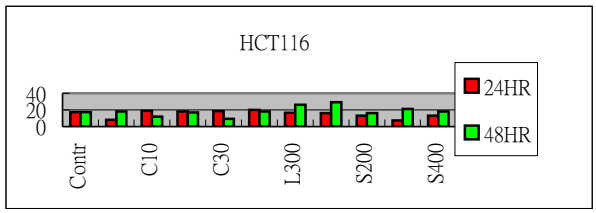


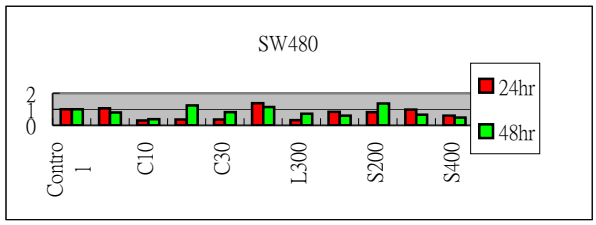




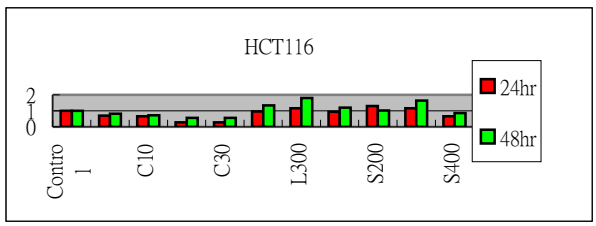
(4). Northern blot for APC, β-catenin and c-myc

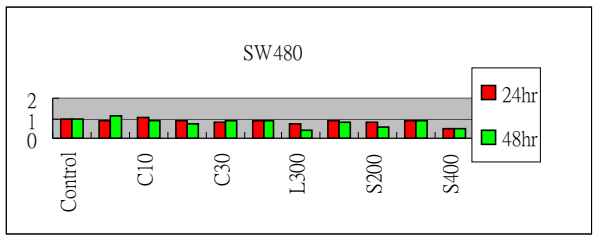
<1>. APC



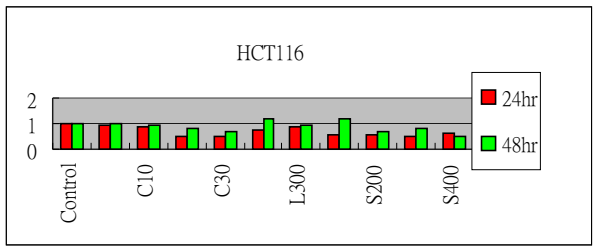


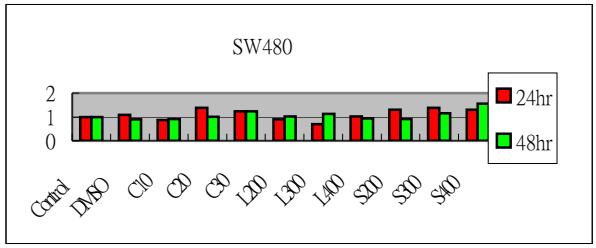
<2>. β-catenin





<3>. c-myc:





(5). Summary of the results:

1. Sulindac still remained the most constant effective (anticancer) agents among these three test compounds.
2. Curcumin has the effects of inducing cancer cells apoptosis and cell cycle paused at G2M phase effects. Sulindac also has the effects of inducing cancer cells apoptosis but cell cycle paused at G1 phase effects. Lonine (Etolodac) has less apoptosis-inducing effects but can pause the cell cycle in G1 phase. All these effects were time and dose dependent.
3. The Northern blot results showed that only c-myc expression decreased in HCT116 after sulindac treated for 48 hours but no significant changes of other genes. Further study still is indicated for confirming the results.

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行政院國家科學委員會補助專題研究計畫成果報告

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※ 比較 Sulindac, COX-2 inhibitors 和 curcumin ※
※ 對大腸癌產生過程中的一系列基因變化的影響 ※
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計畫類別：■個別型計畫　　□整合型計畫
計畫編號：NSC90－2314－B－002－220
執行期間： 90 年 08 月 01 日至 91 年 07 月 31 日

計畫主持人：魏淑?
共同主持人：翁昭旼

本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份
□赴大陸地區出差或研習心得報告一份
□出席國際學術會議心得報告及發表之論文各一份
□國際合作研究計畫國外研究報告書一份

執行單位：台灣大學醫學院附設醫院內科部

中 華 民 國 91 年 10 月 30 日