私立中山醫學院醫學研究所碩士論文
Master Thesis, Institute of Medicine,
Chung Shan Medical and Dental College

指導教授 ： 李宣佑 博士 (Shuan-Yow Li,Ph.D)

抗癌藥物誘發人類淋巴球染色體變異之研究

(1) Daunomycin 誘發正常人類淋巴球染色體斷裂及變異之研究

(2) CCNU 誘發正常人類淋巴球染色體易脆位置頻率與分佈之探討

Studies on Chromosomal Aberrations induced by Antineoplastic Agents in Human Lymphocytes

(1) Chromosomal breakage and aberrations induced by daunomycin in cultured human lymphocytes

(2) Frequency and distribution of CCNU-induced fragile sites in cultured human lymphocytes

研究生 ： 劉蓉錦 (Jung-Chin Liou)

中華民國八十三年七月
July, 1994

本論文爲中山醫學院授予理學碩士學位之必備條件之一，經中山醫學院醫學研究所碩士論文考試委員會審查合格及口試通過。

口試委員

台大醫學院毒理研究所副教授　　郭明良　博士

中山醫學院生物化學研究所教授　　王朝鐘　博士

中山醫學院醫學研究所教授（論文指導教授）　　李宣佑　博士

中華民國八十三年七月

致謝
(Acknowledgements)

說來慚愧，回首我的前半輩子，已過的這兩年研究生生涯才是我眞正體會科學研究的精神與艱辛的開始。很感謝上天給了我這麼一個學習與成長的機會。更難得的是，在這歷練的過程當中，發現了周遭有這許多可愛的人們，使研究的生活不致於苦悶與孤單。

當然，最感謝的是我的指導教授李宣佑博士，由於他的包容、耐心與信任，引發我對於人類遺傳疾病的研究興趣以及一種幾近於無可救藥的使命感。雖然無法確知自己這份熱誠究竟能夠維續多久，但此啓發之恩將永遠令人感念。

此外，要感謝李天翎教授、劉德中教授、陳凌雲教授、謝易修教授、中研院詹崑源教授等幾位師長給與我研究論文寫作上的意見和幫助。特別是李天翎老師的"救火"精神，更令人銘感五內。

還有，蔡姐、Y姐、謝姐、怡鈞、蜜琪、保英、惠萍、淑卿、月君、必明、楊文、姿慧、秋萍、乃鼎、小杜及惠華等細胞遺傳室內的眾兄弟姊妹們，謝謝大家的鼓勵與幫忙。另外，還要向一群可愛的伙伴們：煒翔、祈光、婉瑜、哲青、東霖、秀玲及厚基等道謝，謝謝你們的"有求必應"。

最後，我要感謝我的雙親，一直無怨無悔地支持我，並在漫漫的人生旅途上給我完全的自主。

第一篇 ： Daunomycin 誘發正常人類淋巴球染色體斷裂及變異之研究

Chromosomal breakage and aberrations induced by daunomycin in cultured human lymphocytes

目錄 ：

摘要（Abstract）

本篇研究主要是在探討 daunomycin 此種抗癌藥物的細胞遺傳毒性（genocytotoxicity）如何？ 以及它會對於淋巴球細胞核內的染色體引發那些變異？ 將培養的末稍淋巴球（抽取自五位男性及五位女性，年齡20-29歲）加入 daunomycin 處理作爲實驗組；另外，以 caffeine 作爲致突變作用之強化劑（mutagenic enhancer）。

本實驗結果顯示，daunomycin 處理組出現了大量的染色體斷裂 (chromosomal breakage)，而且其斷裂頻率遠高於對照組（$p<0.025$）。再者，daunomycin 也誘發產生了許多染色體畸變 (chromosomal aberrations)。這些異常包括同源染色體間或非同源染色體間的互相交換 (homologous chromosomal or non-homologous chromosomal interchanges)；同一條染色體內姊妹染色分體的交換 (intrachromosomal exchanges)；同源對染色體間或非同源染色體間的轉位 (homologous or non-homologous chromorsomal translocations) 以及環形染色體 (ring chromosomes) 等。

此外，分析這些畸變染色體之斷裂點 (breakpoints)，發現大部份 daunomycin 所誘發出來的斷裂位置與某些癌細胞異常染色體的斷裂點，以及一些已經定位的致癌基因位置上 (mapped oncogene locations) 相符合。更值得一提的是，大多數對於 daunomycin 敏感的斷裂位置也曾出現在經由不同致突變機制 (mutagenic mechanism) 的其他藥物所誘發異常染色體上。

本篇研究結果意味著：在人類的淋巴球染色體上有某些易脆位置 (fragile sites)，這些易脆位置可能是在細胞遭受致突變劑 (mutagen) 或致癌物 (carcinogen) 攻擊時的靶的 (targets)，或者是基因重組 (gene rearrangement) 的位置。

第一章
前言(Introduction)

近年來許多研究發現在人類的染色體上有許多易脆位置(fragile sites)，這些位置會受遺傳因子與環境因子的影響而產生斷裂(break) (Glover 等人, 1984; Smeets 等人,1986; Sutherland 等人,1982; Sutherland 等人,1984; Sutherland 和 Hecht,1985; Yunis 和 Soreng, 1984; Yunis 等人, 1987)。在正常情況下，這些易脆位置出現斷裂的頻率非常低。但若在細胞的培養液加入某些藥劑處理，則將大大提升易脆位置的表現頻率。這些能夠誘發易脆位置表現的藥劑包括抗代謝劑（例如 fluorodeoxyuridine 和 methotrexate) (Barbi 等人, 1984; Glover, 1981; Yunis 等人, 1987)；DNA 聚合酶抑制劑(例如 aphidicolin) (Glover 等人, 1984; Hecht 等人, 1984; Rao 等人, 1988; Yunis 等人, 1987)；烷基化劑(例如 busulfan) (Yunis 等人, 1987)，以及 DNA 修復抑制劑(例如 caffeine) (Das 等, 1984; Rao 等人, 1988)。

某些抗癌藥物 (antineoplastic agents) 曾經被証實會促使姊妹染色分體交換 (sister chromatid exchange, SCE) 頻率增加許多，如 cyclophosphamide (Huang 和 Furukaga,1978)，5-bromodeoxyuridine (Ishii 和 Bender, 1978)，CCNU (lomustine) (Best 等人, 1988)，mitomycin C (Latt等人, 1974; Raposa 等人, 1978)。 除了 SCE 的增加外，某些抗癌藥物亦會引起染色體之斷裂，例如 bleomycin (Puvirk 和 Austine,1991)。由此可知，許多抗癌藥物亦可能爲致突變物 (mutagens) 或致癌物 (carcinogens)。而致突變物或致癌物均會誘發染色體的斷裂及異常。根據 Yunis 等人(1987) 的研究報告顯示：部份抗癌藥物引發的染色體異常與易脆位置的顯現，其斷裂點和某些癌細胞異常染色體之斷裂點頗爲吻合。

Daunomycin (daunorubicin) 爲一種具有 anthracycline 構造之抗生素，在臨床上常被用以治療白血病 (leukemias)，其作用機轉乃經由嵌入雙股 DNA 的鹼基後，與拓樸異構酶II (topoisomerase II) 形成一個酶-DNA 的共價複合物以抑制拓樸異構酶II 之作用，最後因阻礙受害 DNA 之再接合而導致細胞的死亡。

本篇研究之目的乃在探討 daunomycin 這種抗癌藥物的細胞遺傳毒性 (genocytotoxicity)。由於 daunomycin 的致突變機轉不同於 fluorodeoxyuridine，methotrexate，及 aphidicolin 等藥物，我們想知道是否 daunomycin 也會引起細胞核內染色體的斷裂或其他異常？其造成的染色體斷裂頻率如何？以及其斷裂點在染色體的分佈又如何？ 而我們的結果顯示：在實驗的條件下， daunomycin 會誘發大量的染色體斷裂及多種型式的染色體異常，並且這些斷裂位置也出現在某些癌細胞異常染色體之斷裂點和一些已經定位的致癌基因位置 (locations of mapped oncogenes) 上。

第二章
材料與方法 (Materials and Methods)

2.1 ：實驗材料

抽取自願之10位正常人（5位男性，5位女性，年齡 20-29）之末稍血液

2.2 ：培養液:

RPMI-1640 (GIBCO)

2.3 ：藥品名稱及製備:

Caffeine (最終濃度 2.5 mM)	(SIGMA)
Colcemid (最終濃度 0.05 ug/ml)	(GIBCO)
Daunomycin (最終濃度 0.1 uM)	(SIGMA)
FCS (fetal calf serum)	(GIBCO BRL)
Fixation (methanol:acetic acid = 3:1)	(E.MERK)
Heparin sodium	(B Braun Melsunggen A G)
Hypotonic solution (0.54% KCl)	(E MERK)
PHA (phytohemagglutinin) M form	(GIBCO)
PSN (penicillin, streptomycin, neomycin)	(GIBCO)

2.4 : 儀器 :

Lamina Flow : Bellco Glass Inc. (USA)

Incubator (培養箱):

Forma Scientific Steri-cult incubator
temperature 37℃, Humidity 60-98%,
CO2 control 5%.
Water-Jacketed incubator

Centrifuge (離心機) :

Time and Speed (8 min × 1200 rpm)
Hitachi

Slide warmer (烘片機) :

Fisher

2.5 ： 實驗方法與步驟

2.5.1 ： 各種藥品的處理時間及濃度經 pretest 之處理(見表一)

2.5.2 ： 細胞的培養 (cell culture)

抽取 25 ml peripheral blood + 0.3 ml heparin
↓ 靜置 30 min
取含 buffy coat (lymphocytes) 之血液 0.5 ml
↓
種入含 9.2 ml 之 RPMI-1640 培養液中
(RPMI-1640 含 15% FCS 及 1% PSN)
每個人的血共有 4 種處理，分別爲編號爲
culture 1,2,3,4
↓
加入 0.2 ml PHA (phytohemagglutinin)
↓
置於培養箱(incubator,37℃,60-98% humidity,5%CO2)
↓ 66 h
編號爲 1,2 之 cultures 分別加入 0.1 ml 的
daunomycin (0.1 uM 最終濃度)
↓ 22 h
所有 cultures 均以新鮮之RPMI-1640 洗二次
↓
編號爲 1,4 之 cultures 均加入10 ml RPMI-1640
培養液(含15% FCS 及1% PSN)
編號爲 2,3 之 cultures 則加入9.9 ml 之RPMI-1640
培養液(含 15% FCS 與1%PSN) 和 0.1ml 之
caffeine (2.5mM 最終濃度)
↓ 6 h
收穫細胞 (cell harvest)

2.5.3 ： 收穫細胞與染色體之製備

在收穫前 20 min 將 0.1 ml colcemid (0.05ug/ml)
加入各 cultures
↓

↓

將各組培養的細胞分別倒入離心管

↓離心 8 min, 1200 rpm

倒掉上清液

↓

加入10 ml 的 0.54% KCl 低張溶液

↓16 min

↓離心 8 min

倒掉上清液

↓

緩緩加入 8 ml 固定液（甲醇:冰醋酸=3:1）

↓30 min

↓離心 8 min

加入 8 ml 固定液

↓離心 8min

倒掉上清液

↓

加入 8 ml 固定液

↓5 min

↓離心 8 min

倒掉上清液

↓

加入 0.5 ml 固定液，調勻懸浮液

↓

將懸浮液滴在載玻片上

↓

將載玻片置於烘片機上（37℃）

↓24 h

以 Trypsin-Giemsa staining 方法進行染色

（使用 Wright's stain）

2.5.4 ： 染色體的觀察與分析

在顯微鏡下採隨機方式觀察染色體分散良好的中期細胞(metaphases)，每一種處理均記錄 100 個 metaphases。如有難以分析之染色體變異，則予以照相並排列其核型 (karyotype)。我們依照 ISCN (1985) 的標準來記錄染色體的斷裂點 (breakpoint)。

2.5.5 ： 統計方法

本實驗以 ANOVA (analysis of variance)方法分析這些不同處理條件下的染色體斷裂頻率是否有差異；並以多重比以較檢驗 (multiple comparison tests)來檢定那組別有明顯差異 (Rosner 等人,1990)。

第三章
結果 (Results)

4 種不同培養條件的人類淋巴球（見表 一），其出現的染色體斷裂頻率(frequency of chromosomal breakage) 如表二 所示。由表二，總共有 193 個染色帶至少出現一次以上的斷裂。此外，由表一可以發現 daunomycin 所誘發的染色體斷裂數明顯地增加很多。各組之平均染色體斷裂數(每 100 個中期細胞之染色體斷裂數 ± 標準誤) 如下:

1. Control 組（爲不加 daunomycin 或 caffeine 者）: 1.2 ± 0.4。
2. Daunomycin 組 : 277.3 ± 30.5 。
3. Daunomycin + Caffeine 組 : 404.4 ± 33.9。
4. Caffeine 組 : 12.8 ± 1.7。

圖 一乃展示的在顯微鏡下所見由 daunomycin 誘發出來的染色體斷裂及異常情形。經過配合排列核型 (karyotype) 的分析，而將 daunomycin 所誘發的染色體斷裂點之分佈列於表 三（表三所列乃是該位置出現十次以上斷裂者）。

由表二可見，daunomycin + caffeine 處理組出現之染色體斷裂數較諸 daunomycin 組者顯著高出許多。然而， daunomycin + caffeine 組觀察到 201 個染色帶至少出現一次斷裂。此顯示 caffeine 可強化染色體對 daunomycin 敏感位置的斷裂。

Daunomycin 除了誘發大量的染色體斷裂之外，我們也觀察到許多不同型式的染色體畸變 (chromosomal aberrations)(見圖二)。這些畸變的染色體包括 : 同源染色體互相交換 (homologous chromosome interchanges)（圖 二A），同源染色體轉位 (homologous chromosome translocations)(圖 二B)，非同源染色體互相交換 (non-homologous chromosome interchanges) (圖 二C, 二D)，複合染色體交換 (complex chromosomal interchanges) (圖 二E)，非同源染色體轉位(non-homologous chromosome translocations)(圖 二F)，染色體內姊妹染色分體交換 (intrachromosomal exchanges)（圖 二G），以及環形染色體 (ring chromosomes)（圖 二H）。

經過照相及排列染色體核型之後，分析並整理這些畸變染色體之斷裂點（見 表四）。

另外，這些對 daunomycin 敏感的染色體位置和其他藥物所誘發的相似易脆位置，以及和某些癌細胞異常染色體斷裂點之關係列於表三。

第四章
討論 (Discussion)

近幾年來，在人類染色體上有許多的易脆位置 (fragile sites) 被發現。這些易脆位置會受遺傳因子或某些環境因子的影響而發生斷裂 (Glover 等人,1984; Hecht 和 Sutherland, 1984; Smeets 等人, 1986; Sutherland, 1982. 1985)。在正常情況下，染色體出現斷裂的頻率非常低。但是，如果將培養的細胞暴露於一些藥物時，則可誘發某些染色體易脆位置的表現。這些能引起染色體大量斷裂的藥劑包含抗代謝類的抗癌藥物如 fluorodeoxyuridine 與 methotrexate (Barbi 等人,1984; Glover 等人, 1981; Rao 等人,1988; Yunis 等人,1987)，DNA 聚合酶抑制劑如 aphidicolin (Craig-Holmes 等人, 1987; Glover 等人,1984; Hecht 和 Glover ,1984; Rao 等人, 1988; Yunis 等人, 1987) 烷基化劑如 busulfan (Yunis 等人, 1987)，及 DNA 修復抑制劑如 caffeine (Das 等人,1984; Rao 等人, 1988)。而且，不同的致突變物亦可引發許多相似染色體位置的斷裂 (Daniel 等人,1984; Glover,1981; Rao 等人,1988; Yunis 等人,1987)。

其次，至目前爲止，已知許多人類的白血病 (leukemias)，淋巴腫瘤 (lymphomas) 和一些實質性腫瘤 (solid tumors) 的細胞的異常染色體(如轉位，倒轉，或缺失等) 會出現某些特定的斷裂點 (Berger ,1985等人; Cohen 等人,1979; de Braekeleer 等人,1985; Hecht 和 Sutherland; Le Beau,1988; Mitelman,1990; Takahashi 等人,1988; Trent 等人,1985; Whang-Peng 等人,1982; Yunis,1983)。再者，許多易脆位置也和某些癌細胞染色體斷裂點頗相符合 (de Braekeleer 等人,1985; Hecht 和 Glover,1984; Hecht 和 Sandberg,1988; Le Beau,1988; Yunis,1983; Yunis 和 Soreng 1984; Yunis 等人,1987)。

Daunomycin (daunorubicin) 爲一種具 anthracyclin 結構之抗生素，它可藉由嵌入雙股的 DNA 或經由抑制拓樸異構酶之作用而殺死細胞，因而它常被應用以治療白血病 (Wingard 等人,1991)。本篇實驗的主旨乃在探討此一致突變機轉不同於 fluorodeoxyuridine，methotrexate 和 aphidicolin 等藥物的daunomycin ，其對染色體的影響爲何？以及其對染色體之作用點爲何？

如表二 所示，daunomycin 所誘發的染色體斷裂頻率顯著高於 ($p< 0.025$) 控制組，由此可知 daunomycin 爲一種很強的染色體變異誘發劑 (inducer)。

此外，caffeine 因其具抑制修補受害 DNA 的能力而使藥物所誘發的染色體或 DNA 受損程度可以表現出來。因此，caffeine 常被應用爲致突變作用的強化劑 (mutagenic enhancer)(Kihlman 等人,1982; Rao 等人,1988; Yunis 等人,1987)。表二 顯示: 在經二次換洗之後再加入 caffeine 處理 6 小時的組別，其染色體出現了大量的斷裂數目，同時，我們可以看出 caffeine 顯著地增加了 daunomycin 所誘發的染色體斷裂 (表二)。同時，由表三可見，caffeine 可強化大部份 daunomycin 所誘發的染色體斷裂。但是，亦有幾個染色體位置如 4q24，7p22，9p23，15q15，16p11.2 和 22q13 等可能因 caffeine 而減少斷裂的情形 (如表三 # 號所示)。

由表二亦知，在所觀察的 1000 個 daunomycin 組細胞中總共有 193 個染色體帶 (chromosome bands) 至少出現一次斷裂。在這些 daunomycin 誘發的斷裂位置當中，1p32 之出現最爲頻繁，出現率爲 2.2% (在所記錄的 2773 個 染色體斷裂數中，占 60 個)。其他斷裂位置出現率依次爲 3p14(2.0%)，3p24(1.5%)，11q14(1.5%)，2p13 (1.4%)，6q23 (1.4%)，14q24 (1.4%)，9q22 (1.4%)，1p21 (1.3%)，2q32 (1.3%)，7q31 (1.3%)，3q13(1.2%)，7q21 (1.2%)，4q21 (1.2%)，6q21 (1.2%)，12q24 (1.2%)，8q22 (1.2%)，5p14 (1.1%)，5q31 (1.1%) 及 12p12 (1.1%)(見表三)。以上所述的這些位置的斷裂，僅占了 10 個人之淋巴球經 daunomycin 處理所產生 2773 個 總斷裂數之 27.0% ，亦即，雖然 dauonmycin 可以強烈地誘發染色體的斷裂，但是其斷裂點卻非僅僅集中染色體的少數幾個位置上。

除了引發產生許多染色體的斷裂之外，daunomycin 亦誘發了許多不同型式的染色體畸變 (chromosomal aberrations) 。這些染色體變異包括同源染色體的互換 (homologous chromosomal interchanges)(圖 二A)，同源染色體的轉位 (homologous chromosomal translocations)(圖 二B)，非同源染色體的互換 (non-homologous chromosomal interchanges)(圖 二C,二D)，非同源染色體的轉位 (non-homologous chromosomal translocations)

(圖 二F)，多對染色體的交換 (圖 二E)，染色體內交換 (intrachromosomal exchanges)(圖 二G)，和環形染色體 (ring chromosomes)(圖 二H)。

這些同源染色體互換的圖形顯示同源染色體在有絲分裂 (mitosis) 過程的配對 (pairing) 及重組 (recombination) 的情形。而非同源染色體間的互換圖形，也顯現了有絲分裂時產生轉位或其他染色體變異的過程。在一條染色體內姊妹染色分體的不均衡交換圖形 (unequal intrachromosomal exchange configurations)(圖 二G) 則可解釋爲產生染色體缺失 (deletion) 的一個中間過程。

另外，daunomycin 組的中期細胞出現約 1.2% 的環形染色體(12 ring chromosome/1000 metaphases)，而在 12 個觀察到的環形染色體中，又以第二對染色體出現頻率最高 (約占 42%)。圖 三H 展示環形染色體的產生機制：首先，於一條染色體的不同位置同時產生 2 個斷裂，之後這一段不完整染色體再頭尾接合而形成環狀。

值得一提的是，不論染色體究竟發生了上述何種變異，基本上都是起因於染色體一個或一個以上的位置之斷裂，而在斷裂過程中可能損害了座落於這些位置上的某種基因，妨礙基因正常表現。

表 四 所列乃是經過分析 daunomycin 所誘發的畸變染色體，其斷裂位置的分佈情形。在這些異常染色體的斷裂點中，以 1p32 及 12q24 出現最多，約有 1.2% 的出現率 (12次/1000 細胞)。接著出現率較高者爲 3p14 (1.1%)，5q31 (0.8%)，而 1q31、3q27、5q13、6p23、8q22 與 9q22 等均有 0.7% 的出現率，再者，1p21 和 10p14 出現率則爲 0.6% 。以上所述這些斷裂占 daunomycin 所誘發染色體變異總斷點的 24% 。

染色體斷裂點在 1p32 常見於神經母細胞瘤 (neuroblastoma) 與急性淋巴球白血病 (Acute lymphocytic leukemia)(Mitelman, 1990)。而 3p14 則爲人類染色體上最常見的易脆位置 (Rao 等人,1988; Smeet 等人,1986)，它經常出現在肺小細胞癌 (small cell lung cancer) 及家族性腎細胞癌 (familial renal carcinoma) 之異常染色體。而脊髓發育不良症候群 (myelodysplastic syndrome，非霍奇金式淋巴瘤 (non-Hodgkin's lymphoma) 及慢性淋巴白血病 (chronic lymphocytic leukemia) 等癌細胞之異常染色體也常在 11q14 的位

置出現斷裂 (Mitelman,1990)。其它對 daunomycin 敏感的位置與一些癌細胞染色體斷裂點以及一些其它致突變劑之易脆位置相符者列於表三。

表四 所列乃是一些 daunomycin 誘發的斷裂位置與其他藥物的易脆位置、某些癌細胞異常染色體的斷點、以及一些致癌基因位置 (locations of oncogenes) 的相同處。由表四亦可發現，大部份 daunomycin 所誘發的斷裂點也出現在他種藥物誘發的易脆位置，此外，有許多斷點也與已知的一些癌細胞的斷點相符合。此種相似性是否意味著這些染色體上的易脆位置與致突變作用 (mutagenesis) 或致癌作用 (carcinogenesis) 有關？也許這些染色體上的位置可能是提供了致癌過程中基因重組之處 ，以及某些致突變劑 (mutagens) 的作用的共通靶的 (common targets)。

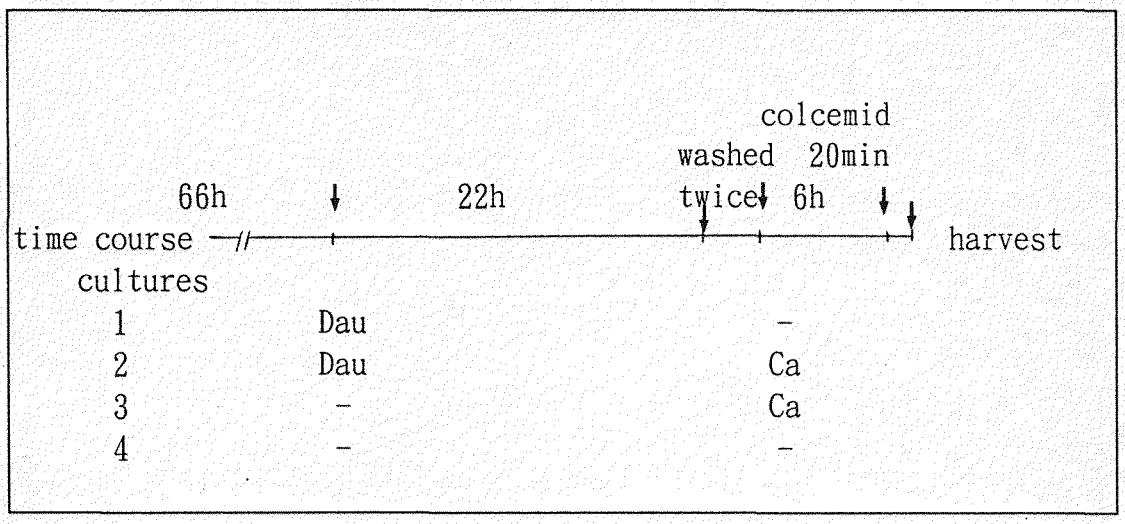
綜合本實驗的結果是：daunomycin 不僅可以強烈地誘發人類淋巴球染色體斷裂及染色體畸變，而其誘發的斷裂位置與某些藥物的易脆位置以及某些癌細胞的染色體斷點頗爲相似。

參考文獻：

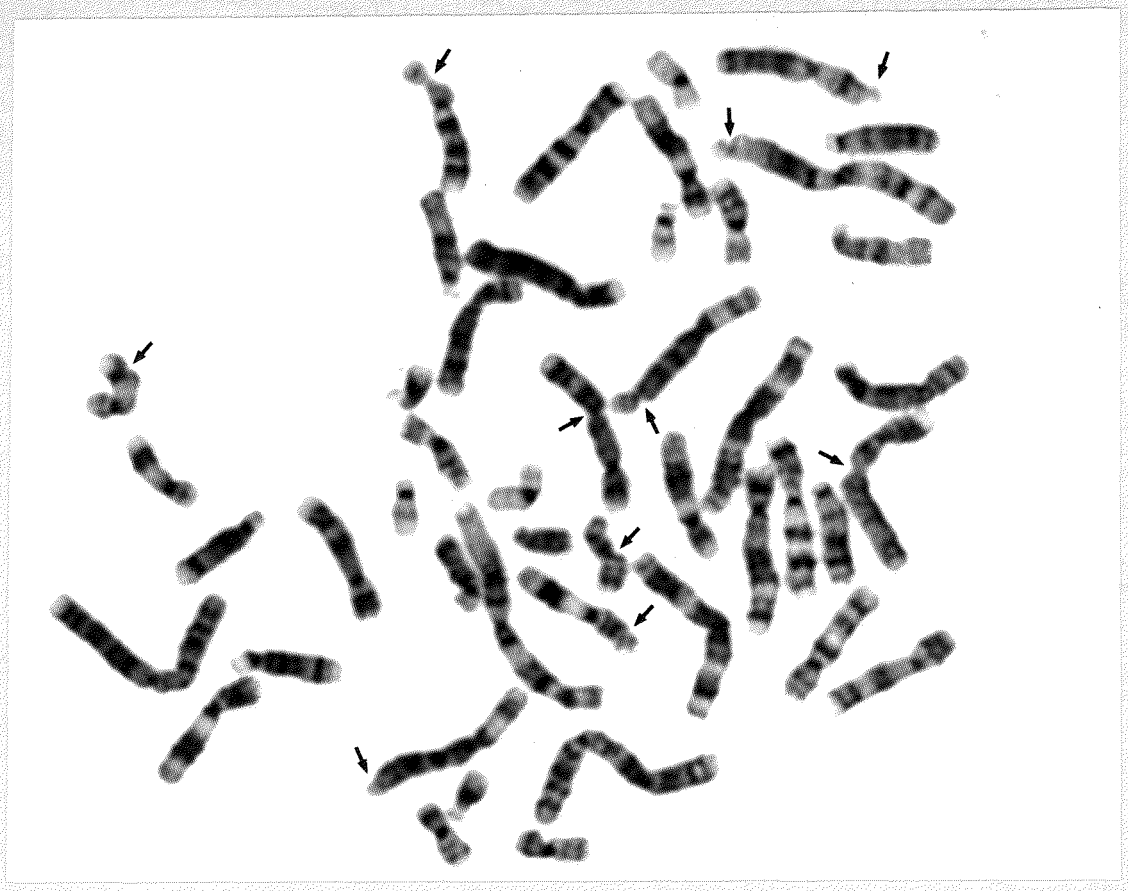
1. Barbi G, Steinbach P, Vogel W : Non-random distribution of methotrexate-induced aberrations on human chromosomes. Detection of further folic acid sensitive fragile sites. Hum Genet 1984; 68: 290-294.
2. Berger R, Bioomfield CD, Sutherland GR: Report of the committee on chromosome rearrangements in neoplasia and on fragile sites. Human Gene Mapping 8, Helsinki Conference. Cytogenet cell Genet 1985; 40:490-535.
3. Craig-Holmes AP, Strong LC, Goodacre A, Pathak S: Variations in the expression of aphidicolin-induced fragile sites in human lymphocyte cultures. Hum Genet 1987; 76: 134-137.
4. Cohen AJ, Li FP, Berg S, Marchetto DJ, Tsai S, Jacobs SC, Brown RS: Hereditary renal-cell carcinoma associated with a chromosomal translocation. N Engl J Med 1979; 301: 592-595.
5. Croci G: BrdU-sensitive fragile sites on long arm of chromosome 16. Am J Hum Genet 1983; 35: 530-533.
6. Daniel A, Ekblom L, Philip S: Constitutive fragile sites 1p31, 3p14, 6q26 and 16q23 and their use as controls for false-negative results with fragile (X). Am J Med Genet 1984; 18: 483-491.
7. Das SK, Lau CC, Pardee AB : Comparative analysis of caffeine and 3-aminobenzamide as DNA repair inhibitors in Syrian baby hamster kidney cells. Mutat Res 1984; 131: 71-79.
8. de Braekeleer M, Smith B, Lin CC: Fragile sites and structural rearrangements in cancer. Hum Genet 1985; 69: 112-116.
9. Glover TW : FudR induction of the X chromosome fragile site: Evidence for the mechanism of folic acid and thymidine inhibition. Am J Hum Genet 1981; 33 : 234-242.
10. Glover TW, Berger C, Coyle J, Echo B: DNA polymerase alpha inhibition by aphidicolin induces gaps and breaks at common fragile sites in human chromosomes. Hum Genet 1984; 67: 136-142.
11. Hecht F, Glover Tw: Cancer chromosome breakpoints and common fragile sites induced by aphidicolin. Cancer Genet Cytogenet 1984; 12: 185-189.

12. Hecht F, Sutherland GR: Detection of the fragile X chromosome and other fragile sites. Clin Genet 1984; 26:301-303.
13. Hecht F, Sutherland GR: Fragile sites and cancer breakpoints. Cancer Genet Cytogenet 1984; 12:179-181.
14. Hecht F, Sandberg AA: Of fragile sites and cancer chromosome breakpoints. Cancer Genet Cytogenet 1988; 31: 1-3.
15. ISCN: An international system for human cytogenetic nomenclature. Birth Defects Orig. Art Ser 21: 46-47, 1985.
16. Jacky PB, Sutherland GR: Thymidylate synthetase inhibition and fragile site expression in lymphocytes. Am J Hum Genet 1983;35: 1276-1283.
17. Kihlman BA, Hansson K, Andersson HC : The effect of post-treatments with caffeine during S and G2 on the frequencies of chromosomal aberrations induced by thiotepa in root tips of Vicia faba and in human lymphocytes in vitro. Mutat Res 1982; 104: 323-330.
18. Le Beau MM: Editorial: chromosomal fragile sites and cancer-specific breakpoints - a moderating viewpoint. Cancer Genet Cytogenet 1988; 31: 55-61.
19. Mark J, Dahlenfors R, Ekedahl C: Cytogenetics of the human benign mixed salivary gland tumor. Hereditas 1983; 99: 115-129.
20. Mitelman F: Catalog of chromosome aberrations in cancer,4th Ed: A John Wiley and Sons Inc, New York,1991.
21. Rao PN, Heerema NA, Palmer CG: Fragile sites induced by FudR, caffeine, and aphidicolin: Their frequency, distribution, and analysis. Hum Genet 1988; 78: 21-28.
22. Rosner B: Fundamentals of biostatistics, Boston. PWS -Kent publishing company , pp 474-526,1990.
23. Schmid M, Klett C, Niederhofer A: Demonstration of a heritable fragile site in human chromosome 16 with distamycin A. Cytogenet Cell Genet 1980; 28: 87-94.
24. Smeets DCFM, Scheres JMJC, Hustinx TWJ: The most common fragile site in man is 3p14. Hum Genet 1986; 72: 215-220.
25. Sutherland GR: Heritable fragile sites on human chromosome VIII. Preliminary population cytogenetic data on the folic-acid-sensitive fragile sites. Am J Hum Genet 1982; 34: 452-458.

26. Sutherland GR: Heritable fragile sites on human chromosomes. IX. population cytogenetics and segregation analysis of the BrdU requiring fragile sites at 10q25. Am J Hum Genet 1982; 34: 753-756.
27. Sutherland GR, Parslow MI, Baker E: New classes of common fragile sites induced by 5-azacytidine and BrdU. Hum Genet 1985; 69: 233-237.
28. Takahashi E, Kaneko Y, Ishihara T,Minamihiisamatsu M, Murata M, Hori T: A new rare distamycin A-inducible fragile site, fra(11)(p15.1), found in two acute nonlymphocytic leukemia (ANLL) patients with t(7;11)(p13;p15). Hum Genet 1988; 80: 124-126.
29. Trent J, Casper J, Merltzer P, Thompson F, Fogh J: Nonrandom chromosome alterations in rhabdomyosarcoma. Cancer Genet Cytogenet 1985; 16: 189-197.
30. Voiculescu I, HausmannC, Wolff G, Back E: A BrdU-requiring fragile site on chromosome 12. Hum Genet 1988;78:183-185.
31. Wang N, Perkins KL: Involvement of band 3p14 in t(3;8) hereditary renal carcinoma. Cancer Genet Cytogenet 1984; 11: 478-481.
32. Whang-Peng J, Kao-shan CS, Lee EC: Specific chromosome defect associated with human small-cell lung cancer: deletion 3p(14-23). Science 1982; 215: 181-182.
33. Wingard LB, Brody TM, Larner J Schwartz A: Individual antineoplastic drugs. In Human Pharmacology- molecular to clinical. London: Wolfe Publishing Limited, pp598-600, 1991.
34. Yunis JJ: The chromosomal basis of human neoplasia. Science 1983; 221: 227-236.
35. Yunis JJ, Soreng AL: Constitutive fragile sites and cancer. Science 1984; 226: 1190-1204.
36. Yunis JJ, Soreng AL, Bowe AE: Fragile sites are targets of diverse mutagens and carcinogens. Oncogene 1987; 1: 59-69.
37. Zhou X, Xu B, Chu C, Xia G, Li N, Sha R: Human chromosome hot points. 1. Hotpoint at 3p14 in three populations. Hum Genet 1984; 67: 249-251.



表一：各種藥品時間處理表。



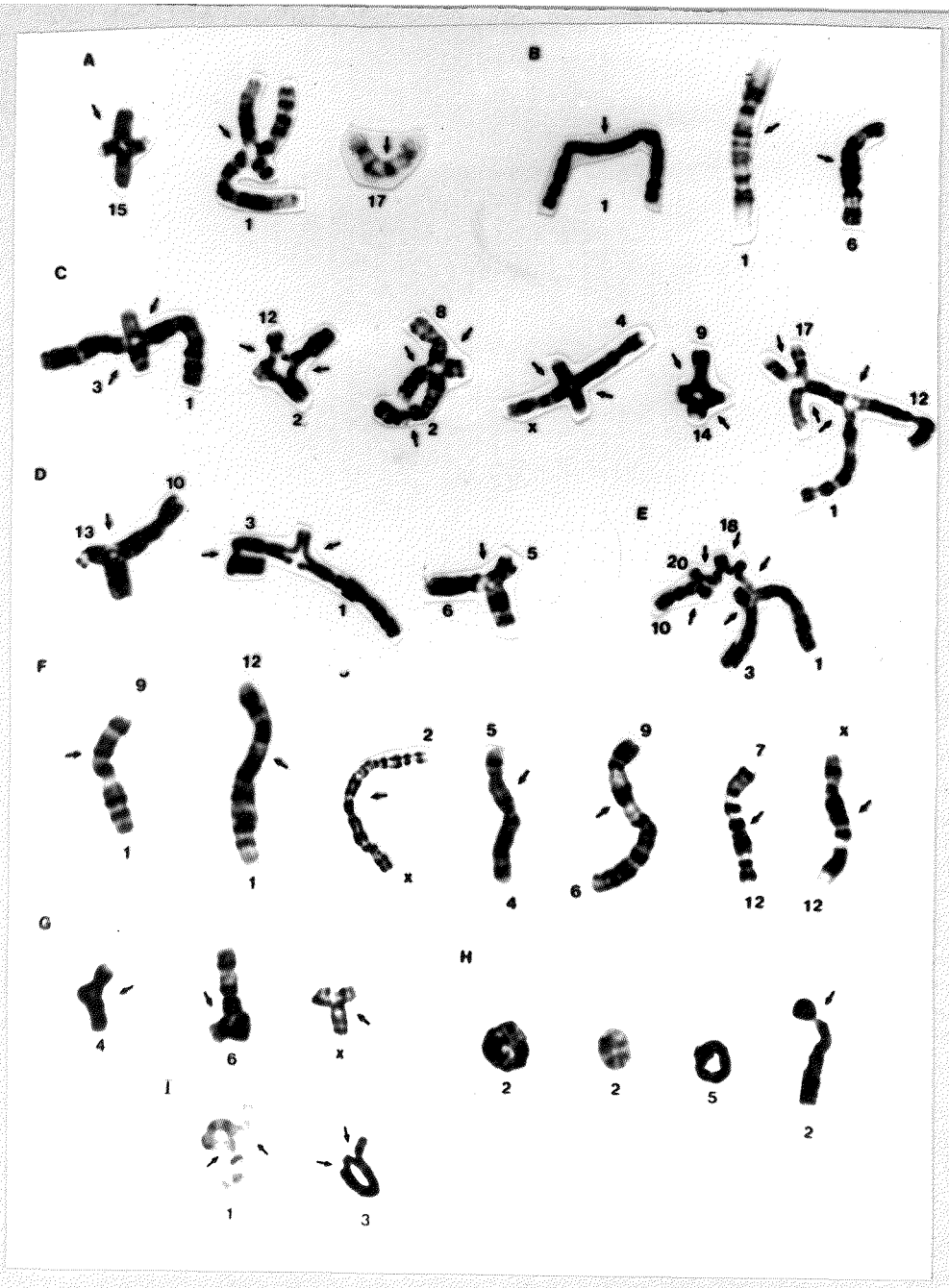
圖一 A. 顯微鏡下所見由 daunomycin 誘發的染色體斷裂情形 (如箭號所示)。

圖一B. Daunomycin 所誘發的染色體斷裂及異常情形 (如箭號所示)。

表二　由 daunomycin，daunomycin 和 caffeine，及 caffeine 所誘發的染色體斷裂頻率之比較。

Culture	treatmant	no.of bands showing breakage	mean breaks/ individual(± SE)	mean breaks/ cell
1	Daunomycin	193	277.3 ± 30.5	2.77
2	daunomycin +caffeine	201	404.4 ± 33.9	4.04
3	caffeine	85	12.8 ± 1.7	0.13
4	untreated	9	1.2 ± 0.4	0.01

a. Daunomycin 的最終濃度爲 0.1 uM。
b.Caffeine 的最終濃度爲 2.5 mM。
c. S E = 標準誤 。
d. $p < 0.025$。



圖二 Daunomycin 自培養之正常人淋巴球所誘發出來的染色體畸變及重組的圖形

A.展示同源染色體之互相交換 (homologous interchanges)，分別記錄爲: cte(15;15)(q15;q15),cte(1;1)(p13;q32) 及 cte(17;17)(q23;q25)。

B.展示雙中心同源染色體之轉位 (dicentric homologous translocations)，分別記錄: dic(1)p32p32), dic(1)(q23q31), dic(6)(q15q21)。

C.四向非同源染色體的互相交換 (quadriradial non-homologous interchange)圖形，分別記錄爲: cte(1;3)(p34;p23),cte(2;12)(q21;q21),cte(2;8)(q31;q22) 和一個2p13 的斷點,cte(4;X)(q31;q22),cte(9;14)(q22;q23), 及一個雙重交換圖形爲 cte(1;12)(p13;q24) 和 cte(1;17)(p32;q21)。

D.三向非同源染色體互相交換（triradial non-homologous interchanges）圖形，記錄爲: cte(10;13)(q26;q14), cte(1;3)(p34;p25) 和一個 3q12 的斷點，及 cte(5;6)(q13;p23)。

E.由 1，3，10，18，20 等不同對染色體所形成的五中心複合體(pentacentric complex)。

F.乃爲非同源染色體轉位（non-homologous translocations）的圖形，記錄爲: t(1;9)(p22;q12),t(1;12)(p34;q22), t(2;X)(q33;q26),t(4;55)(p16;q13),t(6;9)(p23;q22), t(7;12)(q32;q24)，及 t(12;X)(p12;q22)。

G.展示染色體內交換（intrachromosomal exchanges）的圖形，記錄爲: ct del(4)(q21q26),ct del(6)(q155;q23)，及 ct del(X)(p11q13)。

H.乃爲環形染色體（ring chromosomes），分別爲: r(2),r(2), r(5)，及一個斷點在 2p13 的 r(2)。

I.分別展示二個環形染色體的形成過程: 一是同時在 1p34 和 1q32 的位置發生斷裂，爾後二個斷點再癒合；另一則是同時在 3p23 和 3q27 斷裂後又接合，而環產生環形的構造。

表三　對 daunomycin 和 caffeine 敏感的染色體分佈位置，其與某些癌細胞染色體斷點和一些定序的致癌基因位置，及與某些致突變劑誘發表現的染色體易脆位置之關係。

band	Dau	Dau + Ca	Ca	cancer chromosomes with known breakpoints [20,35,36]	oncogene location	mutagen-fragile site
1p21	**	***				FdU; MTX [36]
1p22	*	*		neuroblastoma; breast adenocarcinoma [20,34,36]		Apc [21,36]
1p31	*	*		disseminated-neuroblastoma [20]		
1p32	***	*****		neuroblastoma;ALL [2,20]	L-myc	Apc [3,21,36]
1p34.3	*	**				
1q21	*	*				Apc; Aza; Bus [36]
1q24	*	*				
1q31	*	**				
1q41	*	*				
2p13	**	***		NHL;ALL;B-CLL [2,3,20]		
2p16	*	**				Apc [36]
2p24	*	**		leiomyoma [20,36]	N-myc	FdU; MTX [36]
2q12	*	*				
2q22	*	*				
2q31	*	*				Apc [3,10,11]
2q32	**	**				
2q33	*	**		ANLL [2,20]		
3p12	*	*		malignant fibrous histiocytoma [20]		
3p14	***	****	**	SCLC;ALL;breast adenocarcinoma; familial renal carcinoma;mixed salivary gland tumor; rhabdomyosarcoma [4,13,19,20,29,31,32,34,35]		Apc;Aza;Ca; FdU;MTX [21,27,36,37]
3p21	*	*				
3p24	**	***				Apc [21,36]
3q13.2	**	***				
3q22	*	*		Lennert's lymphoma;CML [2,20]		
3q24	*	*				
3q26.2	*	**		MPS;ANLL [20,36]		
3q27	*	**		NHL [20,36]		
4p15.2	**	***				Apc [36]
4q21	**	**		bilineal ALL/ANLL [34,36]		
4q24	*	#				
4q26	*	**				
4q27	*	**			raf 2	
4q31.3	*	**				
4q33	*	*				
5p14	**	**				Apc [36]
5q12	*	*				
5q13	*	*		refractory anemia; MDS; ALL; ANLL [2,20,36]		
5q15	*	**		ANLL-M3; MDS [20]		
5q21	*	**				
5q31	**	**		ANLL; MDS [20,34,36]		

5q32	*	*				
5q34	*	*		ANLL-M2 [20]	c-fms	
6p23	*	**		ANLL [20]		
6q13	*	*				
6q15	*	**		ovary adenocarcinoma; B-PLL; ALL [20,34,36]		
6q21	**	**		OPA; ALL; PLL; ANLL-M5b [20,36]	ros; slk	
6q22	**	**			c-ras; myb	
6q25	*	**				
7p14	*	**				Apc; FdU [36]
7p22	*	#				
7q11.2	*	*				
7q21	**	**		leiomyoma; ANLL [2,36]		Apc [3,36]
7q22	*	*				
7q31	**	**		ANLL; MDS [20,36]	met; kit	MTX [1,36]
7q32	*	*	*	leiomyoma [20]		Apc; Ca; FdU [21,36]
8p11.2	*	*				
8p22	*	*				
8q21	*	**		MPT [34,36]		
8q22	**	**		ANLL-M2 [20]	c-mos	Apc; FdU; MTX [21,36]
8q23	*	*				
9p12	*	*				
9p21	*	**				
9p23	*	#				
9q12	*	*				Aza [27]
9q22	**	**		ANLL [20]		Apc [36]
10p13	*	**		ANLL [34,36]		
10p14	*	*		ANLL-M4,M5 [20]		
10q21	*	*				
10q23	*	**				
10q25	*	*				BrdU [26]
11p14	*	**		Wilms' tumor [20,34,36]		Apc [21]
11q13	*	*		NHL; CML; PLL; B-CLL; RAEB [20,36]	bcl 1; int 2	Bus; MTX [36]
11q14	**	****	*	MDS; NHL; CLL [20,35,36]		FdU; MTX [36]
11q23	*	*		CML; CLL; ALL; ANLL-M4,M5; MDS; Ewing's sarcoma; neuroepithelioma [20,36]	ets 1	Apc [11,21]
12p12	**	**		B-ALL; CML; ANLL-M5a [20]	k-ras 2	
12q13	*	**		T-cell lymphoma; ALL; salivary gland adenocarcinoma; myxoid liposarcoma [34,35,36]		
12q21	*	**				Bus; FdU; MTX [21,36]
12q24.1	**	***		CML [20]		Apc; BrdU [21,30]
13q13	*	**				Apc [3]
13q21	*	***				
13q22	**	**		NHL; B-CLL; astrocytoma-III,IV [2,20]		
14q21	*	**		ANLL [20]		
14q23	*	*				Apc [3,36]
14q24	**	**		OPA [20,34]		FdU; MTX [1,36]

15q15	*	#				
15q21	*	**				
15q22	*	*		ANLL-M3 [20]		Apc [36]
15q23	*	*				
15q25	*	*				
16p11.2	*	#				Aza; Bus [27,36]
16p13	*	**		AMLEO; ANLL-M4,M5 [20,36]		
16q22	*	*	*	ANLL-M2 [20]		BrdU;Ca;Dist A [21,23,27]
17p12	*	*				Dist A [36]
17q21	*	**		ANLL-M3 [34,36]	erb A1	
17q23	*	**		CML; B-CLL [20]		Apc [21,36]
18q21	*	*				
20q12	*	*				
22q12	*	*	*			
22q13	*	#				
Xp22.3	*	**	*			Apc; Ca; FdU [21,36]
Xq21	*	*				
Xq22	*	*	*			Apc [21,36]

a. 本表所列乃是該位置之斷裂出現超過 10 次或 10 次以上者。
* =10-29 個斷裂， ** =30-49 個斷裂， *** =50-69 個斷裂，
**** =70-89 個斷裂， ***** = 超過 90 個斷裂數者，
# = Caffeine 處理後使 daunomycin 誘發之斷裂數減少者。

b. 縮寫 ：
ALL=acute lymphocytic leukemia; AMLEO=acute myelomonocytic leukemia with eosinophilia; ANLL=acute non-lymphocytic leukemia;B-CLL=B-cell chronic lymphocytic leukemia; B-PLL=B-cell prolymphocytic leukemia; CML=chronic myelomonocytic leukemia; MDS=myelodysplastic syndrome; MPT=mixed parotid gland tumor; MPS=myeloproliferative syndrome; NHL=non-Hodgkin's lymphoma; OPA=ovarian papillary adenocarcinoma; RAEB=refractory anemia with excess of blasts; SCLC=small cell lung cancer.

c. 藥品縮寫：
Apc=aphidicolin; Aza=5-azacytidine; BrdU=bromodeoxyuridine; Bus=busulfan; Ca=caffeine; Dist A=distamycin A; FdU=fluorodeoxyuridine; MTX=methotrexate.

表四　Daunomycin 所誘發之異常染色體 (包括 interchanges 和 translocations)，其斷裂點之出現頻率與分佈情形。

	Dau		
breakpoint	chromatid exchange	translocation	total break
1p13	1q32, 12q24		2
1p21	1p32, 10p14, 15q23	4q26, 5p14	6
1p22		9q12	1
1p31		6q25	1
1p32	1p21, 1p32, 2p13, 3q24, 5p14, 10q25, 12q21, 17q21	1q42, 9p21, 11p14, 13q34	12
1p34	3p23, 3p25 8q22, 10q21	12q22	5
1q21	11q23, 15q22, 16p13, 20q12		4
1q23	1q31	4q31, 6q13, 7q32	4
1q24	14q32		1
1q25	2p24, 3p24, 4q22, 8q21, 12p12		5
1q31	1q23, 3p14, 6q25, 12q24, 12q24, 13q13 15q24		7
1q32	1p13	1q42	2
1q41	4q26, 7p13		2
1q42	11p15, 12q24	1p32, 1q32	4
2p11	19p12		1
2p13	1p32, 15q25		2
2p16	3p14, 8q22, 8q24, 13q21, 17q21		5
2p22	15q22		1
2p23	17p12		1
2p24	1q25, 17q23, Xq22		3
2q13	6q23		1
2q21	4q21, 12q21		2
2q31	5q21, 8q22, 15q21		3
2q32	3p14		1
2q33	6p23, 8q21	Xq26	3
2q35	Xq22		1
2q37		7q22	1
3p12		10q25, Xp11	2
3p13	12p12	5q31, 16p12	3
3p14	1q31, 2p16, 2q32, 5q21, 5q23, 8q22, 12q24, Xq25	4q31, 9p21, 14q31	11
3p23	1p34, 12q24	6p23	3

	Dau		
breakpoint	chromatid exchange	translocation	total break
3p24	1q25, 4q21		2
3p25	1p34		1
3q13	16q23, 17p12		2
3q21	9q34, 18q22		2
3q24	1p32		1
3q25	8p21		1
3q27	8q21, 15q23, 15q23, 16p11, Xq25	12q24, 15q25	7
4p12	11q13, 16p12		2
4p15	9p21	9q12, 10p14, 11q23	4
4p16	5q13		1
4q12	7p15		1
4q21	2q21, 3p24, Xp11		3
4q22	1q25		1
4q24	13q31		1
4q26	1q41	1p21, 12q24	3
4q27	9q22	Xp21	2
4q31	Xq22	1q23, 3p14, 5q31	4
4q35		8q13	1
5p14	1p32, 14q24	8p22, 1p21	4
5p15	Xq26		1
5q11	7q22		1
5q13	4p16, 6p23, 10q22, 13q13, 22q12	1p21, 6q26	7
5q15	8q22	q	1
5q21	2q31, 3p14, 15q21		3
5q23	3p14		1
5q31	13q13, 13q21, 15q22, 15q25, 16q12	3p13, 4q31, 11q13	8
6p23	2q33, 5q13, 9q22, 14q24, 17q21	3p23, 15q15	7
6q13		1q23	1
6q15		6q21	1
6q21	9q22	6q15, 10q25	3
6q23	2q13, 15q22		2
6q25	1q31	1p31	2
6q26		5q13	1
7p13	1q41, 9p24		2
7p14	12q24		1
7p15	4q12		1
7p22		15q26	1
7q22	5q11, 9q22, 16q22	2q37	4

	Dau		
breakpoint	chromatid exchange	translocation	total break
7q31	11q23		1
7q32	12q24	1q23, 14q32 15q22	4
8p21	3q25		1
8p22	8p22	5p14, 10q26	3
8q11	15q15		1
8q13	15q22	4q35	2
8q21	1q25, 2q33, 3q27		3
8q22	1p34, 2p16, 2q31, 3p14, 5q15, 13q31 18q12		7
8q24	2p16	19q13	2
9p21	4p15, 17q23	1p32, 3p14	4
9p24	7p13		1
9q12		1p22, 4p15	2
9q21	10q23, 13q33, 16p12, 19p12		4
9q22	4q27, 6p23, 6q21, 7q22, 14q23, 14q31	9q31	7
9q31	10q25	9q22	2
9q32	10q23, 19p13	14q24	3
9q34	3q21		1
10p14	1p21, 10q26, 11p14, 11q23, 16q22	4p15	6
10q21	1p34, 15q24, 15q24		3
10q22	5q13, Xq25		2
10q23	9q21, 9q32, 13q33		3
10q24	20q12		1
10q25	1p32, 9q31	3p12, 6q21,	4
10q26	10p14, 13q14	8p22	3
11p14	10p14	1p32, Xp22	3
11p15	1q42	15q21	2
11q13	4p12, Xq15	5q31	3
11q23	1q21, 7q31, 10p14	4p15	4
12p12	1q25, 3p13	Xq22	3
12p13	16q23		1
12q13	Xq22		1
12q14	20q12		1
12q21	1p32, 2q21		2
12q22		1p34	1

	Dau		
breakpoint	chromatid exchange	translocation	total break
12q24	1p13, 1q31, 1q31, 1q42, 3p14, 3p23, 7p14,7q32 4q23	3q27, 4q26, 19p13	12
13q13	1q31,5q13, 5q31,14q24		4
13q14	10q26		1
13q21	2p16, 5q31		2
13q31	4q24,8q22 22q12		3
13q33	9q21, 10q23		2
13q34		1p32	1
14q13	Xq21		1
14q21	21q22		1
14q23	9q22, 12q24		2
14q24	5p14, 6p23, 9q32, 13q13		4
14q31	9q22	3p14	2
14q32	1q24	7q32	2
15q15	8q11, 15q15, 17q22, 19p12	6p23	5
15q21	2q31, 5q21	11p15,Xp22	4
15q22	1q21, 2p22, 5q31,8q13	7q32	5
15q23	1p21, 3q27, 3q27		3
15q24	1q31, 10q21, 1q21		3
15q25	2p13, 3q27, 5q31		3
15q26		7p22	1
16p11	3q27		1
16p12	3p13, 4p12, 9q21		3
16p13	1q21		1
16q12	5q31		1
16q22	7q22, 10p14		2
16q23	3q13, 12p13		2
17p12	2p23, 3q13	19q13	3
17q12	17q21, 19q13		2
17q21	1p32, 2p16, 6p23, 17q12		4
17q22	15q15		1
17q23	2p24, 9p21	17q25	3
17q25		17q23	1
18q12	8q22		1
18q22	3q21, 18q22		2
19p12	2p11, 9q21, 15q15		3
19p13	9q32	12q24	2

	Dau		
breakpoint	chromatid exchange	translocation	total breaks
19q13	17q12	8q24,17p12	3
20q12	1q21, 10q24, 12q14, 20q12, 20q12		5
21q22	14q21		1
22q12	5q13, 13q31		2
Xp11	4q21	3p12	2
Xp21		4q27	1
Xp22		11p14, 15q21	2
Xq15	11q13		1
Xq21	14q13		1
Xq22	2p24, 2q35, 4q31, 12q13	12p12	5
Xq25	3p14, 3q27, 10q22		3
Xq26	5p15	2q33	2

附錄 ：

統計分析: 10 個正常人之不同處理的淋巴球，其染色體之斷裂數目。

treatment	daunomycin	daunomycin + caffeine	caffeine	untreated
subject				
1	186	294	7	2
2	348	597	18	0
3	142	303	14	0
4	197	309	9	4
5	316	546	13	1
6	254	359	20	2
7	185	331	8	1
8	342	388	17	1
9	395	433	4	0
10	408	484	18	1

利用 ANOVA (analysis of variance) 統計方法分析不同條件處理之染色體斷裂率如下 ：

	sum of square	degree of freedom	mean of square	F
between	1196003.1	3	398667.7	76.5
within	187645.7	36	5212.4	
total	1383648.8	39	35478.2	

查表得知 $F_{0.025}(3,36) = 3.59$。 故而 $p < 0.025$ 亦即不同處理所誘發的染色體斷裂數有顯著差異。

附表：10 位正常人之淋巴球經不同處理後其染色體的斷裂頻率及分佈。

	Unt	Dau	Dau Ca	Ca
Fra site				
1p12		3	2	
1p13		3	8	
1p21		36	61	1
1p22		12	16	
1p31		15	22	
1p32		60	101	3
1p34.3		24	32	
1p36.1		3	12	
1q12		3	7	
1q21		28	28	
1q22		3	26	
1q24		21	26	
1q25		6	6	
1q31		23	46	1
1q32		8	12	
1q41		27	20	1
1q42		19	5	1
2p11.2		3		
2p12	1			
2p13		39	59	
2p14		4		
2p16		23	42	1
2p21		12	3	
2p22			17	
2p23				
2p24		21	31	
2q11.2		3	2	
2q12	1	11	21	
2q14		3	6	
2q21		4	16	
2q22		19	17	1
2q24				
2q31	2	11	25	
2q32		36	36	4
2q33		21	38	1
2q34				
2q35		9	14	
2q37		5	7	
3p12	3	24	12	
3p13		3	40	
3p14		56	76	36
3p21		20	25	
3p22			3	
3p23				
3p24		41	51	1
3p26		1		
3q11.2		2	2	

	Unt	Dau	Dau Ca	Ca
Fra site				
3q13.2		34	51	
3q21		4		
3q22		16	29	1
3q23				
3q24		13	12	
3q25		8	20	
3q26.2		22	33	
3q27		19	45	
4p12			1	
4p13		2		
4p15.1		6	16	
4p15.2		32	51	3
4p16		6		
4q12		2	3	
4q13				
4q21		33	46	
4q22		8	26	1
4q23				
4q24		13		1
4q26		13	39	
4q27		25	35	2
4q31.3		28	30	
4q32		3	2	1
4q33		13	20	3
5p12				
5p13		1	4	
5p14		31	46	3
5p15		1		2
5q11.2		4		
5q12		14	21	
5q13		14	17	
5q15		26	34	
5q21		29	31	2
5q22				
5q23		3		
5q31		31	41	2
5q32		15	15	
5q33				
5q34		14	15	2
5q35		1		
6p12		7	11	
6p21.1		4	6	
6p22		22	46	
6p23		4		
6p24		1		
6p25				
6q12				
6q13		23	18	
6q15		23	36	2
6q16				
6q21		33	35	2

	Unt	Dau	Dau Ca	Ca
Fra site				
6q22		39	30	2
6q23			5	
6q24		1		
6q25		24	39	
6q26		8	17	2
6q27		4	4	5
7p11.2		8	8	
7p12				
7p13			11	
7p14	1	13	30	1
7p15		1	3	1
7p21		3	34	2
7p22		16		
7q11.2		13	21	
7q21		34	48	2
7q22		13	9	
7q31		36	46	1
7q32		29	24	1
7q33				
7q34		6	3	
7q35			11	
7q36				
8p11.2		11	22	
8p12				
8p21		5	7	
8p22		10	14	
8p23				
8q11		7	15	
8q12				
8q21		14	38	
8q22		32	37	
8q23		12	22	1
8q24.1		8	16	
9p12		12	11	
9p13			1	
9p21		20	30	
9p22				
9p23		16	6	1
9q12		14	12	
9q21		8	27	
9q22		38	46	
9q31		6	21	
9q32		9	13	
9q33	1	3	5	
9q34		8	11	
10p11.2			1	
10p12			7	
10p13		17	30	
10p14		25	31	
10p15		3		
10q11.2				

	Unt	Dau	Dau Ca	Ca
Fra site				
10q21	1	28	29	
10q22		3	7	
10q23		29	34	1
10q24		5	3	
10q25		20	20	
10q26		5	3	
11p11.2		4	3	
11p12		9	12	
11p13			8	
11p14		29	32	2
11p15		3	12	1
11q12		3	7	
11q13		15	28	
11q14		41	86	8
11q21		2	2	
11q22		3	6	
11q23		20	22	
11q24		3	6	
12p12		30	45	
12p13		3	10	
12q12			3	
12q13	1	21	37	
12q14		8	12	
12q15			5	
12q21		23	40	
12q22		7	3	
12q23			2	
12q24.1		33	55	
13q12		1		
13q13		28	38	3
13q14		2	5	
13q21		10	65	2
13q22		32	48	
13q31		3	6	
13q32		14	11	
13q33		7	9	
14q12		6	15	
14q21		23	47	
14q22		2	1	
14q23		20	22	
14q24		39	49	
14q31		3	11	
14q32		4	9	
15q12		1		
15q14		4		
15q15		10	7	
15q21		11	30	
15q22		25	24	
15q23		20	21	1
15q25		14	12	
15q26		5	2	

	Unt	Dau	Dau Ca	Ca
Fra site				
16p11.2		10	2	
16p12	1	3		
16p13.1		19	32	
16q12		5	8	
16q21		9	5	
16q22		17	19	
16q23		7	18	2
17p12		24	25	
17q12		7	7	
17q21		23	36	
17q22				
17q23		27	41	
17q24		2	2	
18p11				
18q12		6	18	
18q21		24	25	2
18q22		4	12	
19p12		3	1	
19p13.3		3	4	
19q13.1		5	5	
20p11.2				
20p12		8	22	
20q11.2			3	
20q12		17	29	
20q13.1		7	6	
21q21		6	11	
21q22		3	4	
22q12		13	21	1
22q13		10		
Xp11		6	5	
Xp21		4	5	1
Xp22.1		4	3	4
Xp22.3		15	30	
Xq13		2	5	
Xq21		12	19	1
Xq22		23	47	1
Xq23		1	3	
Xq25		9	23	
Xq26		6	1	1
Xq27		6	9	
total	12	2773	4044	128

註： Unt = untreated，
Dau = daunomycin，
Ca = caffeine。

第二篇
CCNU 誘發正常人類淋巴球染色體易脆位置頻率與分佈之探討

Frequency and Distribution of CCNU-induced Fragile sites in Cultured Human Lymphocytes

目錄

圖表

摘要 (Abstract)

本篇實驗主要在探討 CCNU (lomustine) 對正常人類淋巴球所誘發染色體之斷裂頻率與易脆位置的分佈。我們並以咖啡因 (caffeine) 爲此致突變作用之強化劑 (mutagenic enhancer)。

我們的結果顯示：在 CCNU 處理組的染色體斷裂數明顯地高於控制組 ($p< 0.005$)，並且 caffeine 的確強化了 CCNU 易脆位置的表現。

我們以出現率占該個人染色體斷裂總數的 4% 或 4% 以上者，爲 CCNU的易脆位置。基於此一標準，歸納出在人類的淋巴球染色體上出現 14 個位置對 CCNU 非常敏感。

此外，大部份 CCNU 所引發的斷裂位置也出現在其他致突變劑 (mutagenic agents) 的易脆位置處。再者，一些對 CCNU 敏感的位置與某些癌細胞異常染色體之斷裂點 (breakpoint) 相同。此一情形意味著：這些在染色體上的易脆位置，可能是在發生致突變作用 (mutagenesis) 及致癌作用 (carcinogenesis) 的重要部位，由於這些斷裂而產生染色體或基因的變異與重組。

第一章
緒論 (Introduction)

染色體上的易脆位置 (fragile sites) 乃是指當細胞在某些培養條件下容易發生斷裂 (break) 的脆弱位置。緊接在發生斷裂之後，便容易產生染色體的缺失(deletion)，轉位 (translocation)，倒轉 (inversion)，環形染色體 (ring chromosome)，以及其他染色體構造上的異常。

在低含量之 folic acid 或 thymidine 的培養條件下，可以誘發一些易脆位置的表現 (Barbi 等人,1984; Glover,1981; Sutherland 等人,1982)。其他可以誘發染色體發生大量斷裂的藥物包括 distamycin A (Hori 等人,1988; Schmid 等人,1984)，bromodeoxyuridine (Sutherland ,1982; Sutherland 等人,1985; Voiculescu 等人,1988)，methotreaxate (Barbi 等人,1984; Yunis 等人,1987)，fluorodeoxyuridine (Rao 等人,1988; Tommerup 等人,1981)，Aphidicolin (Craig-Homes 等人,1987; Glover 等人,1984; Hecht 和 Glover ,1984; Rao 等人,1988)，5-azacytidine (Sutherland 等人,1985)，以及 daunomycin (Liou 和 Li,1994)。此外，各種經由不同致突變機轉的藥物亦可誘發許多相同染色體位置的斷裂 (Daniel 等人,1984; Glover 等人,1984; Yunis 和 Soreng,1984; Yunis 等人,1987)。

CCNU [lomustine; 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea] 爲一種常被應用以治療淋巴腫瘤 (lymphoma) 及某些實質性腫瘤 (solid tumors)，特別是腦瘤 (brain tumor) 的抗癌藥物。它本身爲一種 化基劑，可以嵌入 DNA 內而導致癌細胞的死亡 (Friedman 和 Carter,1978; Walker, 1973; Wingard 等人,1991)。對人類的淋巴球而言，CCNU 曾被証實爲很強的姊妹染色分體交換 (sister chromatid exchange)的誘發劑(inducer)(Best 和Mckenzie ,1988)。

本篇研究的目的在探討 CCNU 對人類淋巴球所誘發的染色體斷裂情形爲何？以及其斷裂位置在染色體上的分佈又如何？我們以咖啡因 (caffeine) 作爲 CCNU 誘發染色體斷裂的強化劑 (enhancer) (Das 等人,1984; Kihlman 等人,1982; Yunis 等人,1987)，並且以出

現率占該個人染色體總斷裂數 4% 或 4% 以上的位置作爲篩選 CCNU 易脆位置的標準 (Rao 等人,1988)。

我們實驗的結果顯示：在人類的淋巴球染色體上有 14 個位置對 CCNU 非常敏感，而且其中幾個位置和其他致突變劑所誘發的染色體斷裂位置也相同。

第二章
材料與方法（Material and Methods)

2.1：實驗材料

抽取自願之正常人的末稍血液（總共8位男性6位女性，年齡 20-28 歲，其中以來自4位男性和2位女性之血液進行 CCNU 濃度測試，另外，來自其他4位男性4位女性之血液則進行誘發染色體易脆位置之用）

2.2 :培養液名稱及製備（見附錄一）

2.3 :藥品名稱及製備（見附錄二）

2.4 :儀器（見附錄三）

2.5 :實驗方法與步驟

2.5.1. CCNU 濃度之測試 ：

來自每個人的血液均有三種培養 (cultures)，在 RPMI-1640 培養液（含 15% FCS 和 1% PSN）中培養 62 小時後，分別以10^{-4}M，10^{-5}M ，10^{-6}M（最終濃度）的 CCNU 處理 24 小時，爾後再收穫細胞以及進行染色體的製作與染色，接著以顯微鏡觀察並記錄各種 CCNU 濃度處理之染色體的斷裂數，以決定下一步實驗所使用 CCNU 的濃度。

在此部份實驗的結果是以 10^{-5}M 之CCNU 的效果較佳（詳細的細胞培養，細胞收穫，染色體製備等步驟見後面敘述）。

2.5.2. CCNU 誘發染色體易脆位置的表現及以 caffeine 強化此致突變作用之實驗步驟 ：

2.5.2.A.細胞的培養及藥品之處理:
(cell culture and treatment of agents)

抽取 10 ml peripheral blood + 0.1 ml heparin
↓ 30 min
取含 buffy coat (lymphocytes) 之血液 0.5 ml
↓
種入含 9.2 c.c 之 RPMI-1640 培養液中
(RPMI-1640 含 15% FCS 及 1% PSN)
每個人的血液共有 4 種處理，分別編號爲
culture A,B,C,D
↓
加入0.2 ml PHA (phytohemagglutinin)
↓
置於培養箱 (incubator,37℃,60-98% humidity,5% CO2)
↓ 62 h
編號爲 A,B 之 cultures 分別加入 0.1 ml 的
CCNU (10 M 最終濃度)
↓ 24 h
所有 cultures 均以新鮮之 RPMI-1640 洗二次
↓
編號爲 A,B 之 cultures 加入 10 ml RPMI-1640
培養液 (含 15% FCS 及 1% PSN)
編號爲 B,C 之 cultures 則加入 9.9 ml 之
RPMI-1640培養液 (含15% FCS 及 1% PSN) 和
0.1 ml 之 caffeine (2.5mM 最終濃度)
↓ 6 h
收穫細胞

2.5.2.B.收穫細胞與染色體之製備：
(cell harvest and preparations of chromosomes)

在收穫前 20 min 將 0.1 ml colcemid (0.05ug/ml)
加入各 cultures
↓
將各組培養的細胞分別倒入離心管
↓ 離心 8 min, 1200 rpm
倒掉上清液

加入 10 ml 的 0.54% KCl 低張溶液
↓16 min
↓離心8 min
倒掉上清液
↓
緩緩加入 8 ml 固定液 (甲醇:冰醋酸=3:1)
↓30 min
↓離心 8 min
加入 8 ml 固定液
↓10 min
↓離心 8 min
倒掉上清液
↓
加入 8 ml 固定液
↓5 min
↓離心 8 min
倒掉上清液
↓
加入 0.5 ml 固定液，調勻懸浮液
↓
將懸浮液滴在載玻片上
↓
將載玻片置於烘片機上 (37°C)
↓24 h
以 Trypsin-Giemsa staining 方法進行染色
(使用 Wright's stain)

2.5.3. 染色體的觀察與分析:

在顯微鏡下採隨機方式觀察染色體分散良好之中期細胞 (metaphases)，而且每一種處理均記錄 100 個metaphases，而所觀察的染色體大約是 400-500 的 band level 。我們依照 ISCN (1985) 的標準來記錄染色體的斷裂點(breakpoint)。

2.5.4. 統計方法 (見附錄四):

本實驗以 ANOVA (analysis of variance) 方法分析這些不同處理條件下的染色體斷裂頻率有否差異 (Rosner,1990)。

第三章
結果 (Results)

CCNU 劑量之評估:

染色體之斷裂頻率與 CCNU 濃度相關，當以 10^{-4}M（最終濃度）之 CCNU 處理 24 小時時，大部份的細胞都死亡；然而當細胞以 10^{-6}M（最終濃度）之 CCNU 同樣處理 24 小時，則所引發的染色體斷裂數目非常低 (23.5± 5.9；每100 個中期細胞的染色體斷裂數 ± 標準差)。而當以 10^{-5}M（最終濃度）之 CCNU 處理 24 小時，此時之染色體斷裂數明顯地增加許多 (135.0± 47.8；每100個中期細胞之染色體斷裂數 ± 標準差)。因此，我們以最終濃度爲 10^{-5}M 的 CCNU 處理 24 小時爲進行本研究的條件。

CCNU 誘發染色體斷裂頻率及染色體斷裂位置之探討 :

圖一所示，乃是 CCNU 所誘發染色體斷裂的情形。而在本實驗中，CCNU 總共引發 135 個的染色帶 (chromosome bands) 至少出現一次斷裂（見表一）。由表一可知，CCNU 組之平均染色體斷裂數爲 94.4±19.6 個(在每 100 個分裂中期細胞之染色體斷裂數±標準差)。

我們以出現率占該個人總斷裂數 4% 或 4% 以上的位置作爲遴選易脆位置的標準（Rao 等人,1988)。基於該標準，總共有 14 個位置符合此條件（見表二)。在此 14 個 CCNU 易脆位置中，有 8 個位置見於 2 個或 2 個以上的個體。

從表二可見，在這 14 個對 CCNU 敏感的位置中，出現最爲頻繁的是 9q12，其出現率占總 755 個斷裂數的 17.0%，並且此 8 個人均有此位置之斷裂。3q26.2 爲出現率次爲頻繁的斷裂點，占總斷裂數的 2.6% 。 其他對 CCNU 敏感的位置且出現率超過總斷裂數之 1% 者分別爲: 5q21.2 (2.4%)，13q21.2 (2.0%)，3p23 (1.7%)，4q31.3 (1.6%)，12q22 (1.5%)，以及 4q23 (1.1%)。總括這 14 個位置的斷裂數目占所有斷裂數的 37.7% 。

圖二所示，乃是 CCNU 誘發的 14 個易脆位置的斷裂情形。

咖啡因 (caffeine) 對誘發染色體異常之影響 ：

Caffeine 常被應用爲一些致突變作用的強化劑 (mutagenic enhancer)(Das 等人,1984;Kihlman 等人,1982; Yunis 等人,1987)。在本實驗裡，以 CCNU 處理 24 小時，繼之經過二次洗滌，再加入 caffeine 處理 6 小時者，其染色體斷裂頻率明顯地高於 ($p< 0.005$) 僅處理 CCNU 者（見表一）。

由 CCNU+Caffeine 所誘發的斷裂點在染色體上的分佈列於表三，此組總共有 13 個位置之斷裂數出現在個人 4% 或 4% 以上。其中以 9q12 出現率爲最高，占此組總斷裂數 1890 之 8.6%。而 3p14 斷點的出現率則爲 4.7% 。此二位置（9q12 和 3p14）的斷裂均見於所有 8 位正常人之染色體中。

其他顯現斷裂數目較多的位置分別是: 3q26.2 (3.6%)，Xp22.3 (2.3%)，以及 4q31.3 (1.1%)（見表三）。表三所列之 CCNU+Caffeine 敏感位置的斷裂數和約占所有斷裂的 25.3%。此一結果顯示: 雖然 caffeine 會劇烈地增強染色體斷裂之誘發，但它並非僅僅影響到染色體的某幾個部位而已。

由 caffeine 處理組之染色體斷裂位置出現率超過個人 4% 以上者列於表四。在 17個 caffeine 引發的易脆位置中，以 3p14 出現率占總數的 20.2% 爲最高，而且此 8 位正常人之染色體均有出現此一斷點。此3p14 的斷點，一直是人類染色體上最常見的易脆位置（Rao 等人,1988; Smeets 和 Scheres,1986)。其他出現率超過總斷裂數 1% 的位置分別是：Xp22，3q26.2，7q32，Xq22，4q27 及 11q14 (見表四)。

表五所列，乃是 CCNU 特有或 caffeine 特有，以及 CCNU 和 Caffeine 的共同易脆位置。在這些位置當中，3q26.2，4q31.3，和 Xp22.3 爲對 CCNU 及 caffeine 之共同敏感位置。

第四章
討論 (Discussion)

近幾年來，在人類的染色體上曾被誘導出許多易脆位置的表現 (expression of fragile sites)。所謂的易脆位置，乃是在染色體上容易受遺傳或外界環境因子影響而發生斷裂的脆弱位置。在低含量 folic acid 或 thymidine 的培養條件下，可以誘發某些染色體位置的斷裂 (Barbi 等人,1984; Glover,1981;Sutherland,1982)。此外，不同的藥物亦可引發一些易脆位置的表現，例如: distamycin A (Hori 等人,1988; Schmid 等人,1980)，bromodeoxyuridine (Sutherland,1982; Sutherland 等人,1985)，methotrexate (Barbi 等人,1984; Yunis 等人,1987)，fluorodexyuridine (Rao 等人,1988)，5-azacytidine (Sutherland 等人,1985)，aphidicolin (Craig-Homes 等人,1987; Glover 等人,1984; Hecht 和 Glover,1984; Rao 等人,1988)，及 daunomycin (Liou 和 Li,1994)。再者，具不同致突變機制的藥物亦可誘發許多相同染色體位置的斷裂 (Daniel 等人,1984; Glover,1981; Yunis 和 Soreng,1984; Yunis 等人,1987)。

CCNU[lomustine;1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea]，爲一種 nitrosourea，它主要是透過造成 DNA 的　基化而產生細胞毒性。並且因其具有脂溶性及能夠穿過血腦障壁 (blood-brain barrier) 的特性 (Bertino 等人,1992; Wingard 等人,1991)，而經常被應用以治療腦腫瘤和枝氣管腫瘤，以及霍金奇氏白血病 (Hodgkin's leukemias) (Fewer 等人,1972; Schein 等人 ,1984; Selawry 等人,1972; Walker 等人,1973)。再者，CCNU 曾被証實能對人類淋巴球誘發大量的姊妹染色分體交換 (sister chromatid exchanges) (Best 等人,1988)。

本篇研究的目的，主要在探討 CCNU 對人類淋巴球之染色體有何影響？它是否會引起染色體的斷裂？以及其斷裂點在染色體上的分佈爲何？而我們的結果顯示，在實驗的條件下，由 CCNU 所誘發的染色體斷裂數明顯地高於控制組的 ($p<0.005$) (見表 一)。其次，除了染色體斷裂之外，並未發現 CCNU 引起其他染色體構造上的異常。而暴露於 daunomycin 的細胞，其染色體則會出現大量的轉位 (translocation)，互相交換 (interchange)，及環形染色體

(ring chromosome) 等異常 (Liou 和 Li,1994)。

由於在細胞處理藥物的同時，可能會產生一些隨機性的斷裂 (random breaks)，因此以斷裂出現率超過該個人 4% 以上的位置，才視爲該藥物之易脆位置 (Rao 等人,1988)。在此選擇標準下，則人類的淋巴球之染色體上有 14 處對 CCNU 敏感的位置 (見表 2)。總計此 14 個位置所發生的斷裂數占總數的 33.6%。

在這些 CCNU 誘發的斷裂位置中，以 9q12 出現最多次，它的出現率占所有斷裂數的 17.0% ，而且都超過每個人總斷裂數的 4% (見表 二)。斷點在 9q12 亦可被 5-azacytidine 和 mytomycin C 等二種藥物所誘發 (Hecht 和 Sandberg,1988;Shaw 和 Cohen,1965; Sutherland 等人,1985)。對人類的染色體而言，9q12 的位置爲組成型異染色質之區段(constitutive heterochromatin region)，其主要由簡單的重覆序列 DNA (repeated sequence DNA) 所組成 (Miklos 和 John,1979; Miklos,1982)。此種組成型異染色質不含有孟德爾氏的基因而且不進行轉錄 (translation) (John,1988)，而且至目前爲止，此種異染色質在生物學上的眞正功能亦不清楚。

CCNU 所誘發次多的斷裂點爲 3q26.2，此位置出現率占總斷裂數的 2.6%，而且在 8 人之中，有 4 人的染色體在 3q26.2 位置斷裂數超過個人之 4% 以上 (見表 二)。某些癌細胞，例如急性非淋巴白血病 (acute nonlymphocytic leukemia) 和脊髓發育不良症候群 (myelodysplastic symdrome) 之異常染色體常見有 3q26.2 的斷點 (Mitelman,1991)。

染色體上 5q21 的位置可因 aphidicolin 的誘發而產生大量的斷裂 (Craig-Holmes 等人,1987;Yunis 等人,1987)。而 13q21.2 的位置對於 bromodeoxyuridine，busulfan，aphidicolin，及 fluorodeoxyuridine (Sutherland,1982; Yunis 等人,1987)。此外，威廉氏腫瘤 (Wilm's tumor) 及視網膜胚細胞瘤 (retinoblastoma) 常見在 13q(13.2-21.2) 區段發生斷裂之異常染色體，其亦含有 13q21.2 的斷點 (Mitelman,1991; Yunis 等人,1987)。而許多卵巢癌細胞常出現 3p13 的染色體斷裂點 (Mitelman,1991; Yunis 等人,1987)。肺小細胞癌則常發生染色體 3p(14-23) 之間的缺失，此亦可見 3p23 的斷裂位置 (Mitelman,1991; Whang-Peng 等人,1982)。

一個易脆位置的表現，究竟是由於某些染色體的位置較容易產生斷裂，或者是因這些位置上受損的 DNA 之修復能力較差所致？咖啡因 (caffeine) 因其具抑制細胞分裂過程中修復受損 DNA之能力，而常被用來強化 (enhance) 某些藥物之致突變作用 (Das 等人,1984; Kihlman 等人,1982; Yunis 等人,1987)。

如表 一 所示，CCNU+Caffeine 處理組的染色體斷裂出現率顯著高於 CCNU 組的。然而， CCNU 與 caffeine 處理的細胞，雖造成其染色體劇烈地斷裂，但就 CCNU 的 14個敏感位置而言，caffeine 並未特別提高其出現率 (見表 二 和表 三)。此種結果可能是因大部份由 CCNU 所誘發的染色體斷裂，都在經洗掉 CCNU 後再培養 6 個小時間已完成修復受損的部份，而其他被吾人所觀察到位置的斷裂，則是因修復能力較差之緣故。因此，當加入 caffeine 處理以破壞受害 DNA 的修復時，則先前被 CCNU 所誘發的染色體斷裂便表現出來了。隨著整個染色體其他不同位置斷裂之增加，相對地便降低了此 14 個 CCNU 易脆位置的比率。

暴露於 caffeine 6 小時的細胞亦出現染色體位置的斷裂 (見表 四)。斷點 3p14 與 Xp22 爲最常見的 caffeine 易脆位置。

表 五 所列，乃是 CCNU 或 caffeine 所特有，以及 CCNU 和 caffeine 所共同的染色體斷裂位置。其中 3q26.2，4q31.3 及 Xp22 等三個染色帶 (chromosome bands) 爲此二種藥物之共同斷裂處。然而， 5p14 及 12q13 此二位置，則僅有在配合處理 CCNU 及 caffeine 時才出現多量斷裂，此顯示這二個易脆位置的表現是基於修復 DNA 的能力被減弱所致。

至今，本身具不同致突變機轉的各種藥物，被証實可以誘發許多染色體上相同位置的斷裂 (Daniel 等人,1984; Glover 等人, 1984; Rao 等人,1988;Yunis 和 Soreng,1984;Yunis 等人,1987)。不同種類的藥物可以誘發相似易脆位置的表現，此一情形提示著這些位置在染色體上可能不同藥物致突變作用 (mutagenesis) 之靶的 (targets)。

此外，已知許多腫瘤細胞具有其特異的染色體構造異常 (specific structural chromosome defects)，包括染色體的轉位 (translocation)，倒轉 (inversion)，缺失 (deletion) 等

(Mitelman,1991)，但這些染色體異常主要都牽涉到 2 個位置上的斷裂。而且，許多致突變劑所誘發的易脆位置和某些癌細胞的畸型染色體之斷裂點頗相吻合 (Hecht,1988; Hecht 和 Glover,1984; Hecht 和 Sandberg,1988; Le Beau 等人,1987; Yunis 和 Soreng,1984; Yunis 等人,1987)。此一現象意味著：在某些致突變或致癌過程之中，可能因這些染色體上的易脆位置發生斷裂而阻斷了某些基因的表現，或者因此造成染色體及基因的重新組合。

參考文獻：

1.Barbi G, Steinbach P, Vogel W : Non-random distribution of methotrexate-induced aberrations on human chromosomes. Detection of further folic acid sensitive fragile sites. Hum Genet 1984; 68: 290-294.

2.Bertino JR: Antineoplastic drugs. In Melmon KL, Merrelli HF, Hoffman BB, Neirenberg Dw eds. Clinical Pharmacology: Basic principles in therapeutics. New York: McGraw-Hill, Inc, pp 617-618, 1992.

3.Best RG, Mckenzie WH: Sister chromatid exchange in human lymphocytes exposed to ascorbic acid and the cancer chemotherapeutic agent 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea. Teratol Carcinogen Muta. 1988; 8: 339-346.

4.Craig-Holmes AP, Strong LC, Goodacre A, Pathak S: Variations in the expression of aphidicolin-induced fragile sites in human lymphocyte cultures. Hum Genet 1987; 76: 134-137.

5.Daniel A, Ekblom L, Philip S: Constitutive fragile sites 1p31, 3p14, 6q26 and 16q23 and their use as controls for false-negative results with fragile (X). Am J Med Genet 1984; 18: 483-491.

6.Das SK, Lau CC, Pardee AB : Comparative analysis of caffeine and 3-aminobenzamide as DNA repair inhibitors in Syrian baby hamster kidney cells. Mutat Res 1984; 131: 71-79.

7.Fewer D, Wilson CB, Boldrey EB: Phase II study of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU, NSC-79030) in the treatment of brain tumors. Cancer Chemother Rep 1972; 56: 421-427.

8.Friedman MA, Carter SB: Serious toxicities associated with chemotherapy. Semin Oncol 1978; 5: 193-202.

9.Glover TW : FudR induction of the X chromosome fragile site: Evidence for the mechanism of folic acid and thymidine inhibition. Am J Hum Genet 1981; 33 : 234-242.

10.Glover TW, Berger C, Coyle J, Echo B: DNA polymerase alpha inhibition by aphidicolin induces gaps and breaks at common fragile sites in human chromosomes. Hum Genet 1984; 67: 136-142.

11.Hecht F, Glover Tw: Cancer chromosome breakpoints and common fragile sites induced by aphidicolin. Cancer Genet Cytogenet 1984; 12: 185-189.

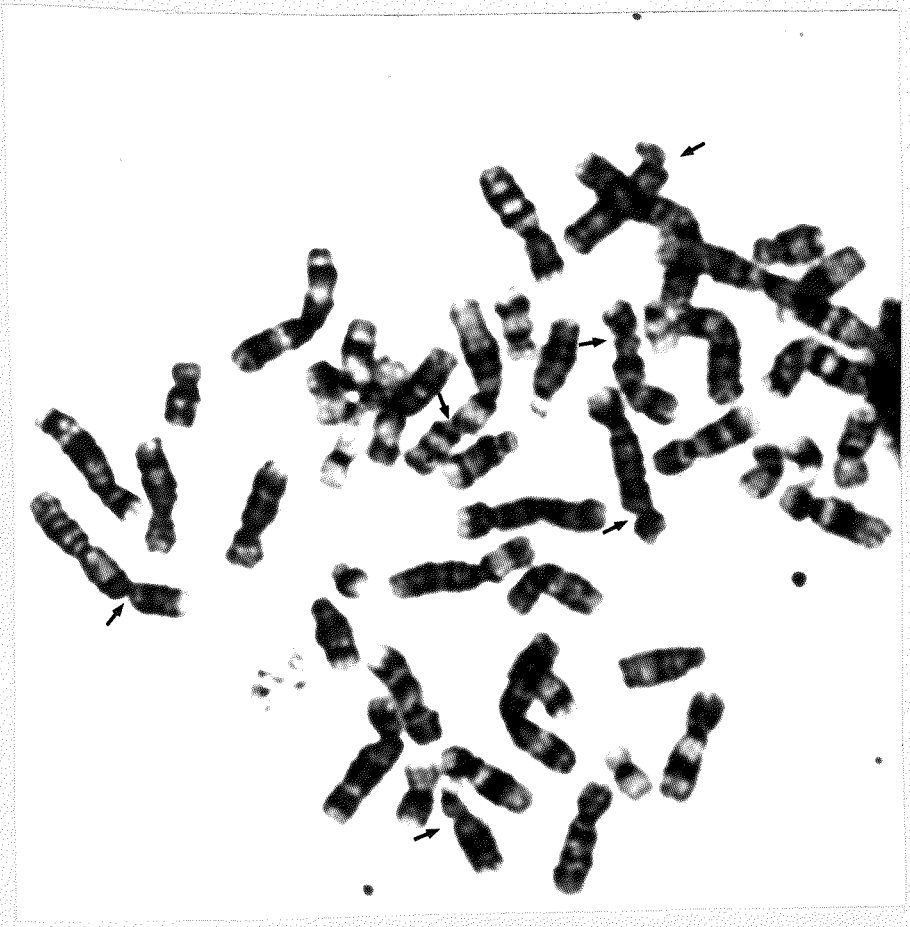
12.Hecht F, Sandberg AA: Of fragile sites and cancer chromosome breakpoints. Cancer Genet Cytogenet 1988; 31: 1-3.
13.Hori T, Takahasi E, Murata M : Nature of distamycin A-inducible fragile sites. Cancer Genet Cytogenet 1988; 34 : 189-194.
14.ISCN: An international system for human cytogenetic nomenclature. Birth Defects Orig. Art Ser 21: 46-47, 1985.
15.John B: The biology of heterochromatin. In: Verma RS ed. Heterochromatin: molecular and structural aspects. Cambridge University, Cambridge, pp 1-147, 1988.
16.Kihlman BA, Hansson K, Andersson HC : The effect of post-treatments with caffeine during S and G2 on the frequencies of chromosomal aberrations induced by thiotepa in root tips of Vicia faba and in human lymphocytes in vitro. Mutat Res 1982; 104: 323-330.
17.Le Beau MM: Editorial: chromosomal fragile sites and cancer-specific breakpoints - a moderating viewpoint. Cancer Genet Cytogenet 1988; 31: 55-61.
18.Liou JC, Li SY: Daunomycin-induced chromosomal aberrations in cultured human lymphocytes. 1994;(submitted).
19.Miklos GLG, John B : Heterochromatin and satellite DNA in man: properties and prospects. Am J Hum Genet 1979; 31: 264-280.
20.Miklos GLG: Sequencing and manipulating highly repeated DNA. In: Dover GA, Flavell RB eds. Genome evolution. Academic, New York: pp 41-68, 1982.
21.Mitelman F: Catalog of chromosome aberrations in cancer,4th Ed: A John Wiley and Sons Inc, New York,1991.
22.Rao PN, Heerema NA, Palmer CG: Fragile sites induced by FudR, caffeine, and aphidicolin: Their frequency, distribution, and analysis. Hum Genet 1988; 78: 21-28.
23.Rosner B: Fundamentals of biostatistics, Boston. PWS-Kent publishing company , pp 474-526,1990.
24.Schein PS, Tew KD, Mathe G: Pharmacology of nitrosourea Antineoplastic chemotherapy (Bukarda B, Karrer K, anti-cancer agents in clinical chemotherapy: Mathe G : eds) vol. 3, Thieme-Strutton, pp 264-282, 1984.
25.Schmid M, Klett C, Niederhofer A: Demonstration of a heritable fragile site in human chromosome 16 with distamycin A. Cytogenet Cell Genet 1980; 28: 87-94.

26. Shaw MW, Cohen MM: Chromosome exchanges in human leukocytes induced by mitomycin C. Genetics 1965; 51: 181-190.
27. Selawry OS, Hansen HH: Superiority of CCNU 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, NSC-79037 over BCNU (1.3-bis 2-chloroethyl-1-nitrosourea); NSC 409962 in treatment of advanced Hodgkin's disease. Proc Am Assoc Cancer Res 1972; 13: 46.
28. Smeets DCFM, Scheres JMJC, Hustinx TWJ: The most common fragile site in man is 3p14. Hum Genet 1986; 72: 215-220.
29. Sutherland GR: Heritable fragile sites on human chromosome VIII. Preliminary population cytogenetic data on the folic-acid-sensitive fragile sites. Am J Hum Genet 1982; 34: 452-458.
30. Sutherland GR: Heritable fragile sites on human chromosomes. IX. Population cytogenetics and segregation analysis of the BrdU requiring fragile sites at 10q25. Am J Hum Genet 1982; 34: 753-756.
31. Sutherland GR, Parslow MI, Baker E: New classes of common fragile sites induced by 5-azacytidine and BrdU. Hum Genet 1985; 69: 233-237.
32. Tommerup N, Poulsn H, Brondum-Nielsen K: 5-Fluoro-2-deoxyuridine induction of the fragile site on Xq28 associated with X-linked mental retardation. J Med Genet 1981; 18: 374-376.
33. Voiculescu I, HausmannC, Wolff G, Back E: A BrdU-requiring fragile site on chromosome 12. Hum Genet 1988;78:183-185.
34. Walker MD: Nitrosoureas in central nervous system tumors. Cancer Chemother. Rep 1973, 4: 21-26.
35. Whang-Peng J, Bunn PA, Kao-Shan CS Lee EC, Carney DN, Gazdar A, Minna JD: A nonrandom chromosomal abnormality, del3p(14-23) in human small cell cancer (CTCL). Cancer Genet Cytogenet 1982; 6: 119-134.
36. Wingard LB, Brody TM, Larner J Schwartz A: Individual antineoplastic drugs. In Human Pharmacology-molecular to clinical. London: Wolfe Publishing Limited, pp593-596, 1991.
37. Yunis JJ, Soreng AL: Constitutive fragile sites and cancer. Science 1984; 226: 1190-1204.
38. Yunis JJ, Soreng AL, Bowe AE: Fragile sites are targets of diverse mutagens and carcinogens. Oncogene 1987; 1: 59-69.

表一： 經 CCNU，CCNU+Caffeine，和 Caffeine 等處理組及控制組的淋巴球，其染色體斷裂頻率之比較。

culture	treatment	no.of bands showing breakage	mean breaks/ individual (±SD)	mean/ cell
1	CCNU	135	94.4 ±19.6	0.94
2	CCNU + Caffeine	154	236.3 ±44.4	2.36
3	Caffeine	92	61.8 ±16.6	0.62
4	Control	11	1.5 ± 1.2	0.02

a. The final concentration of CCNU is 10^{-5} M.
b. The final concentration of Caffeine is 2.5 mM.
c. Cultures not treated with CCNU or Caffeine were served as control.
d. $p < 0.005$。

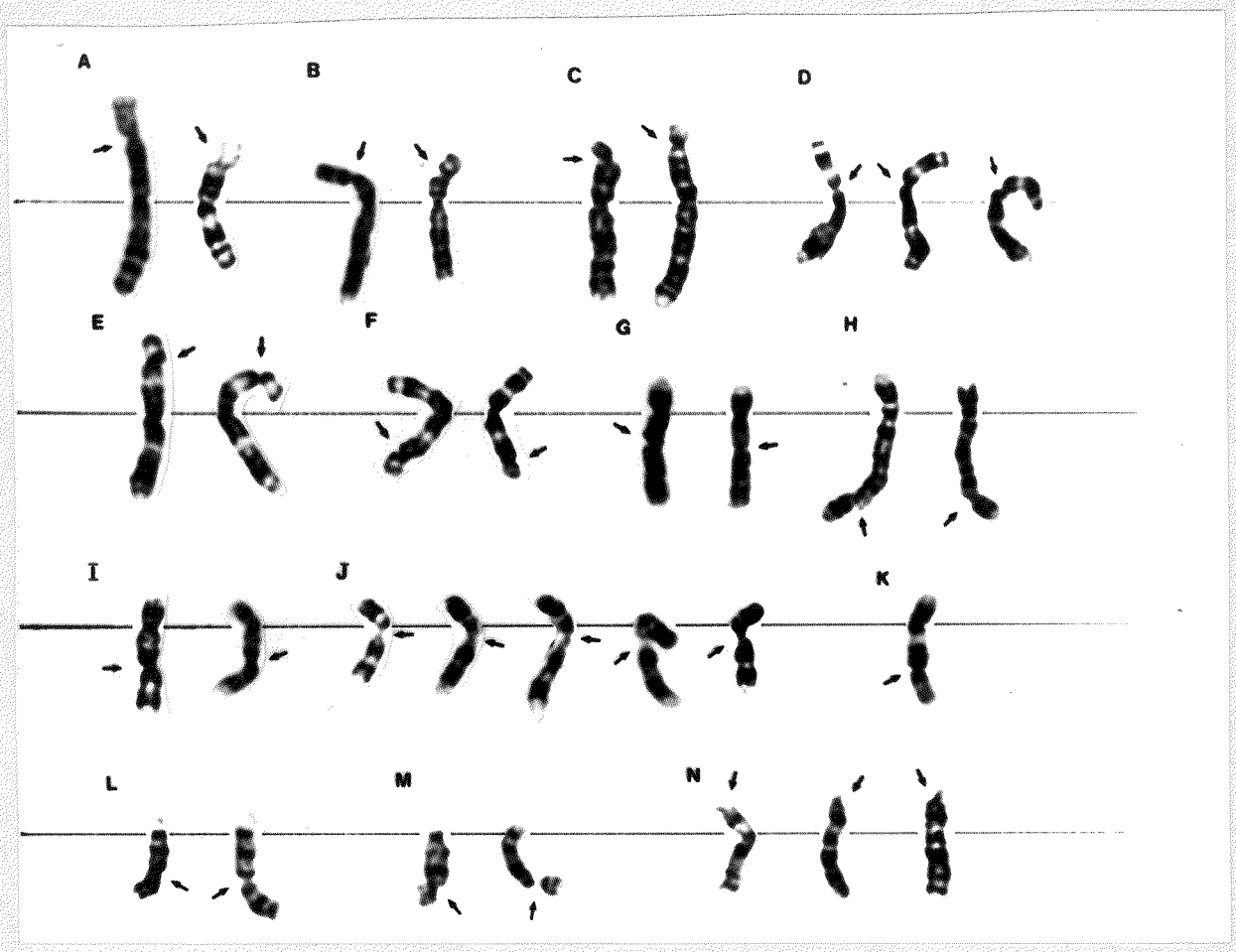


圖一：CCNU 誘發淋巴球染色體斷裂情形。

表二：出現在 CCNU 處理組之染色體斷裂的分析。

subject	1	2	3	4	5	6	7	8	total breaks	total individuals
total breaks	110	90	104	93	69	120	64	105	755	8
breakpoint										
1p34						5 (4.2)[a]			5 (0.6)[b]	1 (12.5)[c]
2p15							4 (6.3)		4 (0.5)	1 (12.5)
2p23			7 (6.7)						7 (0.9)	1 (12.5)
3p13						5 (4.2)			5 (0.6)	1 (12.5)
3p23	5 (4.5)	5 (5.6)			3 (4.3)				13 (1.7)	3 (37.5)
3q26.2	5 (4.5)	4 (4.4)	6 (5.8)					5 (4.8)	20 (2.6)	4 (50.0)
4q23						5 (4.2)	3 (4.7)		8 (1.1)	2 (25.0)
4q31.3	7 (6.4)	5 (5.6)							12 (1.6)	2 (25.0))
5q21.2		7 (7.8)	6 (5.8)			5 (4.2)			18 (2.4)	3 (37.5)
9q12	22 (20.0)	17 (18.9)	18 (17.3)	22 (23.7)	15 (21.7)	22 (18.3)	12 (18.7)	20 (19.0)	128 (17.0)	8 (100)
12q22						6 (5.0)		5 (4.8)	11 (1.5)	2 (25.0)
13q21.2	6 (5.5)		5 (4.8)	4 (4.3)					15 (2.0)	3 (37.5)
13q31		4 (4.4)							4 (0.5)	1 (12.5)
Xp22.3		4 (4.4)							4 (0.5)	1 (12.5)
14[d]									254 (33.6)	

註：a. 占該個人總斷裂數的百分比。
b. 占所有人總斷裂數的百分比。
c. 出現該易脆位置的人次比率。
d. 斷裂點總數。



圖二： 14 個對 CCNU 敏感之人類淋巴球的染色體位置。
這些斷點分別爲： A. 1p34; B. 2p15; C. 2p23; D. 3p13;
E. 3p23; F. 3q26.2; G. 4q23; H. 4q31.3; I. 5q21.2;
J. 9q12; K. 12q22; L. 13q21.2; M. 13q31; N. Xp22.3。
（染色體斷裂點乃依據 1985 年 ISCN 標準所作的記錄）

表三： CCNU+Caffeine 組的染色體斷裂點之分析。
（註腳見表二）

subject	1	2	3	4	5	6	7	8	total breaks	total individuals
total breaks	269	210	268	230	231	239	149	294	1890	8
breakpoint										
1p31						10 (4.2)[a]	6 (4.0)		16 (0.8)[b]	2 (25.0)[c]
1q23				10 (4.3)					10 (0.5)	1 (12.5)
3p14	15 (5.6)		13 (4.9)	12 (5.2)	16 (6.9)	12 (5.0)	9 (6.0)	12 (4.1)	89 (4.7)	7 (87.5)
3q23							6 (4.0)		6 (0.3)	1 (12.5)
3q26.2	12 (4.5)	9 (4.3)	15 (5.6)		10 (4.3)		10 (6.7)	12 (4.1)	68 (3.6)	6 (75)
4q31.3			10 (3.7)		11 (4.8)				21 (1.1)	2 (25.0)
5p14			12 (4.5)						12 (0.6)	1 (12.5)
9q12	25 (9.2)	12 (5.7)	15 (5.6)	24 (10.4)	26 (11.3)	27 (11.3)	10 (6.7)	24 (8.2)	163 (8.6)	8 (100)
12q13	12 (4.5)								12 (0.6)	1 (12.5)
12q22								13 (4.4)	13 (0.7)	1 (12.5)
13q21.2		10 (4.8)					6 (4.0)		16 (0.8)	2 (25.0)
13q31						10 (4.2)			10 (0.5)	1 (12.5)
Xp22.3		12 (5.7)	10 (3.7)	10 (4.3)	11 (4.8)				43 (2.3)	4 (50.0)
13[d]									479 (25.3)	

表四： Cafeine 誘發之染色體斷裂分析。
（註腳見表二）

Subject	1	2	3	4	5	6	7	8	total breaks	total individuals
total breaks	61	41	80	80	67	59	35	71	494	8
breakpoint										
1p31	4 (6.6)[a]								4 (0.8)[b]	1 (12.5)[c]
1q23								4 (5.6)	4 (0.8)	1 (12.5)
2q32								3 (4.2)	3 (0.6)	1 (12.5)
3p14	20 (32.8)	8 (19.5)	17 (21.3)	14 (17.5)	13 (19.4)	10 (16.9)	6 (17.2)	12 (16.9)	100 (20.2)	8 (100.0)
3q13		2 (4.9)							2 (0.4)	1 (12.5)
3q26.2	3 (4.9)				3 (4.5)		2 (5.7)	4 (5.6)	12 (2.4)	4 (50.0)
4q31.3						3 (5.1)		3 (4.2)	6 (1.2)	2 (25.0)
5q31						3 (5.1)			3 (0.6)	1 (12.5)
6p23						3 (5.1)			3 (0.6)	1 (12.5)
6q15						3 (5.1)			3 (0.6)	1 (12.5)
6q26			3 (3.8)						3 (0.6)	1 (12.5)
7q32			4 (5.0)		3 (4.5)			5 (7.0)	12 (2.4)	3 (37.5)
11q14	4 (6.6)	2 (4.9)							6 (1.2)	2 (25.0)
11q21			4 (5.0)	5 (6.3)		3 (5.1)			12 (2.4)	3 (37.5)
16q23			4 (5.0)						4 (0.8)	1 (12.5)
Xp22		4 (9.8)	5 (6.3)	4 (5.0)	7 (10.4)	3 (5.1)	2 (5.7)		25 (5.1)	6 (75.0)
Xq22			4 (5.0)		4 (6.0)		3 (8.6)		11 (2.2)	3 (37.5)
17[d]									213 (43.1)	

表五： CCNU 與 Caffeine 特有或共有染色體斷裂位置。

treatment	sites
unique to CCNU	1p34,2p15,2p23,3p13,3p23,4q23,5q21.2 9q12,12q22,13q21.2,13q31
common to CCNU and caffeine	3q26.2, 4q31.3, Xp22
unique to caffeine	1p31,1q23,3p14,2q32,3q13,5q31,6p23, 6q15,6q26,7q32,11q14,11q21,16q23, Xq22

附錄

附錄一：培養液名稱及製備

RPMI-1640 GIBCO

附錄二：藥品名稱及製備

Caffeine (2.5 mM) SIGMA
CCNU (10 M) SIGMA
Colcemid GIBCO
FCS (fetal calf serum) GIBCO BRL
Fixation (methanol: acetic acid=3:1) E.Merk
Heparin sodium B Braun Melsungen A G
Hypotonic solution (0.54% KCl) E.MERK
PHA (phytohemagglutinin) M form GIBCO
PSN (penicillin, streptomycin,neomycin) GIBCO

附錄三：儀器

1. Lamina Flow : Bellco Glass Inc. (USA)

2. Incubator（培養箱）:
   Forma Scientific Steri-cult incubator
   temperature 37°C, Humidity 60-98%,
   CO2 control 5%.
   Water-Jacketed incubator

3. Centrifuge（離心機）: Hitachi
   Time and Speed (x 1200 rpm)

4. Slide warmer (烘片機) : Fisher

附錄四：統計分析

A. 此 8個人經不同處理之染色體斷裂數目。

	CCNU	CCNU+Caffeine	Caffeine	Control
1	110	269	61	3
2	90	210	41	2
3	104	268	80	0
4	93	230	80	2
5	69	231	67	0
6	120	239	59	3
7	64	149	35	1
8	105	294	71	1

B. 統計分析：

	sum of square	degree of freedom	mean of square	F
between	238013.1	3	79337.7	120.5
within	18428.9	28	658.2	
total	256442.0	31		

F 0.005(3,28)=5.32 ； 故而 p < 0.005，
亦即各組之染色體斷裂數有顯著差異。

附表一： CCNU 所誘發的染色體斷裂位置及出現次數。

site/num of c	1	2	3	4	5	6	7	8	total
1p12	3	2			1	2		1	9
1p13	1								1
1p21		1		1				1	3
1p31	1					2	2		5
1p32	2			1					3
1p34			2	1		5		1	9
1p36	1						1		2
1q12				2					2
1q21								1	1
1q23		2	1	1		2	1	3	10
1q25	2		2		1				5
1q31	1		1	1			1		4
1q32	1							1	2
1q41		1			1				2
1q42	1		1						2
1q44					1				1
2p11	1				2			1	4
2p12			1						1
2p13	1	1	2				2	2	8
2p15	2			1		2	4	2	11
2p21		2	4						6
2p23	3	1	7	2	2	1	1	3	20
2q13				1			1		2
2q21		1						1	2
2q23				1			1		2
2q32					1				1
2q33	2						4	2	8
2q35	3	1			1				5
3p12					2				2
3p13	1		3	1		5			10
3p14	2			1					3
3p21				1			2	4	7
3p23	5	5	3	1	1		1	3	19
3q12			1				1		2
3q13					1			1	2
3q21						3		4	7
3q23		2		2	3		1	2	10
3q26.2	5	4	6	2	1	2	1	5	26
4p12							1		1
4p15	2		2	2	1	4	1		12
4q12					1		1		2
4q13	1							2	3
4q23	1		2	1		5	3	2	14
4q27			3					2	5
4q31.3	7	5		2	1				15
4q33				2		1		1	4
5p13			1		1				2
5p14	1		1		1				3
5p15		1							1
5q12								1	1
5q13							1	3	4
5q15	1	2	2	4	2	3	1	1	16
5q21.2		7	6	2	1	5	2	2	25
5q31	1		2		1	2		2	8
5q33				1	2	3			6
6p21		1							1
6p23					1	1			2

6q13	1					2			3
6q15	1	3			2	1		1	8
6q21					1				1
6q23						1			1
6q26	2		1		1	3			7
6q27							1		1
7p11	1								1
7p12			2						2
7p13					1	1			2
7p14			1	2				1	4
7p21		2							2
7q21			1		1				2
7q22		1							1
7q31	1		2	1	2	4		1	11
7q32		1							1
7q34				2		3			5
8p21		1							1
8q11				1					1
8q21				2					2
8q22			2	2	1	4	1	2	12
8q23	1								1
9p13				1					1
9p22	1			1					2
9q12	22	17	18	22	15	22	12	20	148
9q21							2	1	3
9q22	1	1			1		1	1	5
9q31			2				1		3
9q32				1	1				2
9q34		2						1	3
10p12				2	1		2		5
10p15						1			1
10q21						1			1
10q22		1	1			1			3
10q23	1			3	1	1	1		7
10q25					2				2
10q26			1						1
11p11		1							1
11p14	1		2		2				5
11p15								3	3
11q13								1	1
11q14		2	2		1	2		1	8
11q21	1			1			1		3
11q23	1								1
12p12		2	1					1	4
12q13	3	1			2	1	2	2	11
12q15	2						1		3
12q22			1	1		6		5	13
12q24	2					3			5
13q13				1				2	3
13q21.2	6	2	5	4	1	1	1	1	21
13q31	4	4		2					10
13q32			1						1
13q33				1					1
14q12			1	1					2
14q13		1							1
14q21	1			1		2		1	5
14q24							1		1
14q31			1			3			4

15q12			1						1
15q15				1	1				2
15q22	1					1		1	3
15q25				2					2
16p12	1					1			2
16q21			1						1
16q23		1				1	1		3
17p12								1	1
17q21			1						1
17q22						2			2
17q23	1			1	1	1			4
18q12				1	1			1	3
18q21						1			1
18q22			1	1				2	4
Xp22.3	1	4				1	1		7
Xq21		1		1					2
Xq22		1	1						2
Xq24			1				1	1	3
Xq25		2							2
Xq26	1					1			2
total	110	90	104	93	69	120	64	105	755

附表二： CCNU + Caffeine 所誘發的染色體斷裂位置及出現次數。

site \ subject	1	2	3	4	5	6	7	8	total
1p12						1		2	3
1p13			1	1		1			3
1p21	1	1	1	2		1	1	2	9
1p31	2	3	5	5	3	10	6	9	43
1p32	4	1	4	1			3	2	15
1p34	2	6	3	1	1	2	2	1	18
1p36		1	1		3	1	3	2	11
1q12	1								1
1q21			1	1					2
1q22						1			1
1q23	1	1	3	10	4	2	3	2	26
1q25	1	1							2
1q31	3	2	2	3	2	1	1	4	18
1q32			1						1
1q41	1	1	2		1	2	2	2	11
1q42	1		1		1				3
1q44						2			2
2p11				1					1
2p13		2	2	1	2	1	4	2	14
2p15	2	4	6	3	3	1	3	4	26
2p23	3	2	5	1	4	4	4	4	27
2q13	2			1			3	1	7
2q21		3		2	2				7
2q23	1	4	2	5	3	1	1	3	20
2q31	1	2	1			1			5
2q32	5	1		5	3		1	4	19
2q33		1	2					6	9
2q35	2	3	1	1	1	1	1	3	13
3p12							1	2	3
3p13	1			4	2	2			9
3p14	15	5	13	12	16	12	9	12	94
3p21		1							1
3p23	4	1	3	4	5	8	2	5	32
3q13		2	4		1	2	2	1	12
3q21	1			2					3
3q23	2		1		4	2	6	9	24
3q26.2	12	9	15	6	2	10	10	12	76
4p15	5	2	2	5	2	3	1	1	21
4q12	2	1	2	1				1	7
4q13	3		2	1	2	2		1	11
4q21		1	1						2
4q23	2	2	2	4	2	2	1	4	19
4q25	2		4	1	1				8
4q27	4	4	1	1		1	1	8	20
4q31	1	1	10	2	11	3		2	30
4q33	3	4	4	3	4	2		8	28
5p13					1				1
5p14	3	4	12	2	3	5	3	6	38
5p15	1				1				2
5q11	1								1
5q12		1							1
5q13			1					2	3
5q15	6	1	2	3	1	4	2	6	25
5q22	6	4	8	1	5	1	2	1	28
5q31	1	1	2	3	4	2		2	15
5q33	2	1	1	4	2	2	1	3	16
6p21		1	1	1			1		4

6p23	3		2	1	4	3	3	2	18
6q13	2		1		1	5	1		10
6q15						2			2
6q21	5	3	6	2	5	7		4	32
6q23	4	2	1	1		3		2	13
6q24						1			1
6q25	1		1	1		4			7
6q26								2	2
6q27	2	1	3	2	2	3		1	14
7p11			1						1
7p12					1	2		1	4
7p13					1				1
7p14		1		1	1			2	5
7p21	2		3	1	1	5		1	13
7q11	1		1	1	1			1	5
7q21	1	3						4	8
7q22	1	1				3		2	7
7q31	2	1	3	1	1	5	1	3	17
7q32	2	1	3	4	5	1	1	3	20
7q34		1	1	1					3
8p11	1								1
8p12			1					1	2
8p21			1	1					2
8p22	1					3		2	6
8q11	1	1							2
8q21	4	3	1	2	1				11
8q22	1	1		3	1	3		2	11
8q23	1	2			2		1	3	9
8q24		1	2	2					5
9p13					1				1
9p22	2	3	2			5	1	4	17
9q12	25	18	15	24	26	27	10	18	163
9q21				1		1		2	4
9q22		1	2					2	5
9q31		2	1			1			4
9q32	4	3	2	4	1	4		3	21
10p11		1		1					2
10p12		2			1	1	1	2	7
10p14	1	2	1	2	1	1	1		9
10q21		3					2	4	9
10q22	2								2
10q23	2	1	2	1	1		1	1	9
10q25	1	3		1	1	1	1	3	11
10q26	1	3		1	1	2		2	10
11p11						1			1
11p14	3	2	3			1	2	5	16
11q13	1	2	4		3	1	1	1	13
11q14	1			2	2	1	1	1	8
11q21	3	1	6	6	3	1	3	6	29
11q23	3		2	1		1	1	1	9
12p11	1								1
12p12		1		1	2	1	1		6
12q13	12		1		1		1	1	16
12q15	5	4	7	3	6	4	5	1	35
12q22	4		3	4	1	3	1	13	29
12q24	5	1	2	1	1	2	2		14
13q12	1					1			2
13q13	1	1	1		1		2	2	8

13q21.2	8	10	5	6	4	3	6	7	49
13q31	4	2	4	1	4	10	1	4	30
13q32			1	2	3			1	7
13q33	2								2
14q11	2								2
14q13	2		1		2	2		1	8
14q21	3	2	2	3	1	1		3	15
14q23		1			1	1	2	1	6
14q24		1		2		1		1	5
14q31	2	1		3	2	1		2	11
15q13	1							1	2
15q15			3	2	1	2			8
15q22	3		1	1	2	2	2	1	12
15q24		2							2
15q25					2	1	2	3	8
16p12				2					2
16p13	1								1
16q13					1	2		1	4
16q21				1					1
16q23		2	2	1	1	1	1		8
17p12								1	1
17q12					1				1
17q21	2		1						1
17q23		1	1	2		1		2	7
18q12	1	2	1				1	2	7
18q21	3	1		2	2				8
18q22			1		2	1	2	1	7
19q13	1								1
20p12				1				1	2
20q13								2	2
21q21				1					1
22q12	1	2	1						4
Xp11					2				2
Xp22.3	2	12	10	10	11	1	2	5	53
Xq22	6	4	5	2	5	2	5	5	34
Xq24		4			2	1			7
Xq25		1	4	4				1	10
Xq26	2	1	2	2					7
Xq27			3	1			1	1	6
total	269	210	268	230	231	239	149	294	1890

附表三： Caffeine 所誘發的染色體斷裂位置及出現次數。

site	1	2	3	4	5	6	7	8	total
1p21			3		1				4
1p31	4		1	2	2	1			10
1p32			1						1
1q12						1			1
1q21					1				1
1q23		1						4	5
1q25								1	1
1q31		1							1
1q44	2								2
2p13				1		2			3
2p15	1			1	2	1	1	1	7
2p23				1		2	1	1	5
2q21			1		1				2
2q23				2	1	2		2	7
2q31	1								1
2q32	1		2	1	2		1	3	10
2q35				1			1		2
3p12	2							1	3
3p14	20	8	17	14	13	10	6	12	100
3p23	1		1	2				2	6
3q13	1	2	1						4
3q21		1							1
3q23					2	1	1		4
3q26	3		2	2	3		2	4	16
4p13		1				2			3
4p15		1	2		1	1	1		6
4q12				1	1			1	3
4q13								1	1
4q23				1		1	1		3
4q25		1							1
4q27	1	1		2	1		1		6
4q31	1				1	3		3	8
4q33								1	1
5p14	1	1		1	2	1	1	1	8
5q15				3	1	1		1	6
5q22	1		2	1	2	1		1	8
5q31	1			3				1	5
5q33				1			1		2
6p23	1					3		1	5
6q15			1	2		3			6
6q21				2					2
6q26			3	1					4
6q27	1	1		1					3
7p11								1	1
7p14		1				1			2
7p21								1	1
7q21					1				1
7q31			1		1				2
7q32	1	1	4	2	3		1	5	17
8p22	1								1
8q21	1		2	2				1	6
8q22			2				1	1	4
8q23		1		1					2
9p22	1				1	1			3
9q12	1	1	2	3	4		1	5	17
9q21				1					1
9q32			1		1				2

10p12				1					1
10p14			1						1
10q21						2			2
10q23				2		2		1	5
11p12		1							1
11p14	2		1	1	1		1	1	7
11q13		1							1
11q14	4	2		1		2			9
11q21	2	1	4	5	2	3	1	1	19
11q23								1	1
12p12	1		1					1	3
12q13		1						3	4
12q15	1		1	2	1	2			7
12q22		1							1
12q24		1							1
13q12			1						1
13q13						2	1	1	4
13q21		1	3						4
13q31					1		2		3
13q32						1			1
14q13			1					1	2
14q24			1	1					2
14q31		1							1
15q15		1		1	2			1	5
16q23		1	4	2			1		8
17q21						1	1		2
17q23				1		1			2
18q12	1	1		2			1	1	6
18q21			2			1		1	4
18q22			1						1
19q13				1					1
Xp22.3	1	4	5	4	7	3	2	1	27
Xq22	1	1	4	1	4	1	3	1	16
Xq25			1		1		1		3
total	61	41	80	80	67	59	35	71	494

CSG51-05

J Genet Mol Biol 1994; 5:
Published by the Chinese Society of Genetics, Taipei, Taiwan, ROC

DAUNOMYCIN-INDUCED CHROMOSOMAL BREAKAGE AND ABERRATIONS OF CULTURED HUMAN LYMPHOCYTES

JUNG-CHIN LIOU AND SHUAN-YOW LI

The genocytotoxic effect of daunomycin was studied in cultured lymphocytes from ten healthy individuals. We used caffeine as mutagenic enhancer. Our results showed that daunomycin could induce high frequency of chromosome breakage ($p<0.025$) and numerous chromosomal aberrations as well. The major types of chromosomal aberrations included interchanges between homologous or non-homologous chromosomes, intrachromosomal exchanges, translocations, dicentric chromosomes, and ring chromosomes. Further, many of the daunomycin-induced break sites were common to the cells exposed to diverse mutagenic agents. In addition, most of the daunomycin-sensitive sites were coincident with the specific cancer chromosome breakpoints and some of mapped oncogenes locations. This study indicates that there exists some fragile sites within the chromosomes which are sensitive to daunomycin and other agents. Such fragile sites may be involved in mutagen-induced disruption of active DNA sequences that are critical in promoting tumorgenesis and other genetic defects.

Keywords: daunomycin; chromosomal aberrations; fragile sites; specific cancer breakpoints

The fragile sites are specific sites in chromosomes that breakage can be increasingly induced under certain culture conditions. Culture medium containing low concentrationof folic acid or thymidine induces the expression of some fragile sites [1,9,16,25]. Fragile sites are also inducible by mutagenic agents such as fluorodeoxyuridine [9,21,36], methotrexate [1,36], aphidicolin [3,10,11,21,36], busulfan [36], caffeine [7,21], bromodeoxyuridine [5,26,27,30], distamycin A [23,28], and 5-azacytidine [27]. Besides, diverse mutagens which have different action mechanisms are able to induce many similar fragile sites [6,9,21,36]. At present, many types of leukemia, lymphoma and solid tumor cells are known to have specific structural chromosomal defects, primarily involving translocations, deletions and inversions with two precise breakpoints [2, 4, 8, 13, 14, 18, 19, 20, 28, 29,31, 32, 34, 35]. In addition, several studies have shown that these mutagen-sensitive sites are correlated with specific cancer chromosome breakpoints [8,11,13,14,18,20,28,35,36].

Daunomycin (daunorubicin) is the antibiotics usually used to treat leukemias. Its anthracycline structure will intercalate into the double-stranded DNA to destroy the neoplastic cells, or it may inhibit topoisomerase 11 by stablizing a covalent enzyme-DNA complex and thus disrupt the rejoining of DNA breaks of cancer cells [33].

Corresponding author: Dr.Shuan-Yow Li, Cytogenetics Laboratory, Chung Shan Medical and Dental College, Taichung, Taiwan (R.O.C.)
Cytogenetics Laboratory, Chung Shan Medical and Dental College, Taichung,Taiwan (Republic of China)

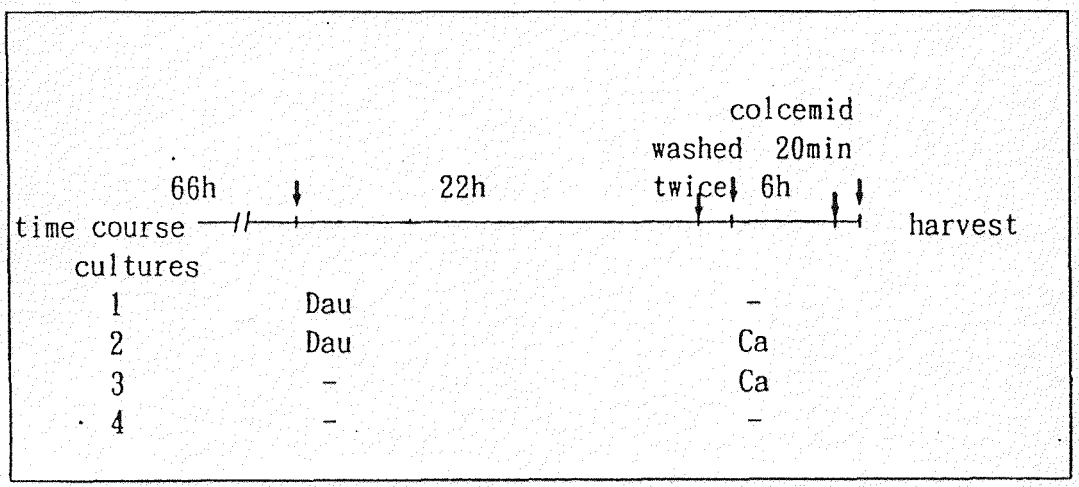
This study was aimed at detecting the frequency and distribution of daunomycin-inducible fragile sites in cultured human lymphocytes. We applied caffeine as a mutagenic enhancer [17,36]. Our results demonstrate that daunomycin shows a potent effect in induction of chromosome breaks and different types ofchromosomal aberrations. Many of the daunomcin-sensitive sites are similar to other mutagenic agents fragile sites. Furthermore, most of the daunomycin-sensitive sites are correspondent to the breakpoints of certain cancer abnormal chromosemes and locations of some oncogenes. These phenomena may be helpful to explain why structural chromosomal rearrangements often present when cells are exposed to mutagens and carcinogens.

MATERIALS AND METHODS

Peripheral blood from ten healthy individuals (five males and five females, 20-29 years of age) were cultured in RPMI-1640 medium. The medium was supplemented with 15% FCS (fetal calf serum, GIBCO.), 1% PSN antibiotics mixture (penicillin, streptomycin and neomycin, GIBCO), and 2% PHA (phytohemagglutinin, GIBCO). Four separate cultures (1,2,3,4) (see Table 1) from each individual were set up and incubated for four days. Before harvest, cultures 1 and 2 of each subject were exposed to daunomycin (0.1 uM final concentration, Sigma). Twenty two hours later, all cultures were washed twice with fresh RPMI-1640 medium. After washing, caffeine (2.5mM final concentration,Sigma) was added to cultures 2 and 3 for the last 6 hours of incubation. Mitotic divisions were arrested by treatment with colcemid (0.05 ug/ml, GIBCO) for the last 20 min of incubation. Then all cells were treated with 0.075 M hypotonic KCl for 16 min, and they were fixed by three changes of 3:1 methanol glacial acetic acid fixative. Cells in fresh fixative were dropped onto clean slides. The prepared chromosomes were Trypsin-G banded with Wright's stain. Cells from cultures which were not treated with daunomycin or caffeine were served as control.

One hundred metaphases from each treatment were analyzed by microscope, or by karyotyping. The band level of observed chromosomes were ranging from 400 to 500 bands. The chromosome breakpoints were recorded according to the international system for cytogenetic nomenclature (ISCN)[15].

Table 1. The four different treatments of lymphocytes from each individual.



Dau (daunomycin, 0.1 uM final concentration),
Ca (caffeine, 2.5 mM final concentration).
Colcemid was used to arrest cells in metaphases.

Table 2. Comparsion of chromosome breakage frequency induced by daunomycin, daunomycin with caffeine,and caffeine.

Culture	treatmant	no.of bands showing breakage	mean breaks/ individual(±SE)	mean breaks/ cell
1	Daunomycin	193	277.3±30.5	2.77
2	daunomycin+caffeine	201	401.4±33.9	4.04
3	caffeine	85	12.8±1.7	0.13
4	untreated	9	1.2±0.4	0.01

a. The final concentration of daunomycin is 0.1 uM.

b. The final concentration of caffeine is 2.5 mM.

c. Cultures not treated with daunomycin or caffeine were served as control.

d. SE = standard error.

e. $P<0.025$.

Analysis of variance [22] was done to analyze the frequency of daunomycin-inducible chromosome breaks.

RESULTS

The four different treatments of lymphocyte cultures are listed in Table 1.

As shown in Table 2, the frequency of chromosome breakage induced by daunomycin is very high (277.3±30.5, mean breaks 100 metaphases±standard error). There are 193 chromosome bands showing breakage at least once in one or more individuals (Table 2). Table 3 lists the frequency and distribution of daunomycin-inducible chromosome breaks.

The frequency of chromosome breakage obtained from cultures receiving the treatments of daunomycin with caffeine is noticeably high in comparison with that treated with daunomycin alone (Table 2). However, as seen in Table 2, there are 201 chromosome bands having breakage in daunomycin with caffeine metaphases. This implicates that although the expression of breakage frequency is drastically enhanced by caffeine, the locations of chromosome breakage are not increased accordingly.

Table 3 also summarizes the relation of daunomycin-sensitive sites to the known specific cancer chromosome breakpoints and locations of mapped oncogenes, and to the fragile sites induced by other mutagenic agents.

In addition to breaks, large numbers of chromosomal rearrangements also present in cells exposed to daunomycin (Fig. 1). The major types of chromosomal aberrations are homologous chromosomal interchanges (Fig. 1A), homologous dicentric chromosomes (Fig. 1 B), non-homologous chromosomal interchanges (Fig. 1 C, 1 D), complex chromosome interchanges (Fig. 1 E), non-homologous chromosomal translocations (Fig. 1 F), intrachromosomal exchanges (Fig. 1G), and ring chromosomes (Fig. 1 H).

The breakpoints of these daunomycin-induced abnormal chromosomes were karyotyped and analyzed. Table 4 lists the structural abnormalities (including interchanges and translocations) with frequency more than 1.0% of the total 403 recorded chromosome breakpoints.

DISCUSSION

Recently, a large number of fragile sites have been identified in human chromosomes [10,12,24,25,26,27]. Fragile sites are the points in chromosomes where they preferentially show breaks, either spontaneously or after expoure to certain mutagenic agents. These mutagens include antifolate agents (such as fluorodeoxyuridine and methotrexate) [1,9,21,36], DNA polymerase alpha inhibitors

Fig. 1 The major types of daunomycin-induced chromosomal aberrations.

A shows inter-homologous chromosome exchanges, and are interpreted as: cte(15;15)(q15;q15),cte(1;1)(p13;q32) and cte(17;17)(q23;q25).

B shows homologous dicentric chromosomes: dic(1)p32p32), dic(1)(q23q31), dic(6)(q15q21).

C shows quadriradial interchange of non-homologous chromosomes: cte(1;3)(p34;p23), cte(2;12)(q21;q21), cte(2;8)(q31;q22) with a break at 2p13, cte(4;X)(q31;q22), cte(9;14)(q22;q23), double interchanges in chromosome 1-cte(1;12)(p13;q24) and cte(1;17)(p32;q21).

D is triradial interchange of non-homologous chromosomes: cte(10;13)(q26;q14), cte(1;3)(p34;p25) with a break at 3q12, and cte(5;6)(q13;p23).

E is a pentacentric complex in chromosomes 1,3,10,18 and 20.

F shows non-homologous translocations: t(1;9)(p22;q12), t(1;12)(p34;q22), t(2;X)(q33;q26), t(4;5)(p16;q13), t(6;9)(p23;q22), t(7;12)(q32;q24), and t(12;X)(p12;q22).

G shows intrachromosomal exchanges: ct del(4)(q21q26), ct del(6)(q15 q23), and ct del(X)(p11q13).

H is ring chromosomes: r(2), r(2), r(5), and a ring 2 chromosome with a breakpoint at 2p13.

I shows chromosomes with double breaks: chromosome 1 at p34 and q32, chromosome 3 at p23 and q27, both forming ring-like configuration. (The breakpoints were recorded according to ISCN, 1985)

Table 4. The breakpoints in the interchanged and translocated chromosomes observed in daunomycin metaphases.

breakpoint	chroinatid exchange	translocation	total break	breakpoint	chroinatid exchange	translocation	total break
1p21	1p32,10p14, 15q23	4q26, 5p14	5	6p23	2q33, 5q13, 9q22,14q24, 17q21	3p23, 15q15	7
1p32	1p21, 1p32, 2p13, 3q24, 5p14,10q25, 12q21,17q21	1q42, 9p21, 11p14, 13q34	12	8q22	1p34,2p16, 2q31,3p14, 5q15,13q31 18q12		7
1p34	3p23,3p25 8q22,10q21	12q22	5	9q22	4q27,6p23, 6q21,7q22, 14q23,14q31	9q31	7
1q25	2p24,3p24, 4q22,8q21, 12p12		5	10p14	1p21,10q26, 11q23, 16q22	4p15	6
1q31	1q23,3p14, 6q25,12q24, 12q24,13q13 15q24		7	11.p14			
2p16	3p14,8q22, 8q24,13q21, 17q21		5	12q24	1p13,1q31, 1q31,1q42, 3p14,3p23, 7p14,7q32 4q23	3q27,4q26, 19p13	12
3p14	1q31,2p16, 2q32,5q21, 5q23,8q22, 12q24, Xq25	4q31,9p21, 14q31	11	15q15	8q11,15q15, 17q22,19p12	6p23	5
3q27	8q21,15q23, 15q23,16p11, Xq25	12q24, 15q25	7	15q22	1q21,2p22, 5q31,8q13	7q32	5
5q13	4p16,6p23, 10q22,13q13, 22q12	1p21,6q26	7	20q12	1q21,10q24, 12q14,20q12, 20q12		5
5q31	13q13,13q21, 15q22,15q25, 16q12	3p13,4q31, 11q13	8	Xq22	2p24,2q35, 4q31,12q13	12p12	5

This table only shows the breakpoints with frequency more than 1.0% of the total recorded chromosomal abnormalities.

(such as aphidicolin) [3,10,11,21,36], alkylating agents (such as busulfan) [36], and DNA repair inhibitors (such as caffeine) [7,21]. Many similar chromosome fragile sites are induced by diverse mutagens [6,9,21,35,37]. So far, certain chromosome defects are consistently associated with some types of human cancer [2,4,8,13,14,18,20,28,29,32,34]. Further, many fragile sites are correlated with specific cancer chromosome breakpoints and some oncogene locations [8,11,14,18,28,34,37].

Daunomycin (daunorubicin) is an anthracycline antibiotics and is clinically used to treat leukemias. It is able to intercalate into the double-strand DNA to kill neoplastic cells, or it may inhibit topoisomerase II by stablizing a covalent enzyme-DNA complex [33]. The present study is designed to detect the frequency and distribution of daunomycin-sentive sites in human lymphocytes.

As shown in table 2, the frequency of daunomycin-induced chromosome breakage is significantly higher than that of control ($p<0.025$). A total number of 193 chromosome

Table 3. Locations of daunomycin and caffeine sensitive sites,and their relation to specific cancer chromosome breakpoints and mapped oncogenes and to certain mutagen-inducible fragile sites.

band	Dau	Dau + Ca	Ca	cancer chromosomes with known breakpoints [20,35,36]	oncogene location	mutagen-fragile site
1p21	**	***				FdU; MTX [36]
1p22	*	*		neuroblastoma; breast adenocarcinoma [20,34,36]		Apc [21,36]
1p31	*	*		disseminated-neuroblastoma [20]		
1p32	***	*****		neuroblastoma;ALL [2,20]	L-myc	Apc [3,21,36]
1p34.3	*	**				
1q21	*	*				Apc;Aza;Bus [36]
1q24	*	*				
1q31	*	**				
1q41	*	*				
2p13	**	***		NHL;ALL;B-CLL [2,3,20]		
2p16	*	**				Apc[36]
2p24	*	**		leiomyoma [20,36]	N-myc	FdU; MTX [36]
2q12	*	*				
2q22	*	*				
2q31	*	*				Apc [3,10,11]
2q32	**	**				
2q33	*	**		ANLL [2,20]		
3p12	*	*		malignant fibrous histiocytoma [20]		
3p14	***	****	**	SCLC;ALL;breast adenocarcinoma; familial renal carcinoma;mixed salivary gland tumor; rhabdomyosarcoma [4,13,19,20,29,31,32,34,35]		Apc;Aza;Ca; FdU;MTX [21,27,36,37]
3p21	*	*				
3p24	**	***				Apc [21,36]
3q 13.2	**	***				
3q22	*	*		Lennert's lymphoma;CML [2,20]		
3q24	*	*				
3q26.2	*	**		MPS;ANLL [20,36]		
3q27	*	**		NHL [20,36]		
4p15.2	**	***				Apc[36]
4q21	**	**		bilineal ALL/ANLL [34,36]		
4q24	*	#				
4q26	*	**				
4q27	*	**			raf 2	
4q31.3	*	**				
4q33	*	*				
5p14	**	**				Apc[36]
5q12	*	*				

Table 3. (continued)

5q13	*	*		refractory anemia; MDS; ALL; ANLL [2,20,36]		
5q15	*	**		ANLL-M3; MDS [20]		
5q21	*	**				
5q31	**	**		ANLL; MDS [20,34,36]		
5q32	*	*				
15q34	*	*		ANLL-M2 [20]	c-fms	
6p23	*	**		ANLL [20]		
6q13	*	*				
6q15	*	**		ovary adenocarcinoma; B-PLL; ALL [20,34,36]		
6q21	**	**		OPA; ALL; PLL; ANLL-M5b [20,36]	ros; slk	
6q22	**	**			c-ras; myb	
6q25	*	**				
7p14	*	**				Apc; FdU [36]
7p22	*	#				
7q11.2	*	*				
7q21	**	**		leiomyoma; ANLL [2,36]		Apc [3,36]
7q22	*	*				
7q31	**	**		ANLL; MDS [20,36]	met; kit	MTX [1,36]
7q32	*	*	*	leiomyoma [20]		Apc; Ca; FdU [21,36]
8p11.2	*	*				
8p22	*	*				
8q21	*	**		MPT [34,36]		
8q22	**	**		ANLL-M2 [20]	c-mos	Apc; FdU; MTX [21,36]
8q23	*	*				
9p12	*	*				
9p21	*	**				
9p23	*	#				
9q12	*	*				Aza [27]
9q22	**	**		ANLL [20]		Apc [36]
10p13	*	**		ANLL [34,36]		
10p14	*	*		ANLL-M4,M5 [20]		
10q21	*	*				
10q23	*	**				
10q25	*	*				BrdU [26]
11p14	*	**		Wilms'tumor [20,34,36]		Apc [21]
11q13	*	*		NHL; CML; PLL; B-CLL; RAEB [20,36]	bcl 1; int 2	Bus; MTX [36]
11q14	**	****	*	MDS; NHL; CLL [20,35,36]		FdU; MTX [36]
11q23	*	*		CML; CLL; ALL; ANLL-M4,M5; MDS; Ewing's sarcoma; neuroepithelioma [20,36]	ets 1	Apc [1 1,21]

Table 3. (continued)

12p12	**	**		BALL; CML; ANLL-M5a [20]	k-ras 2	
12q13	*	**		T-cell lymphoma; ALL; salivary gland adenocarcinoma; myxoid liposarcoma [34,35,36]		
12q21	*	**				Bus;FdU; MTX [21,36]
12q24.1	**	***		CML [20]		Apc; BrdU [21,30]
13q13	*	**				Apc [3]
13q21	*	***				
13q22	**	**		NHL; B-CLL; astrocytoma-III,IV [2,20]		
14q21	*	**		ANLL [20]		
14q23	*	*				Apc [3,36]
14q24	**	**		OPA [20,34]		FdU; MTX [1,36]
15q15	*	#				
15q21	*	**				
15q22	*	*		ANLL-M3 [20]		Apc [36]
15q23	*	*				
15q25	*	*				
16p11.2	*	#				Aza; Bus [27,36]
16p13	*	**		AMLEO; ANLL-M4,M5 [20,36]		
16q22	*	*	*	ANLL-M2 [20] BrdU;Ca;Dist A		[21,23,27]
17p12	*	*				Dist A [36]
17q21	*	**		ANLL-M3 [34,36]	erb A1	
17q23	*	**		CML; B-CLL [20]		Apc [21,36]
18q21	*	*				
20q12	*	*				
22q12	*	*	*			
22q13	*	#				
Xp22.3	*	**	*			Apc; Ca; FdU [21,36]
Xq21	*	*	*			
Xq22	*	*	*			Apc [21,36]

a. This table presents the daunomycin-induced break sites with frequency more than 1 0 breaks. * =1 0-29 breaks. ** =30-49 breaks. *** =50-69 breaks. **** =70-89 breaks. ***** =break number more than 90. # =break number lower than 1 0.

b. Abbreviations: ALL=acute lymphocytic leukemia; AMLEO=acute myelomonocytic leukemia with eosinophilia; ANLL=acute non-lymphocytic leukemia;B-CLL=B-cell chronic lymphocytic leukemia; B-PLL=B-cell prolymphocytic leukemia; CML=chronic myelomonocytic leukemia; MDS=myelodysplastic syndrome; MPT=mixed parotid gland tumor; MPS=myeloproliferative syndrome; NHL=non-Hodgkin's lymphoma; OPA=ovarian papillary adenocarcinoma; RAEB=refractory anemia with excess of blasts; SCLC=small cell lung cancer.

c. Apc=aphidicolin; Aza=5-azacytidine; BrdU=bromodeoxyuridine; Bus=busulfan; C: caffeine; Dist A=distamycin A; FdU=fluorodeoxyuridine; MTX=methotrexate.

bands showing breakage at least once in one or more of the ten individuals (Table 2).

Among these daunomycin-induced break sites, band 1p32 is the most frequent one, accounting for 2.2% of the total 2773 breaks. Other break sites induced by daunomycin in decreasing order are 3p14 (2.0%), 3p24 (1.5%), 11q14 (1.5%), 2p13(1.4%), 6q23(1.4%), 14q24(1.4%), 9q22(1.4%), 1p21(1.3%), 2q32(1.3%), 7q31(1.3%), 3q13(1.2%), 7q21(1.2%), 4q21(1.2%), 6q21(1.2%), 12q24(1.2%), 8q22(1.2%), 5p14(1.1%), 5q31(1.1 %), and 12p12(1.1%) (Table 3). All these sites mentioned above constitute 27% of the total 2773 breaks in the ten individuals.

Break at 1 p32 is also present in neuroblastoma and acute lymphocytic leukemia [20]. Band 3p14 is known to be the most common fragile sites in human [6,24,37], and such chromosome breakpoint usually presents in small cell lung carcinoma, familial renal carcinoma, mixed salivary gland tumor, acute lymphocytic leukemia, breast carcinoma, and rhabdomyosarcoma [4, 13, 19, 20, 29, 31, 32, 34, 35]. The induction of this break site (3p14) can be enhanced proportionally by certain culture conditions such as exposure cells to caffeine [17,24]. Band 11 q 14 is a breakpoint appearing in myelodysplastic syndrome, non-Hodgkin's lymphoma and chronic lymphocytic leukemia cells [20]. Breakpoint 2p13 is usually seen in non-Hodgkin's lymphoma, acute lymphocytic leukemia and B-cell lymphocytic leukemia [3,20]. Band 6q23 is the location of oncogene c-ras and myb [34,36]. Site 14q24 usually appears in ovarian papillary adenocarcinoma [20,34]. The other daunomycin-sensitive sites that correlate with cancer chromosome breakpoints or oncogene locations are listed in Table 3.

Caffeine, by inhibiting the DNA repair of replicating cells, is generally used as a mutagenic enhancer [7,17,36]. The number of chromosome breaks in the treatment of daunomycin with caffeine metaphases are much more than that of the daunomycin alone (Table 2). There are 201 chromosome bands presenting breakage in cells exposure to daunomycin with caffeine (Table 2). Such result indicates that although caffeine significantly enhances the induction of chromosome breakage, it does not proportionally increase the expression of fragile sites. Caffeine itself can induce the expression of certain fragile sites such as 3p14,16q22,Xp22, and Xq22 [21]. However, a possible repression of sites 4q24, 7p22, 9p23, 15q15, 16p11.2, and 22p13 is noted when caffeine is used in combination with daunomycin (Table 3).

There are also many types of structural chromosomal aberrations observed in daunomycin metaphases except for breaks (Fig. 1). The interchanges between homologous chromosomes (Fig. 1A) implicate the existence of somatic pairing and recombination during mitosis. The interchanges between non-homologous chromosomes (Fig. 1 C, 1 D) imply the transitional stage of forming the translocations and other structural aberrations. Unequal intrachromosomal exchange configurations (Fig. 1G) can be interpreted as an intermediate step pertaining to chromosomal deletions, duplications or inversions. Besides, there are approximate 1.1 % of ring chromosomes in daunomycin-treated cells (Fig. 1 H). Thirty percent of the total 43 ring chromosomes occurs at chromosome 2. A possible process of ring chromosome formation is shown in Fig. 1 1, the rejoining of two breaken sites at' the different points in the same chromosome leads to the ring configuration.

Table 4 shows the breakpoints in an frequency of more than 1.0% of the analyzed interchanged and translocated chromosomes. In these chromosomal lesions, many of the break sites such as 1p32, 3p14, 3q27, 5q13, 5q31, 6p23, 8q22, 9q22, 10p14, 12q24 and 15q22 are seen in certain cancer chromosome defects (Table 3).

Our results demonstrate that daunomycin has a potent effect in the induction of chromosome breaks as well as chromosomal structural defects. Most of the daunomycin-sensitive sites also appear in fluorodeoxyuridine, methotrexate, aphidicolin and other mutagens. The consistence of particular chromosome breakpoints in certain tumor cells implicates the plausible relationship between

these sites and the tumor formation. All of the findings together suggest that such fragile sites in human chromosomes may be the common spots of mutagenesis and carcinogenesis.

ACKNOWLEDGEMENTS

We are grateful to Dr. Te-Chung Liu and Dr. Tien-Ling Lee for their reviews of the manuscript and valuable suggestions, and to Chin-Chu Tsai, Chin-Hwa Yet and Wei-Hsiung for their techinical assistance. We would also like to thank Kun-Yen Jan, Ph.D., Institute of Zoology, Academia Sinica, for reviewing this manuscript. This study was supported by a grant of the National Science Council, Republic of China (NSC-81-0418-B040-501).

REFERENCES

1. Barbi G, Steinbach P, Vogel W: Non-random distribution of methotrexate-induced aberrations on human chromosomes. Detection of further folic acid sensitive fragile sites. Hum Genet 1984; 68: 290-294.
2. Berger R, Bloomfield CD, Sutherland GR: Report of the committee on chromosome rearrangements in neoplasia and on fragile sites. Human e Mapping 8, Helsinki Conference. Cytogenet Cell Genet 1985; 40:490-535.
3. Craig-Holmes AP, Strong LC, Goodacre A, Pathak S: Variations in the expression of aphidicolin-induced fragile sites in human lymphocyte cultures. Hum Genet 1987; 76: 134-137.
4. Cohen AJ, Li FP, Berg S, Marchetto DJ, Tsai S, Jacobs SC, Brown RS: Hereditary renal-cell carcinoma associated with a chromosomal translocation. N Engl J Med 1979; 301: 592-595.
5. Croci G: BrdU-sensitive fragile sites on long arm of chromosome 16. Am J Hum Genet 1983; 35: 530-533.
6. Daniel A, Ekblom L, Philip S: Constitutive fragile sites 1p31, 3p14, 6q26 and 16q23 and their use as controls for false-negative results with fragile (X). Am J Med Genet 1984; 18: 483-491.
7. Das SK, Lau CC, Pardee AB: Comparative analysis of caffeine and 3-aminobenzamide as DNA repair inhibitors in Syrian baby hamster kidney cells. Mutat Res 1984; 131: 71-79.
8. de Brackeleer M, Smith B, Lin CC: Fragile sites and structural rearrangements in cancer. Hum Genet 1985; 69: 112-116.
9. Glover TW: FudR induction of the X chromosome fragile site: Evidence for the mechanism of folic acid and thymidine inhibition. Am J Hum Genet 1981; 33: 234-242.
10. Glover TW, Berger C, Coyle J, Echo B: DNA polymerase alpha inhibition by aphidicolin induces gaps and breaks at common fragile sites in human chromosomes. Hum Genet 1984; 67: 136-142.
11. Hecht F, Glover Tw: Cancer chromosome breakpoints and common fragile sites induced by aphidicolin. Cancer Genet Cytogenet 1984; 12: 185-189.
12. Hecht F, Sutherland GR: Detection of the fragile X chromosome and other fragile sites. Clin Genet 1984; 26:301-303.
13. Hecht F, Sutherland GR: Fragile sites and cancer breakpoints. Cancer Genet Cytogenet 1984; 12:179-181.
14. Hecht F, Sandberg AA: Of fragile sites and cancer chromosome breakpoints. Cancer Genet Cytogenet 1988; 31: 1-3.
15. ISCN: An international system for human cytogenetic nomenclature. Birth Defects Orig. Art Ser 21: 46-47, 1985.
16. Jacky PB, Sutherland GR: Thymidylate synthetase inhibition and fragile site expression in lymphocytes. Am J Hum Genet 1983;35: 1276-1283.
17. Kihlman BA, Hansson K, Andersson HC: The effect of post-treatments with caffeine during S and G2 on the frequencies of chromosomal aberrations induced by thiotepa in root tips of Vicia faba and in human lymphocytes in vitro. Mutat Res 1982; 104: 323-330.
18. Le Beau MM: Editorial: chromosomal fragile sites and cancer-specific breakpoints - a moderating viewpoint. Cancer Genet Cytogenet 1988; 31: 55-61.
19. Mark J, Dahlenfors R, Ekedahl C: Cytogenetics of the human benign mixed salivary gland tumor. Hereditas 1983; 99: 115-129.
20. Mitelman F: Catalog of chromosome aberrations in cancer,4th Ed: A John Wiley and Sons Inc, New York, 1 99 1.
21. Rao PN, Heerema NA, Palmer CG: Fragile sites induced by FudR, caffeine, and aphidicolin: Their frequency, distribution, and analysis. Hum Genet 1988; 78: 21-28.
22. Rosner B: Fundamentals of biostatistics, Boston. PWS -Kent publishing company, pp 474-526,1990.
23. Schmid M, Klett C, Niederhofer A: Demonstration of a heritable fragile site in human chromosome 16 with distamycin A. Cytogenet Cell Genet 1980; 28: 87-94.

24. Smeets DCFM, Scheres JMJC, Hustinx TWJ: The most common fragile site in man is 3p14. Hum Genet 1986; 72: 215-220.
25. Sutherland GR: Heritable fragile sites on human chromosome VIII. Preliminary population cytogenetic data on the folic-acid-sensitive fragile sites. Am J Hum Genet 1982; 34: 452-458.
26. Sutherland GR: Heritable fragile sites on human chromosomes. IX. population cytogenetics and segregation analysis of the BrdU requiring fragile sites at 10q25. Am J Hum Genet 1982; 34: 753-756.
27. Sutherland GR, Parslow MI, Baker E: New classes of common fragile sites induced by 5-azacytidine and BrdU. Hum Genet 1985; 69: 233-237.
28. Takahashi E, Kaneko Y, Ishihara T, Minamihiisamatsu M, Murata M, Hori T: A new rare distamycin A-inducible fragile site, fra(11)(p 15. 1), found in two acute nonlymphocytic leukemia(ANLL)patients with t(7;11)(p13;p15). Hum Genet 1988; 80: 124-126.
29. Trent J, Casper J, Merltzer P, Thompson F, Fogh J; Nonrandom chromosome alterations in rhabdomyosarcoma. Cancer Genet Cytogenet 1985; 16: 189-197.
30. Voiculescu 1, HausmannC, Wolff G, Back E: A BrdU-requiring fragile site on chromosome 12. Hum Genet 1988;78:183-185.
31. Wang N, Perkins KL: Involvement of band 3p14 in t(3;8) hereditary renal carcinoma. Cancer Genet Cytogenet 1984; 11: 478-481.
32. Whang-Peng J, Kao-shan CS, Lee EC: Specific chromosome defect associated with human small-cell lung cancer: deletion 3p(14-23). Science 1982; 215: 181-182.
33. Wingard LB, Brody TM, Larner J Schwartz A: Individual antineoplastic drugs. In Human Pharmacology- molecular to clinical. London: Wolfe Publishing Limited, pp598-600, 1991.
34. Yunis JJ: The chromosomal basis of human neoplasia. Science 1983; 221: 227-236.
35. Yunis JJ, Soreng AL: Constitutive fragile sites and cancer. Science 1984; 226: 1190-1204.
36. Yunis JJ, Soreng AL, Bowe AE: Fragile sites are targets of diverse mutagens and carcinogens. Oncogene 1987; 1: 59-69.
37. Zhou X, Xu B, Chu C, Xia G, Li N, Sha R: Human chromosome hot points. 1. Hotpoint at 3p14 in three populations. Hum Genet 1984; 67: 249-251.

CSG51.02

J Genet Mol Biol 1994; 5: 27-31
Published by the Chinese Society of Genetics, Taipei, Taiwan, ROC

FREQUENCY AND DISTRIBUTION OF CCNU-INDUCED FRAGILE SITES IN CULTURED HUMAN LYMPHOCYTES

JUNG-CHIN LIOU, AND SHUAN-YOW LI

The frequency and localization of CCNU-induced fragile sites was studied in cultured human peripheral lymphocytes. We used caffeine as the mutagenic enhancer. Our results showed that there was a significantly higher frequency ($p < 0.005$) of chromosomal breakage in CCNU-treated metaphases than in controls. A frequency of 4% or more of total breaks was applied to eliminate apparent random breaks that were observed. Based on this criteria, there existed 14 CCNU-sensitive sites on human lymphocyte chromosomes. Most of the CCNU-induced fragile sites are also present in cells which were exposed to other mutagenic agents. Furthermore, some of these CCNU-sensitive sites were coincident with certain cancer chromosome breakpoints. Therefore, such fragile sites on chromosomes may be the hot spots of mutagenesis and carcinogenesis.

Keywords: CCNU, chromosomal fragile site, chromosomal breakage, mutagens

Chromosomal fragile sites are weak points on chromosomes that have a tendency to break under certain culture conditions. The breaks lead to the subsequent formation of chromosomal deletions, translocations, inversions, ring chromosomes, and other rearrangements. Fragile sites are inducible in low concentration of folic acid or thymidine culture medium [1,9,32]. They also can be induced by. distamycin A [14,28] bromodeoxyuridine [33,34], methotrexate [1,42], fluorodeoxyuridine [25,36] aphidicolin [4,10,11,25,42], 5-azacytidine [34,37], busulfan [42], and daunomycin [21]. Besides, diverse mutagens which act through different molecular mechanisms can induce many similar fragile sites[5,10,41,42].

CCNU[lomustine; 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea], an alkylating agent, is frequently used to treat lymphoma as well as certain solid tumors, in particular brain tumors[8,38,40]. The alkylation of CCNU to DNA is the major cause of cell death during antineoplastic therapy[40]. It has been proved to be a potent sister chromatid exchange (SCE) inducer in human lymphocytes[3].

This study was designed to examine the frequency and localization of CCNU-induced fragile sites in cultured human peripheral lymphocytes. In addition, we used caffeine to enhance the expression of CCNU-induced break sites[6,17,25,42]. Using a criteria of a frequency of 4 % or more of total break sites in one individual to eliminate random breaks that were observed[25]. Our results showed that there exist 14 CCNU fragile sites on human lymphocytes. Some of them are also seen in other mutagen-induced fragile sites.

MATERIALS AND METHODS

To test the cytotoxicity and the most

Cytogenetics Laboratory, Chung Shan Medical and Dental College, Taichung, Taiwan, Republic of China

effective concentration of the CCNU in the induction of chromosomal breakage, we carried out a large number of preliminary experiments on lymphocytes from five young individuals. Then a concentration of 10^{-5} M CCNU was chosen as the most appropriate concentration for the following studies. We applied caffeine to enhance the induction of CCNU fragile sites[42].

Peripheral blood from eight healthy adults (four males and four females) were cultured in RPMI-1640 medium. The medium was supplemented with 15% fetal calf serum (GIBCO.), 1% PSN antibiotics mixture (penicillin, streptomycin and neomycin, GIBCO), and 2% PHA (phytohemagglutinin, GIBCO)[20]. There were four cultures (1,2,3,4) (see Table 1) from each individual, and they were incubated for 96 h. Before harvrst, CCNU (10^{-5}M final concentration, Sigma) was added to the cultures 1 and 2. Twenty four hours later, all cultures were washed twice with fresh RPMI-1640 medium. Then the cultures 2 and 3 were exposed to caffeine (2.5mM final concentration, Sigma) for the last 6 h of incubation. Mitotic divisions were arrested by treatment with colcemid (0.05 ug/ml, GIBCO) for the last 20 min of incubation. Then all cells were treated with 0.075 M hypotonic KCl for 16 min, and they were fixed by three changes of 3 : 1 methanol : glacial acetic acid fixative. Cells in fresh fixative were dropped onto clean slides. The chromosomes were Trypsin-G banded with Wright's stain[19] The prepared chromosomes were at 400- 500 band level.

One hundred metaphases from each treatment were randomly scored with microscope. The chromosomal breakpoints were recorded according to the international system for cytogenetic nomenclature[15]. Analysis of variance and multiple comparison tests[26] were done to compare the frequency and spectrum of CCNU-induced fragile sites.

RESULTS

Assessment of CCNU dose

The frequency of chromosomal breakage was correlated with the CCNU concentrations. When 10^{-4} M CCNU was added to the cultures for 24 h, most of the cells were killed. However, when 10^{-6}M CCNU was introduced for the same duration, the occurrence of chromosomal breaks was too low (23.5±5.9, mean breaks per individual ± SD). When cells were exposed to 10^{-5}M CCNU for 24 h, there was an obvious increase of breakage (135.6± 47.8) (data not shown). Therefore, a treatment of 10^{-5}M CCNU for 24 h before cell harvest was used as the standard protocol for this study.

Frequency and Localization of CCNU-induced fragile sites

There were 135 chromosome bands showing breakage at least once in one or more individuals (Table1). The frequency of chromosomal breakage induced by CCNU alone was not very high (94.4±19.6). Moreover, we did not find any chromosomal structural aberrations in CCNU metaphases other than

Table 1. Comparison of chromosomal breakage induced by CCNU, CCNU with Caffeine, and Caffeine.

culture	treatment	no.of bands showing breakage	mean breaks/ individual (±SD)	mean breaks/ cell
1	CCNU	135	94.4 ± 19.6	0.94
2	CCNU + Caffeine	154	236.3 ± 44.4	2.36
3	Caffeine	92	61.8 ± 16.6	0.62
4	Control	11	1.5 ± 1.2	0.02

a. The final concentration of CCNU is 10^{-5} M.

b. The final concentration of Caffeine is 2.5 mM.

c. Cultures not treated with CCNU or Caffeine were served as control.

d. $P<0.005$.

請作者自訂"題標"

Frequency and distribution of

Table 2. Analysis of CCNU-inducible chromosomal breakage

subject	1	2	3	4	5	6	7	8	total breaks	total individuals
breaks	110	90	104	93	69	120	64	105	755	8
breakpoint										
1p34						5 (4.2)[a]			5 (0.6)[b]	1 (1 2.5)[c]
2p15							4 (6.3)		4 (0.5)	1 (12.5)
2p23			7 (6.7)						7 (0.9)	1 (12.5)
3p13					5 (4.2)				5 (0.6)	1 (12.5)
3p23	5 (4.5)	5 (5.6)		3 (4.3)					13 (1.7)	3 (37.5)
3q26.2	5 (4.5)	4 (4.4)	6 (5.8)					5 (4.8)	20 (2.6)	4 (50.0)
4q23						5 (4.2)	3 (4.7)		8 (1.1)	2 (25.0)
4q3l.3	7 (6.4)	5 (5.6)							12 (1.6)	2 (25.0)
5q2l.2		7 (7.8)	6 (5.8)		5 (4.2)				18 (2.4)	3 (37.5)
9ql2	22 (20.0)	17 (18.9)	18 (17.3)	22 (23.7)	15 (21.7)	22 (18.3)	12 (18.7)	20 (19.0)	128 (17.0)	8 (100)
12q22						6 (5.0)		5 (4.8)	11 (1.5)	2 (25.0)
13q2l.2	6 (5.5)		5 (4.8)	4 (4.3)					15 (2.0)	3 (37.5)
13q3l		4 (4.4)							4 (0.5)	1 (12.5)
Xp22.3		4 (4.4)							4 (0.5)	1 (12.5)
14[d]									254 (33.6)	

a. Numbers in parenthesis represent % of total breaks in the individual.
b. Numbers in parenthesis represent % of total breaks in all individuals.
c. Numbers in parenthesis represent % of individuals.
d. Total number of breakpoints.

breaks.

In order to justify the presence of a fragile site in an individual, a frequency of 4% of total breaks was applied to eliminate the random breaks[25] Under this criteria, there were 14 or 10.4% (14/135) that occurred with 4% or more of total breaks in one or more subjects (Table 2). Among these 14 fragile sites, 6/14 (42.9%) expressed more than 4% of frequency in one person. The other 8 sites were present in two or more individuals. The most frequent fragile site was 9ql2 with 17.0% of the 755 total breaks, and this site appeared in all of the eight persons. Band 3q26.2 was the second most common site, accounting for 2.6% of the total breaks. Other sites of breakage induced by CCNU in

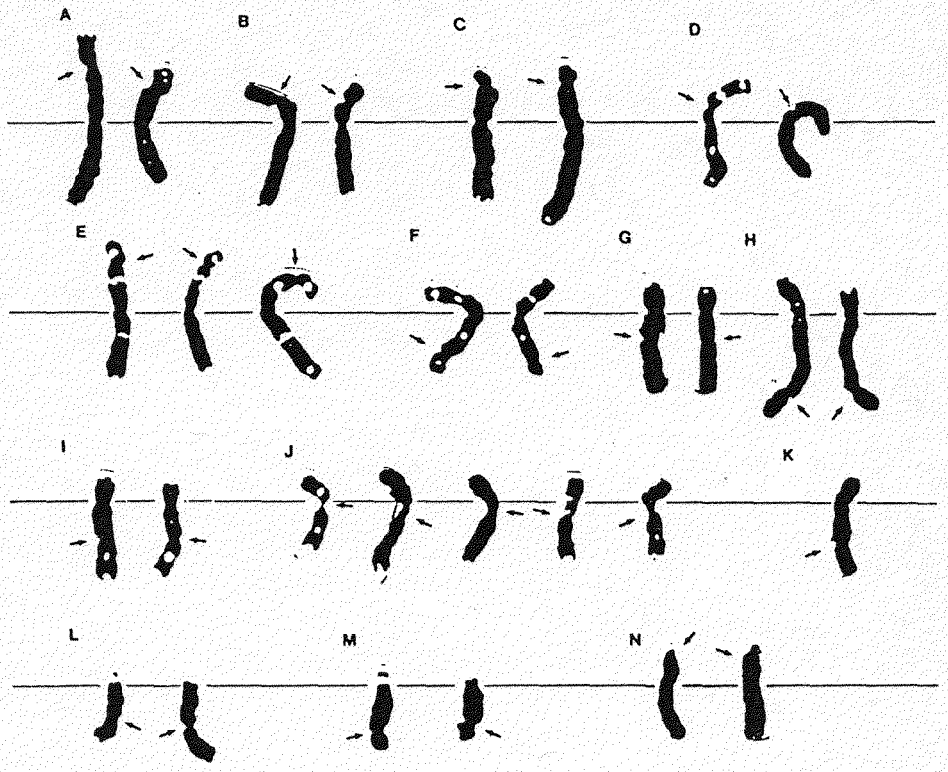


Fig.1. A typical example of CCNU-induced fragile sites in chromosomes of cultured human peripheral lymphocytes. The breakpoints are at : Band 1p34 (A), band 2p15 (B), band 2p23 (C), band 3p13 (D), band 3p23 (E), band 3q26.2 (F), band 4q23 (G), band 4q31.3 (H), band 5q21.2 (I), band 9q12 (J), band 12q22 (K), band 13q21.2 (L), band 13q31 (M), and band Xp22.3 (N). (The identification of chromosome breakpoints was according to ISCN,1985).

decreasing order were: 5q21.2 (2.4%), 13q21.2 (2.0%), 3p23 (1.7%), 4q31.3 (1.6%), 12q22 (1.5%) and 4q23 (1.1 %) (Table 2). All these 14 sites constituted 33.6% of the total fragile sites in the eight individuals. A gallery of CCNU-induced fragile sites was shown in Figure 1.

Enhancement of caffeine in induction of fragile sites

Caffeine was usually used as a mutagen enhancer [6,17,42]. After washing twice, the cells were treated with caffeine for 6 h. Our results showed that the frequency of chromosomal breakage in CCNU+Caffeine-treated metaphases was significantly higher ($p<0.005$) than that in CCNU-induced metaphases (Table 1). The distribution of CCNU+Caffeine-induced breakage was listed in Table 3. Of the 13 fragile sites, band 9q12 was present most frequently and accounted for 8.6% of the 1890 total breaks. Band 3p14 had a frequency of 4.7% in total breaks. Both these sites were seen in all of the eight subjects. Other sites exhibiting breaks in decreasing order were 3q26.2 (3.6%), Xp22.3 (2.3%), and 4q31.3 (1.1 %) (Table 3). All of these 13 sites represented only 25.3% of the total breaks. Thus, although caffeine powerfully enhanced the induction of chromosomal breakage, it did not only work on certain sites of the chromosomes.

Table 4 summarized the fragile sites which were found in caffeine-treated metaphases. Of

Table 3. Analysis of CCNU+Caffeine-inducible chromosomal breakage.

(Footnotes see Table 2)

subject	1	2	3	4	5	6	7	8	total breaks	total individuals
total breaks	269	210	268	230	231	239	149	294	1890	8
breakpoint										
1p31						10	6		16	2
						(4.2)[a]	(4.0)		(0.8)	(25.0)[c]
1q23				10					10	1
				(4.3)					(0.5)	(12.5)
3p14	15		13	12	16	12	9	12	89	7
	(5.6)		(4.9)	(5.2)	(6.9)	(5.0)	(6.0)	(4.1)	(4.7)	(87.5)
3q23							6		6	1
							(4.0)		(0.3)	(12.5)
3q26.2	12	9	15		10		10	12	68	6
	(4.5)	(4.3)	(5.6)		(4.3)		(6.7)	(4.1)	(3.6)	(75)
4q31.3			10		11				21	2
			(3.7)		(4.8)				(1.1)	(25.0)
5p14			12						12	1
			(4.5)						(0.6)	(12.5)
9q12	25	12	15	24	26	27	10	24	16	3 8
	(9.2)	(5.7)	(5.6)	(10.4)	(11.3)	(11.3)	(6.7)	(8.2)	(8.6)	(100)
12q13	12								12	1
	(4.5)								(0.6)	(12.5)
12q22								13	13	1
								(4.4)	(0.7)	(12.5)
13q21.2		10					6		16	2
		(4.8)					(4.0)		(0.8)	(25.0)
13q31					10				10	1
					(4.2)				(0.5)	(12.5)
Xp22.3		12	10	10	11				43	4
		(5.7)	(3.7)	(4.3)	(4.8)				(2.3)	(50.0)
13[d]									479	
									(25.3)	

Table 4. Analysis of caffeine-induced chromosomal breakage (Footnotes see Table 2)

Subject	1	2	3	4	5	6	7	8	total breaks	total individuals
total breaks	61	41	80	80	67	59	35	71	494	8
breakpoint										
1P31	4 (6.6)[a]								4 (0.8)[b]	1 (12.5)[c]
1q23								4 (5.6)	4 (0.8)	1 (12.5)
2q32								3 (4.2)	3 (0.6)	1 (12.5)
3P14	20 (32.8)	8 (19.5)	17 (21.3)	14 (17.5)	13 (19.4)	10 (16.9)	6 (17.2)	12 (16.9)	100 (20.2)	8 (100.0)
3q13		2 (4.9)							2 (0.4)	1 (12.5)
3q26.2	3 (4.9)				3 (4.5)		2 (5.7)	4 (5.6)	12 (2.4)	4 (50.0)
4q31.3						3 (5.1)		3 (4.2)	6 (1.2)	2 (25.0)
5q31						3 (5.1)			3 (0.6)	1 (12.5)
6p23						3 (5.1)			3 (0.6)	1 (12.5)
6q15						3 (5.1)			3 (0.6)	1 (12.5)
6q26			3 (3.8)						3 (0.6)	1 (12.5)
7q32			4 (5.0)		3 (4.5)			5 (7.0)	12 (2.4)	3 (37.5)
11q14	4 (6.6)	2 (4.9)							6 (1.2)	2 (25.0)
11q21			4 (5.0)	5 (6.3)		3 (5.1)			12 (2.4)	3 (37.5)
16q23			4 (5.0)						4 (0.8)	1 (12.5)
XP22		4 (9.8)	5 (6.3)	4 (5.0)	7 (10.4)	3 (5.1)		2 (5.7)	25 (5.1)	6 (75.0)
Xq22		4 (5.0)		4	(6.0)	3	(8.6)	11	3 (2.2)	(37.5)
17[d]								213 (43.1)		

Table 5 Unique and common fragile sites due to CCNU and caffeine.

treatment	sites
Unique to CCNU	1p34,2p15, 2p23, 3p13, 3p23,4q23, 5q21.2,9q12,12q22, 13q21.2, 13q31
common to CCNU and caffeine	3q26.2, 4q31.3, Xp22
unique to caffeine	1p3l, 1q23, 3p14, 2q32, 3q13, 5q31, 6p23, 6q15, 6q26, 7q32,11q14, 11q21, 16q23, Xq22

the detected 17 fragile sites, band 3pl4 were present in all of the eight persons and showed 20.2% of the total breaks. It had been known to be the most common fragile site[31]. The Xp22, 3q26.2, 7q32, Xq22, 4q27,and 11ql4 sites expressed at a frequency more than 1 % of total breaks and in more than one individual. Fragile sites that were unique or common to CCNU and caffeine were listed in Table 5. Of the total 30 sites, the 3q26.2, 4q31.3, and Xp22.3 are commonly present in CCNU and caffeine treatments(Table 5).

DISCUSSION

Recently, a large number of fragile sites have been recognized on human chromosomes. Culturing cells in medium containing low concentration of folic acid or thymidine is one way to induce the expression of certain fragile sites[1,9,32]. Fragile sites are also inducible by diverse agents such as distamycin A [14,28], bromodeoxyuridine [33,34], methotrexate [1,42], busulfan[42], fluorodeoxyuridine [25,36], 5-azacytidine[34,37], aphidicolin [4,10,11,25,42], and daunomycin[21]. Besides, diverse mutagens which act through different molecular mechanisms are able to induce many similar fragile sites[5,9,41,42].

CCNU[lomustine; 1-(2-chroloethyl)-3-cyclohexyl-l-nitrosourea, a nitrosourea, is able to alkylate with DNA to destroy the neoplastic cells. An unsual feature of this agent is its lipid solubility and its ability to penetrate the blood-brain barrier[2,40]. It is effective in treatment of human brain tumors and bronchial tumors as well as in Hodgkin's disease[7,27,30,37]. CCNU has exhibited a potent effect in induction of sister chromatid exchanges in human lymphocytes[3]. The present study is aimed to study the frequency and distribution of CCNU-induced chromosomal breakage.

Our results show that under the experimental conditions used, there is a significantly higher frequency ($p < 0.005$) of chromosomal breakage in CCNU-treated metaphases than in controls (Table1). Moreover, there are no any other chromosomal structural aberrations in the observed CCNU metaphases except for breaks. Large quantities of chromosomal abnormalities, such as translocations, inter- and intra-chromosomal exchanges and ring chromosomes, have been found in cells which were exposed to daunomycin or busulfan[21,42]. Since agents that induce fragile sites may also produce a high number of random breaks, a frequency of 4% or more of total breaks of one individual is used as a means of delineating fragile sites from random breakage[25]. Based on this criteria, there exist 14 CCNU-inducible fragile sites in cultured human lymphocytes (Table 2).

Among these CCNU-sensitive sites, band 9ql2 is the most frequently present one. It accounts for 17% of the total breaks and is seen in all of the eight individuals. The breakpoint 9ql2 has also been shown as an 5-azacytidine- and mitomycin C-inducible fragile sites[13,29]. On human chromosomes, the 9q12 site is a constitutive heterochromatin region which consists of simple sequence repeated DNA [22,23,24]. The constitutive heterochromatin contains no Medelian genes and is never transcribed [16], and so far none of definite function of it has been established. Site 3q26.2 constituted 2.6% of the total breaks, is the second most frequent fragile sites and is present in 50% of the eight subjects. Some acute nonlymphocytic leukemia and myelodysplastic syndrome cells have chromosomal aberrations with breakpoint at 3q26 [24]. Site 5q2l is also an aphidicolin-inducible fragile site [4,42] The 13q2l.2 band is sensitive to bromodeoxyuridine, busulfan, aphidicolin and fluorodeoxyuridine[33,42]. The Wilms'tumor and retinoblastoma cells usually have the chromosome complement of del 13q(13.2-21.2) with a breakpoint at 13q2l.2[24,42]. Many ovarian cancer cells possess the 3pl3 breakpoint[42]. Small cell lung cancer shows frequent presence of a deletion in 3p (14-23) with a break site of 3p23[24,39,42]. All of these 14 CCNU-induced fragile sites account for 33.6% of the total breaks (Table 2).

In consideration of possible mechanisms of the expression of fragile sites, we used caffeine to detect whether the presence of fragile site is due to preferential breakage in these

points or to the results of differential repair of DNA damage. Caffeine, by inhibiting the DNA repair capacity of replicating cells, is generally used as a mutagenic enhancer[6,17,42]. As shown in Table 1, the frequency of chromosomal breakage in CCNU+Caffeine-treated cells is noticeably higher than in CCNU alone. However, although the frequency of breakage is highly induced by combination of CCNU with caffeine, caffeine itself does not show potent effect in induction of the 14 CCNU-sensitive sites (Table2, Table 3). This can be interpreted as most of the CCNU-induced break sites are repaired during the 6 h of incubation after the two washings, and the observed fragile sites are the results of impairment of damaged DNA. With the increase of breakage showing in other sites, the ratio of such above mentioned 14 CCNU fragile sites decreases.

Exposure of cells to caffeine alone produces several fragile sites (Table 4). Sites 3pl4 and Xp22 are the most common two of caffeine-inducible fragile sites[25]. Table 5 shows the unique and common sites sensitive to CCNU and caffeine. The common sites of these two agents are 3q26.2, 4q31.3 and Xp22. On the other hand, sites 5p 1 4 and 12q 1 3 are only induced under combination of CCNU and caffeine. This indicates that the expression of such sites is due to reduced repair ability.

At present, many similar fragile sites are found to be induced by diverse agents which act through different mutagenic mechanisms [5,10,25,41,42]. The similarity of induced fragile sites by different compounds suggests that these regions on chromosomes play the general targets during mutagenesis. Besides, many types of neoplastic cells are known to have specific structural chromosome defects, primarily involving a reciprocal translocation with two breakpoints[24]. Furthermore, the majority of these cancer chromosome aberrations share breakpoints with those mutagen-inducible fragile sites[38,41,42]. The consistence of mutagen-sensitive sites with certain cancer chromosome breakpoints implicates that there is a possible causal relationship between the two[1 1, 1 3,18,41,42]. Since a chromosome band may contain 1,000 or more kilo base pairs, the genes may not necessarilly be within the position of observed cytogenetic breakpoints. However, it is possible that these fragile sites on chromosomes provide the regions for chromosome and gene rearrangements during the malignant process.

ACKNOWLEDGEMENTS

We are grateful to Dr. Tien-Ling Lee for his review of the manuscript and valuable suggestions, and to all of the members in the cytogenetics laboratory of Chung Shan Medical and Dental College for their techinical assistance. This study was supported by a grant of the National Science Council, Republic of China (NSC-81-0418-B040-501).

REFERENCES

1. Barbi G, Steinbach P, Vogel W: Non-random distribution of methotrexate-induced aberrations on human chromosomes. Detection of further folic acid sensitive fragile sites. Hum Genet 1984; 68: 290-294.
2. Bertino JR: Antineoplastic drugs. In Melmon KL, Merrelli HF, Hoffman BB, Neirenberg Dw eds. Clinical Pharmacology: Basic principles in therapeutics. New York: McGraw-Hill, Inc, pp 617-618, 1992.
3. Best RG, Mckenzie WH: Sister chromatid exchange in human lymphocytes exposed to ascorbic acid and the cancer chemotherapeutic agent 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea. Teratol Carcinogen Muta 1988; 8: 339-346.
4. Craig-Holmes AP, Strong LC, Goodacre A, Pathak S: Variations in the expression of aphidicolin-induced fragile sites in human lymphocyte cultures. Hum Genet 1987; 76: 134-137.
5. Daniel A, Ekblom L, Philip S: Constitutive fragile sites 1p3 1, 3pl4, 6q26 and 16q23 and their use as controls for false-negative results with fragile (X). Am J Med Genet 1984; 18: 483-491.
6. Das SK, Lau CC, Pardee AB : Comparative analysis of caffeine and 3-aminobenzamide as DNA repair inhibitors in Syrian baby hamster kidney cells. Mutat Res 1984; 131: 71-79.
7. Fewer D, Wilson CB, Boldrey EB: Phase 11 study of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU, NSC-79030) in the treatment of brain tumors. Cancer Chemother

Rep 1972; 56: 421-427.
8. Friedman MA, Carter SB: Serious toxicities associated with chemotherapy. Semin Oncol 1978; 5: 193-202.
9. Glover TW: FudR induction of the X chromosome fragile site: Evidence for the mechanism of folic acid and thymidine inhibition. Am J Hum Genet 1981; 33: 234-242.
10. Glover TW, Berger C, Coyle J, Echo B: DNA polymerase alpha inhibition by aphidicolin induces gaps and breaks at common fragile sites in human chromosomes. Hum Genet 1984; 67: 136-142.
11. Hecht F, Glover Tw: Cancer chromosome breakpoints and common fragile sites induced by aphidicolin. Cancer Genet Cytogenet 1984; 12: 185-189.
12. Hecht F, Sutherland GR: Detection of the fragile X chromosome and other fragile sites. Clin Genet 1984; 26:301-303.
13. Hecht F, Sandberg AA: Of fragile sites and cancer chromosome breakpoints. Cancer Genet Cytogenet 1988; 31: 1-3.
14. Hori T, Takahasi E, Murata M : Nature of distamycin A-inducible fragile sites. Cancer Genet Cytogenet 1988; 34 189-194.
15. ISCN: An international system for human cytogenetic nomenclature. Birth Defects Orig. Art Ser 21: 46-47, 1985.
16. John B: The biology of heterochromatin. In: Verma RS ed. Heterochromatin: molecular and structural aspects. Cambridge University, Cambridge, pp 1-147, 1988.
17. Kihlman BA, Hansson K, Andersson HC : The effect of post-treatments with caffeine during S and G2 on the frequencies of chromosomal aberrations induced by thiotepa in root tips of Vicia faba and in human lymphocytes in vitro. Mutat Res 1982; 104: 323-330.
18. Le Beau MM: Editorial: chromosomal fragile sites and cancer-specific breakpoints - a moderating viewpoint. Cancer Genet Cytogenet 1988; 31: 55-61.
19. Li SY, Tsai CC, Chou MY, Lin JK: A cytogenetic study of mentally retarded school children in Taiwan with special reference to the fragile X syndrome. Hum Genet 1988; 79: 292-296.
20. Li SY, Lin JK: Differential bleomycin susceptibility in cultured lymphocytes of fragile X patients and normal individuals. Hum Genet 1990; 85: 267-271.
21. Liou JC, Li SY: Daunomycin-induced chromosomal aberrations in cultured human lymphocytes. 1994;(submitted).
22. Miklos GLG, John B : Heterochromatin and satellite DNA in man: properties and prospects. Am J Hum Genet 1979; 31: 264-280.
23. Miklos GLG: Sequencing and manipulating highly repeated DNA. In: Dover GA, Flavell RB eds. Genome evolution. Academic, New York: pp 41-68, 1982.
24. Mitelman F: Catalog of chromosome aberrations in cancer,4th Ed: A John Wiley and Sons Inc, New York, 1999년. 추천
25. Rao PN, Heerema NA, Palmer CG: Fragile sites induced by FudR, caffeine, and aphidicolin: Their frequency, distribution, and analysis. Hum Genet 1988; 78: 21-28.
26. Rosner B: Fundamentals of biostatistics, Boston. PWS-Kent publishing company, pp 474-526,1990.
27. Schein PS, Tew KD, Mathe G: Pharmacology of nitrosourea Antineoplastic chemotherapy (Bukarda B, Karrer K, anti-cancer agents in clinical chemotherapy: Mathe G : eds) vol. 3, Thieme-Strutton, pp 264-282, 1984.
28. Schmid M, Klett C, Niederhofer A: Demonstration of a heritable fragile site in human chromosome 16 with distamycin A. Cytogenet Cell Genet 1980; 28: 87-94.
29. Shaw MW, Cohen MM: Chromosome exchanges in human leukocytes induced by mitomycin C. Genetics 1965; 51: 181-190.
30. Selawry OS, Hansen HH: Superiority of CCNU 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, NSC-79037 over BCNU (1.3-bis 2-chloroethyl-1-nitrosourea); NSC 409962 in treatment of advanced Hodgkin's disease. Proc Am Assoc Cancer Res 1972; 13: 46.
31. Smeets DCFM, Scheres JMJC, Hustinx TWJ: The most common fragile site in man is 3pl4. Hum Genet 1986; 72: 215-220.
32. Sutherland GR: Heritable fragile sites on human chromosome VIII. Preliminary population cytogenetic data on the folic-acid-sensitive fragile sites. Am J Hum Genet 1982; 34: 452-458.
33. Sutherland GR: Heritable fragile sites on human chromosomes. IX. Population cytogenetics and segregation analysis of the BrdU requiring fragile sites at 10q25. Am J Hum Genet 1982; 34: 753-756.
34. Sutherland GR, Parslow MI, Baker E: New classes of common fragile sites induced by 5-azacytidine and BrdU. Hum Genet 1985; 69: 233-237.
35. Tharapel AT, Summitt RL: Minor chromosome variations and selected heterochromorphisms in 200 unclassifiable mentally retarded patients and 200 normal controls. Hum Genet 1978; 41: 121-130.
36. Tommerup N, Poulsn H, Brondum-Nielsen K: 5-Fluoro-2-deoxyuridine induction of the fragile site on Xq28 associated with X-linked mental retardation. J Med Genet 1981; 18: 374-376.
37. Voiculescu I, HausmannC, Wolff G, Back E: A

BrdU-requiring fragile site on chromosome 12. Hum Genet 1988;78:183-185.
38. Walker MD: Nitrosoureas in central nervous system tumors. Cancer Chemother. Rep 1973, 4: 21-26.
39. Whang-Peng J, Bunn PA, Kao-Shan CS Lee EC, Carney DN, Gazdar A, Minna JD: A nonrandom chromosomal abnormality, del3p(14-23) in human small cell cancer (CTCL). Cancer Genet Cytogenet 1982; 6: 119-134.
40. Wingard LB, Brody TM, Larner J Schwartz A: Individual antineoplastic drugs. In Human Pharmacology-molecular to clinical. London: Wolfe Publishing Limited, pp593-596, 1991.
41. Yunis JJ, Soreng AL: Constitutive fragile sites and cancer. Science 1984; 226: 1190-1204.
42. Yunis JJ, Soreng AL, Bowe AE: Fragile sites are targets of diverse mutagens and carcinogens. Oncogene 1987; 1: 59-69.