植治療実験において腫瘍細胞の増殖を抑制したことから、子宮頸癌に対するあらたな分子標的治療の候補として臨床応用が期待される。

F．健康危険情報

特にありません。

G．研究発表

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H．知的財産権の出願・登録状況(予定も含む)

1. 特許取得

特になし。

2. 実用新案登録

特になし。

3. その他

特になし。

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II. 分担研究報告書

4．頸癌化学放射線療法における CDDP 認容性に関する検討

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研究要旨

本邦婦人に対する子宮頸癌初回シスプラチン（CDDP）併用化学放射線療法(CCRT)及び子宮頸癌術後補助 CCRT における併用 CDDP 量の認容性を検証するため、phase 1 dose escalation study を行った。この結果、初回 CCRT 及び補助 CCRT のいずれにおいても週分割投与法では 40 mg/m², 月分割投与法では 80mg/m² が maximum tolerated dose であった。従って本邦婦人に対する CCRT における併用 CDDP の推奨投与量は週分割投与法では 30mg/m², 6 courses であり、月分割投与法では 75mg/m², days 1,29 と判定された。

A．研究目的

CDDP 併用化学放射線療法（CCRT）は米国 GOG, RTOG, SWOG 等の臨床研究組織における第 3 相比較試験から、臨床進行期 Ib2 以上の子宮頸癌初回治療における有効性ならびに術後 high-risk 頸癌に対する補助療法としての有効性が報告され、本邦においても臨床適応が開始されている。しかし海外の CCRT における放射線線量は本邦における治療線量より過量の線量が照射されており、さらに併用される CDDP 量は週分割投与法では 40mg/m², 6 courses であり月分割等用法では 50-70mg/m², 2 courses とされており、CCRT の本邦婦人への適応の安全性は確認されていない。そこで本邦婦人に対する CCRT の認容性を検証するため併用 CDDP 量の推奨用量決定のための臨床第 1 相試験を行った。

B．研究方法

子宮頸癌の診断にて初回 CCRT 治療が行われる患者及び子宮頸癌術後補助 CCRT 治療が行われる患者を対象として、週分割投与法では 30 mg/m² を開始量として 40 mg/m²,50 mg/m² に、月分割投与法では 50mg/m² を開始量として 60 mg/m², 70 mg/m², 75 mg/m², 80 mg/m² に dose escalation を行った。また dose limiting toxicity は治療開始基準をみたさない 1 週間以上の治療延期に設定した。なお本試験は近畿大学 IRB における承認を得た。

C．研究成績

1．４５名の患者（初回 CCRT２５名・補助 CCRT２０名）が登録された。
2．初回 CCRT における併用 CDDP 量は 40 mg/m²、補助 CCRT においては 80 mg/m² が maximum tolerated dose であった。
3．本邦婦人に対する CCRT における推奨 CDDP 量は、週分割投与法で 30 mg/m²、月分割投与法では 75 mg/m² であった。

D．考　察

海外で行われた臨床試験の成績は高い evidence として本邦においても臨床応用がなされる傾向にあるが、化学療法や化学放射線療法については総ての試験成績について本邦婦人への認容性が確認されているとは言いがたい。

今回の phase 1 dose escalation study の結果、CCRT において海外で適応される週分割投与法の CDDP 量は本邦婦人には over dose であることが確認され、本研究班において採用された CDDP 投与量は安全に施行可能であることが確認された。

従って、本邦においても本邦婦人への認容性が確認された放射線線量と併用 CDDP 量によ

る CCRT の有効性の再検証が必要であると考えられるが、現在のところ、骨盤リンパ節転移陰性症例に対する補助療法の必要性ならびに骨盤リンパ節転移陽性症例に対する CCRT の有効性については全く不明であり、本研究班において遂行されている臨床第 3 相比較試験は対象症例が少ないものの極めて有用な試験であると考えられる。

E. 結　論

本邦婦人に対する本邦における放射線照射線量を用いた CCRT での推奨併用 CDDP 量は、週分割投与法で 30 mg/m^2, 6 courses、月分割投与法では 75 mg/m^2, 2 courses であった。今後臨床第 3 相試験による CCRT の有効性の再検証が必要であると考えられた。

F. 健康危険情報

特にありません。

G. 研究発表

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H. 知的財産権の出願・登録状況(予定も含む)

1. 特許取得

特になし。

2. 実用新案登録

特になし。

3. その他

特になし。

厚生労働省科学研究費補助金（がん臨床研究事業）
II. 分担研究報告書

5．婦人科癌における遺伝子多型解析

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研究要旨

ヒトゲノム計画の完了とともに、各種疾病の原因遺伝子の特定やその機能解析が加速度的に進められており、様々な遺伝子診断や分子標的治療への展開が期待されている。これを達成するための有力な手段として、近年注目されているのが遺伝子多型解析である。ヒトゲノムDNAは各個人でほぼ共通であるが、我々の姿形や体質が少しずつ異なっているように、DNA の塩基配列には人により若干の相違がある。遺伝子多型(genetic polymorphism) とは、ある塩基の変化が人口の 1%以上の頻度で存在するものと定義されており、通常末梢血リンパ球 (germ line) DNA を用いて解析される。これまでに高血圧、糖尿病などの生活習慣病、膠原病や癌などの慢性難治性疾患において、発症関連遺伝子の SNP 解析が行われ、各疾患の予防や予後管理に役立てられてきている。

A．研究目的

本研究では、現在も集積されつつある germ line DNA 検体を用いて、より大きな cohort での遺伝子多型解析を進め、癌発症リスクおよび癌の発育・進展や転移・再発との関連性にも焦点をあわせ、GST、p53、HER2、Fas 遺伝子多型が婦人科癌患者の予後管理に役立つか否かを検討する。さらに、germ line DNA に加えて各患者の手術摘出病理標本から microdissection により抽出した癌組織 DNA を用いて抗癌剤代謝関連遺伝子多型を調査し、臨床的な化学療法奏功度あるいは副作用出現状況との比較から、オーダーメイド治療の可能性を模索する。一方、遺伝子多型解析結果と遺伝子発現や機能との相関を明らかにするために、培養細胞を用いた in vitro 実験を行い、GST、Fas 遺伝子の deletion や SNP と癌細胞増殖能や浸潤能、アポトーシスある　いは抗癌剤感受性との関連性を基礎的に検討する。以上から得られる婦人科癌における遺伝子多型に関する知見は、今後の癌発症予防や追跡管理あるいは個々の患者に応じた至適薬剤の投与など、婦人科癌における遺伝子診断や個別化医療の推進を目的とした。

B．研究方法

本研究では末梢血リンパ球、細胞病理標本、培養細胞より抽出した DNA 検体を用いた遺伝子解析が中心となる。GST isoform (GSTM1、GSTT1)の deletion は multiplex PCR 法で、p53 codon 72、HER2 codon 655、Fas promoter –670 の SNP は PCR-RFLP 法で解析する。また、genotyping の妥当性は SSCP、direct sequence 等で検証する。その他の抗癌剤代謝関連遺伝子多型も同様の方法で解析する。一方、遺伝子多型解析結果と遺伝子発現や機能との相関については、培養細胞を用いた in vitro 実験を行うが、細胞増殖能は MTT assay、浸潤能は haptoinvasion assay、アポトーシスは TUNEL 法で評価する。また翻訳領域の deletion や転写調節領域の SNP が遺伝子発現に与える影響については、Light Cycler を用いた Real-Time PCR 法で定量的に解析する。

C．研究成績

子宮頸癌、体癌、卵巣癌患者から同意を得て採取した末梢血リンパ球や細胞病理標本を用いて遺伝子多型解析を行ってきた。その結果、GST isoform (GSTT1) の deletion あるいは Fas promoter –670 の SNP (A/G)が頸癌発生に、p53 codon 72 の SNP (G/C)が体癌発生に関与

することを見い出した(1-6)。特に、Fas promoter –670 の GG genotype は頚癌の発症リスクを2.56倍に高める点で注目される(表1)。現在、細胞病理標本、培養細胞より抽出したDNA 検体を用いた遺伝子解析を進めている最中である。

D．E．考察と結論

以上の結果より、GST、p53、HER2、Fas遺伝子多型が婦人科癌の発症リスクのみならず、癌の発育・進展や予後管理に役立つ可能性が示唆された。このことは、婦人科癌におけるオーダーメイド治療の可能性を示唆するものと考えられる。

F．健康危険情報

特にありません。

G．研究発表

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H．知的財産権の出願・登録状況(予定も含む)

1. 特許取得

特になし。

2. 実用新案登録

特になし。

3. その他

特になし。

資料3

表1 Fas promoter -670 遺伝子型と子宮頸癌発生リスク

Fas promoter -670 polymorphism	Control subjects	Cancer patients	OR (95% CI)
Genotype			
AA	23 (24.2%)	15 (18.1%)	1.00 (referent)
GA	54 (56.8%)	38 (45.8%)	1.08 (0.49 to 2.37)
GG	18 (18.9%)	30 (36.1%)	2.56 (1.08 to 6.10)
Allele			
A	100 (52.6%)	68 (41.0%)	1.00 (referent)
G	90 (47.4%)	98 (59.0%)	1.60 (1.05 to 2.43)

厚生労働省科学研究費補助金（がん臨床研究事業）
II. 分担研究報告書

6．婦人科腫瘍の治療法改善に関する検討

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研究要旨

子宮頸癌の治療成績を改善するためには、予後不良な患者を選別して、適切な治療を行うことである。本研究課題は３つの課題を検討した。(1)子宮頸部扁平上皮癌では、BED10の増加と予後との関連は否定的であるが、局所進行子宮頸部腺癌では両者に相関関係が認めらた。十分な投与線量により頸部腺癌の生存率は扁平上皮癌と同等の結果が示唆される。(2)CPT-11 と NDP の併用化学療法は、重篤な副作用のない安全な治療法であり、十分な奏効率が得られた。予後不良で放射線の効果が低いとされる腺癌での奏効率が高く大きく評価される。(3)転移リンパ節の解析から、体癌におけるセンチネルリンパ節は内腸骨節と閉鎖節であると結論した。臨床進行期Ｉ期、高分化型類内膜腺癌、筋層浸潤がごくわずかである場合には、PAN と PLN 郭清を内腸骨節と閉鎖節の郭清で代用することが可能かもしれない。

A．研究目的

子宮頸癌の治療成績を改善するためには、予後不良な患者を選別して、適切な治療を行うことである。本研究課題は３つの課題を検討した。すなわち、(1) 子宮頸部腺癌 IIIb 期の放射線治療成績—生物学的等価線量を用いた検討、(2)子宮頸癌に対する Neoadjuvant chemotherapy(NAC)の有効性の検討、(3)リンパ節転移の経路の解析である。

(1)では局所進行子宮頸部腺癌の根治放射線治療成績は、扁平上皮癌と比較して不良である。」その原因として、頸部腺癌は放射線感受性が低いにもかかわらず適切な投与線量が明らかになっていないことが考えられる。そこで生物学的等価線量（Biological effective dose: BED）を用いて、子宮頸部腺癌に対する投与線量が予後に与える影響を検討することを目的とした。(2)では進行子宮頸癌における NAC の有用性が報告されている。NAC にはシスプラチンをはじめとする多くの化学療法薬が使用されているが、未だ標準的投与法は確立されていない。そこで、塩酸イリノテカン（CPT-11）とネダプラチン(NDP)を用いた NAC の治療成績を検討し、本治療法の頸癌治療における意義を考察する。(3)では子宮体癌におけるリンパ節転移経路を明らかにし、体癌のセンチネルリンパ節を明らかにすること。リンパ節転移と他の臨床病理学的予後因子との相関を明らかにすることとした。

B．研究方法

(1) 子宮頸部腺癌 IIIb 期の放射線治療成績—生物学的等価線量を用いた検討では、1976 年 8 月から 200 年 11 月までに北里大学病院において根治的放射線治療を施行した子宮頸部腺癌 IIIb 期 13 例を対象とした。対象症例の年齢の中央値は 58 歳であった。全例で外照射と高線量率腔内照射を併用した。総投与線量は、α/β を 10 とし BED10 を用いて算出した。なお、BED10 算出の際の評価点は、外照射では照射野中心から照射野辺縁までの 1/2 の点とし、腔内照射では A 点とした。

(2) 子宮頸癌に対する Neoadjuvant chemotherapy(NAC)の有効性の検討では、2003 年 1 月から 2004 年 12 月の 2 年間に治療を開始した 15 例を対象とした。年齢は 29 歳から 63 歳に分布し、平均 48.3 歳であった。進行期は Ib2 期 3 例、II 期 9 例、III 期 1 例、IV 期 2 例で、組織型は扁平上皮癌 5 例、腺癌 6 例、腺扁平上皮癌 4 例であった。CPT-11 : 50mg/m^2 を day 1, 8, 15 に、NDP:

60 mg/m^2 を day 1 に投与し、4 週間隔で 1～3 コース施行した。効果判定には、MRI による頸部の腫瘍径を用いた。

(3) リンパ節転移の経路の解析は、1971 年から 1998 年の間に骨盤リンパ節(PLN)郭清を含む手術を施行した 342 例を対象とした。うち 49 例では、リンパ節の腫大・深い筋層浸潤・卵巣転移・腹腔細胞診陽性・組織型が漿液性腺癌や明細胞腺癌であったために、傍大動脈節(PAN)の郭清も追加した。リンパ節転移の状態を解析し、臨床病理学的予後因子と比較検討した。

C．研究成績と考察

(1) 全体の 5 年生存率は 51%であった。BED10 ＞100 と＜100 とで比較すると、前者の 5 年生存率は 57%であったのに対し、後者は 30%であった。

(2) CR2 例、PR8 例で、奏効率は 67%であった。組織型別の奏効率は、扁平上皮癌 60%、腺癌 83%、腺扁平上皮癌 50%であった。1, 2, 3 コース投与した症例が各 5 例であったが、奏効率はそれぞれ 40%、60%、100%と、コースを重ねることによって奏効率の上昇が見られた。9 例にダウンステージが見られ、13 例で広汎性子宮全摘出術を施行することができた。なお、重篤な副作用は経験しなかった。

(3) PLN 転移は 46 例に観察された。PAN 郭清も施行した 49 例中 11 例に PAN 転移があり、うち 6 例は PAN 単独転移であった。342 例における PLN または PAN 転移率は 52 例、15.2%であった。この 52 例における転移リンパ節は 99 個であり、PAN が 11, 総腸骨節が 13、外腸骨節が 19、内腸骨節が 29、閉鎖節が 22、そけい上節が 4、仙骨節が 1 であった。PLN 片側または PAN の単独転移は 47 例（55 側）に観察され、うち 9 例では対側 PLN に複数転移を認めた。4 例は両側 PLN に、1 例は片側 PLN にのみ複数転移を認めた。すなわち、複数転移は 14 例、18 側に観察された。片側 PLN または PAN に単独転移例（55 側）における転移リンパ節は、内腸骨節と閉鎖節が各 30.9%, 外腸骨節が 18.2%, PAN が 10.9%を占めていた。複数転移が見られた症例では、88.9%が内腸骨節または閉鎖節に転移を認めた。PAN に単独転移が見られた 6 例は、深い筋層浸潤を有する明細胞腺癌 2 例と腺扁平上皮癌、G2 類内膜腺癌、G3 類内膜腺癌、癌肉腫各 1 例であった。子宮傍結合織転移は 23 例(29 側)に見られたが、うち 16 例はリンパ節転移も認めた。同側のリンパ節に転移している症例の頻度は、対側への転移頻度より高率であった。リンパ節転移頻度は、臨床進行期が進んでいる症例、腺扁平上皮癌・漿液性腺癌・明細胞腺癌・癌肉腫の症例、筋層浸潤が深い症例、リンパ管侵襲のある症例、頸部浸潤例、付属器転移例、子宮傍結合織転移例、腹腔細胞診陽性例で優位に高頻度であった。

E．結　論

子宮頸部扁平上皮癌では、BED10 の増加と予後との関連は否定的であるが、局所進行子宮頸部腺癌では両者に相関関係が認められた。また、BED10＞100 の十分な投与線量で治療することにより、頸部腺癌の生存率は扁平上皮癌と同等の結果が得られるものと考えられた。

CPT-11 と NDP の併用化学療法は、重篤な副作用のない安全な治療法であり、十分な奏効率が得られたことから、NAC に使用する併用化学療法の候補の一つとなりうる。特に、予後不良で放射線の効果が低いとされる腺癌での奏効率が高かった点は、大きく評価される。投与コース数は、２－３コースが推奨される。

転移リンパ節の解析から、体癌におけるセンチネルリンパ節は内腸骨節と閉鎖節であると結論した。臨床進行期 I 期、高分化型類内膜腺癌、筋層浸潤がごくわずかである場合には、PAN と PLN 郭清を内腸骨節と閉鎖節の郭清で代用することが可能かもしれない。子宮体癌におけるリンパ節転移経路は、閉鎖節または内腸骨節を経由するものが多くを占めている。この中には、子宮傍結合織転移を有するものと有しないものとがある。まれに PAN への単独転移が経験される。

F．健康危険情報

特になし。

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H. 知的財産権の出願・登録状況(予定も含む)

1. 特許取得

特になし。

2. 実用新案登録

特になし。

3. その他

特になし。

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III. 研究成果の刊行に関する一覧

著書

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	ページ

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IV. 研究成果の刊行物・別冊

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Caffeine Sensitizes Nondividing Human Fibroblasts to X Rays by Inducing a High Frequency of Misrepair

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Kawata, T., Ito, H., Saito, M., Uno, T., Okayasu, R., Liu, C., Kan'o, M., George, K. and Cucinotta, F. A. Caffeine Sensitizes Nondividing Human Fibroblasts to X Rays by Inducing a High Frequency of Misrepair. *Radiat. Res.* 164, 509–513 (2005).

Caffeine sensitizes cells to ionizing radiation, and this effect is believed to be associated with the disruption of DNA damage-responsive cell cycle checkpoints, which is controlled by ATM. Recent studies suggest that misrejoining of DSBs is one of the underlying mechanisms of AT cell hyper-radiosensitivity. In this study, we investigated the effects of caffeine and radiation on nongrowing G_0 normal human fibroblast cells by determining cell survival and scoring aberrations in calyculin A-induced G_2 chromosomes. Results from the cell survival study indicate that after X-ray exposure G_0 cells were sensitized by 24 h treatment with caffeine. Analysis of chromosome aberrations using FISH (fluorescence *in situ* hybridization) revealed a high frequency of aberrant cells and color junctions in the caffeine-treated cells. Since most DNA repair in nongrowing G_0 cells is believed to result from nonhomologous end joining (NHEJ), caffeine may influence the fidelity of the NHEJ pathway in irradiated G_0 cells.

INTRODUCTION

Ataxia telangiectasia (AT) is a human autosomally recessive syndrome characterized by cerebellar ataxia, telangiectases, immune dysfunction, genomic instability, and a high cancer incidence (*1, 2*). AT cells are abnormally sensitive to ionizing radiation (*3, 4*), as can be seen from many indicators, including an increased level of chromosome aberrations in cells after exposure (*5–8*). The diverse clinical features in individuals affected by AT and the complex cellular phenotypes are all linked to the functional inactivation of a single gene (*ATM*, AT mutated) (*9*). Several theories have been proposed for the underlying processes involved in this radiation hypersensitivity, including loss of cell cycle arrest (*10–13*), inappropriate TP53-mediated apoptosis (*13*), DNA or chromosomal repair deficiency (*5, 6, 14–21*), impaired accuracy of DNA or chromosome break rejoining (*22–24*), and both repair deficiency and impaired accuracy of break rejoining (*25*).

Caffeine is well known as an efficient inhibitor of two key checkpoint-regulating proteins, ATM and ATR (*26, 27*), and has been shown to sensitize cells to ionizing radiation (*28–33*). Ionizing radiation induces DNA damage, which triggers cell cycle checkpoint activation and consequently cell cycle arrest. Cell cycle arrest is believed to provide an extended time for cells to repair DNA damage before they undergo cell division. Since the checkpoint defects induced by caffeine are reminiscent of the checkpoint defects seen in AT cells, this disruption of checkpoints is assumed to result in the radiosensitizing effects produced by this chemical. Therefore, most previous studies have been performed using exponentially growing cells to study the influence of caffeine on cell cycle checkpoints, with relatively few studies focusing the effects of caffeine on nongrowing G_0 cells. However, Iliakis and coworkers (*34*) have clearly demonstrated the radiosensitizing effects of caffeine on nongrowing cells using a colony formation assay. Natarajan and coworkers (*35*) also reported that chromosome aberrations in G_0 lymphocytes treated with caffeine after irradiation have a tendency to increase, although individual differences in sensitivity were observed. These results indicate that caffeine has biological effects other than those that affect cell cycle checkpoints. If ATM is inhibited by caffeine, an increased induction of misrejoining will be observed in G_0 cells that have been treated with caffeine after irradiation, as was observed in AT cells (*22–25*).

In the present study, we investigated the effect of caffeine on cell survival and chromosome break repair in normal human fibroblast cells in G_0. We assessed the fidelity of chromosome break repair by scoring aberrations in FISH (fluorescence *in situ* hybridization)-painted chromosomes.

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MATERIALS AND METHODS

Cells

Cells of the normal human fibroblast cell lines AG1522 and AG1523 were obtained from the NIA cell repository. Low-passage AG1522 and AG1523 cells were grown in minimum essential medium (MEM) supplemented with 15% fetal bovine serum. Cells were plated into T-25 flasks at 25% confluence and grown for 5 days before being irradiated in the confluent state. The cell density was measured using a Coulter counter at the time of irradiation and after 24 h incubation. There were approximately 1.5×10^6 cells per T-25 flask at the time of irradiation, and no significant increase in this cell density was detected 24 h later, indicating that most cells were not cycling. The percentage of cells in G_0/G_1 phase was measured by cytofluorometry and was 93% for each cell line.

Irradiation and Chemicals

X irradiations were performed with an MBR-1520R-3 (Hitachi, Japan) generator operated under 150 kVp and 20 mA with a 1-mm aluminum filter. The dose rate was about 2 Gy/min. Flasks were kept on ice before irradiation, and all the irradiations were carried out at room temperature. Caffeine (Wako Chemicals, Japan) was dissolved in PBS and added to the cell cultures just after irradiation. The concentration of caffeine was adjusted to 5 m*M* or 10 m*M*.

Colony Formation

Confluent cells were exposed to X rays (0–8 Gy) and then were allowed to repair at 37°C for 24 h with either 5 or 10 m*M* caffeine or without caffeine. After incubation, the medium was removed and the cells were washed twice with PBS to remove the caffeine. Cells were then trypsinized and plated onto 100-mm-diameter plastic dishes containing caffeine-free medium to determine colony formation capacity. The cell suspension was counted using a Coulter counter, and the number of cells seeded was adjusted to yield 100 colonies per 100-mm dish. Surviving cells were determined from the number of colonies containing a minimum of 50 cells.

Chromosome Aberrations

A chemically induced PCC (premature chromosome condensation) technique with calyculin A was used to collect chromosomes in the G_2 and mitotic phases of the cell cycle (*24, 36, 37, 38*). Calyculin A can induce PCC effectively in G_2 phase of the cell cycle. After exposure of confluent cells to 6 Gy of X rays, cells were returned to the incubator for 24 h for repair with or without caffeine. After 24 h, the medium was removed from the flasks and the cells were washed twice with PBS as described above. Cells were then trypsinized and transferred from a T-25 flask to a T-75 flask to allow growth. When nongrowing AG1522 cells were exposed to 6 Gy, the G_2/metaphase index was found to peak at around 36 h after subculture (*24*). We therefore elected this time for collecting the first cell cycle postirradiation G_2/metaphase chromosomes. After incubation for 36 h after subculture, calyculin A (Wako Chemicals; final concentration 50 n*M*) was added and the cells were incubated for 30 min at 37°C to allow chromosome condensation. After treatment with calyculin A, cells were transferred to a tube and centrifuged for 5 min at 2000 rpm. The pellet was carefully resuspended in 8 ml of 75 m*M* KCl and incubated at 37°C. After 20 min, 2 ml of freshly prepared fixative solution (methanol:glacial acetic acid = 3:1 vol/vol) was slowly added to the solution, and the tubes were centrifuged again. A final wash and fixation in fresh fixative was completed before the cells were dropped onto a glass slide. Cells were aged overnight at 37°C on a slide warmer and then hybridized *in situ* with fluorescent DNA whole probes 1 (Green) and 3 (Orange) (Vysis). Cells were counterstained with DAPI, and chromosome aberrations were viewed with a Zeiss Axioskop fluorescence microscope. Aberrations in FISH-painted chromosomes were analyzed in one sample of cells irradiated with each dose.

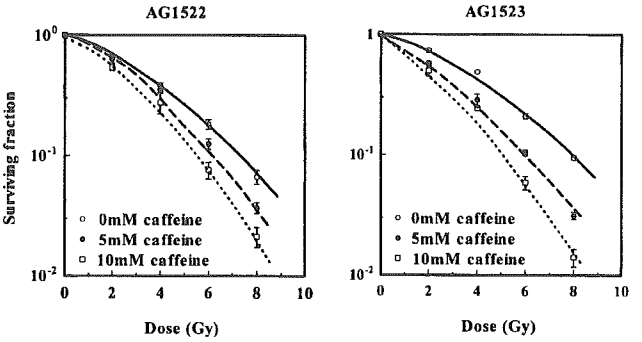

FIG. 1. Survival curves for nongrowing AG1522 and AG1523 cells treated with 0, 5 or 10 m*M* caffeine after irradiation. Each point represents the mean and the standard error (SE) of three independent experiments.

Scoring of Chromosome Aberrations

We measured the number of color junctions per cell as a simple parameter representing the frequency of chromosome misrejoining and the number of excess painted fragments to represent non-rejoined breaks. These excess fragments, which would presumably include both interstitial and terminal-type deletions, were included in one category of deletions. Although terminal deletions are true unrejoining breaks, most interstitial deletions are likely to form rings through rejoining of their broken ends. However, we were forced to include both types of deletions due to the difficulty of discriminating them in FISH-painted chromosomes without the use of telomere probes. Bicolor junctions originate from misrejoining of either a FISH-painted chromosome and a DAPI-stained chromosome or two FISH-painted chromosomes. A reciprocal interchange between a FISH-painted chromosome and DAPI-stained chromosome, i.e. dicentrics and translocations, would therefore contain two bicolor junctions, and an incomplete interchange would contain one bicolor junction. Each painted chromosome was treated independently, and a few exchanges involved exchanges between the painted chromosomes. Therefore, a reciprocal exchange between two FISH-painted chromosomes was measured as four bicolor junctions and an incomplete exchange as two bicolor junctions. The percentage of aberrant cells, which gives a direct measurement of the extent of chromosome damage, was calculated as the ratio of the number of aberrant cells and the total number of cells scored. The total numbers of cells scored were 297 (6 Gy, 0 m*M* caffeine) and 269 (6 Gy, 10 m*M* caffeine) for AG1522 cells and 210 (6 Gy, 0 m*M* caffeine) and 135 (6 Gy, 10 m*M* caffeine) for AG1523 cells.

RESULTS

Similar to what was seen previously (*34*), caffeine had no cytotoxic effects on nonirradiated cells up to concentrations of 10 m*M* (data not shown). Figure 1 shows survival data for AG1522 and AG1523 cells exposed to 0, 5 or 10 m*M* caffeine after irradiation at various doses; a concentration-dependent cell killing effect is demonstrated.

Figure 2 shows an example of aberrations in AG1522 cells exposed to 10 m*M* caffeine after irradiation. The figure clearly shows a color junction originating from chromosome 1 and 3. Figure 3a–c shows the number of color junctions per cell and deletions per cell and the percentage of aberrant cells for AG1522 and AG1523 cells with or without caffeine treatment. In cells treated with 10 m*M* caffeine, the percentage of aberrant cells and the number of color junctions per cell are almost twice as high as yields in cells

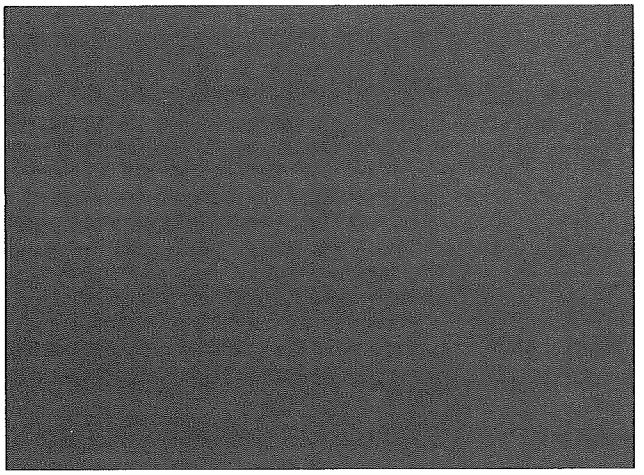

FIG. 2. Example of chromosome aberrations in AG1522 cells after exposure to 6 Gy. Chromosome 1 was painted with a Spectrum Green probe and chromosome 3 with Spectrum Orange probe. Spreads were counterstained with DAPI. Color junction between chromosome 1 and 3 is clearly visible.

without caffeine treatment, while the frequency of deletions per cell was similar for caffeine-treated and nontreated samples. Although chromosome-type aberrations increased in samples treated with caffeine, no significant increase was found in the induction of chromatid-type breaks in either type of cells treated with caffeine after irradiation (data not shown).

DISCUSSION

Our experiments were designed to determine the effects of caffeine on radiation-induced chromosomal damage and repair in nongrowing G_0 human fibroblast cells. Since chromosome aberrations are believed to reflect DSB repair, aberrations in FISH-painted chromosomes indicate the effect of caffeine on DSB repair. To eliminate the effects arising from cell cycle checkpoints, confluent cells were irradiated and allowed to repair for 24 h at 37°C with or without caffeine.

The chromosomal repair process in G_0 can be assessed directly in interphase cells using the conventional fusion PCC technique (*39–42*). Kovacs *et al.* (*42*) irradiated nongrowing AG1522 cells and compared PCC 24 h after irradiation with that in cells that had reached first metaphase after exposure and found that there were fewer in aberrations at metaphase, particularly unrejoined breaks, indicating the importance of scoring aberrations in prematurely condensed chromosomes for assessing the repair at G_0. However, fusion PCC is relatively difficult and the PCC index is low. In the present study, we elected to score aberrations in G_2 chromosomes condensed by calyculin A instead of directly scoring G_0 chromosomes. A recent study comparing the frequency of aberrations in calyculin A-induced G_2 PCC with aberrations in G_0 fusion PCC demonstrated that the frequencies of aberrations were similar (*43*). This indicates that a G_2/M-phase cell cycle delay results in the decreased level of aberrations observed in metaphase and therefore that yields found in the G_2 cells are reflective of the yields of misrepaired DSBs or chromosome breaks formed in G_0.

In mammalian cells, two major repair pathways are known to be involved in the repair of DNA breaks: homologous recombination (HR) and nonhomologous end joining (NHEJ) (*44*). HR, which requires the presence of homologous sequences in a homologous chromosome or in a sister chromatid, is precise (relatively error-free) and is important for the repair of DSBs in late-S and G_2 phase, while the NHEJ pathway is error-prone and mutagenic and is the predominant repair process during G_0, G_1 or early S phase (*45, 46*). In the present study, since nongrowing G_0 cells were irradiated and allowed to repair for 24 h before

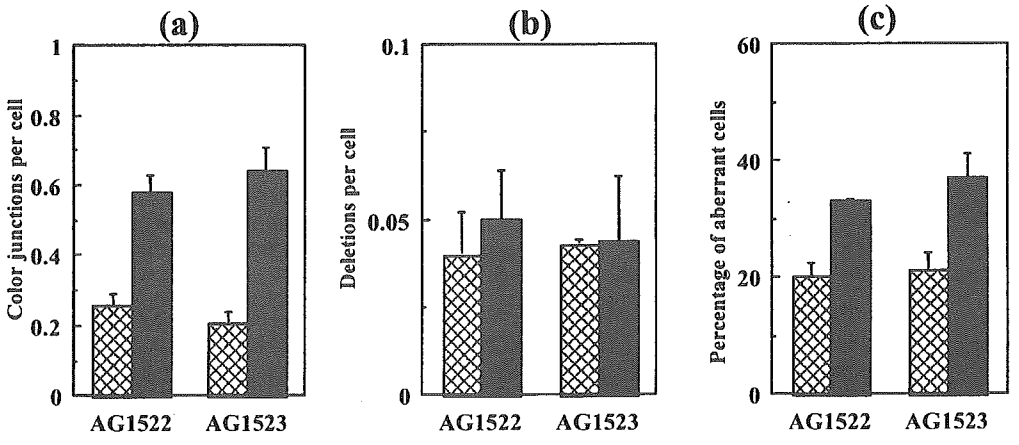

FIG. 3. The effects of caffeine on the induction of color junctions per cell, deletions per cell, and the percentage of aberrant cells. The hatched bar shows the results for samples treated with 0 m*M* caffeine after 6 Gy irradiation. The black bar indicates results for samples treated with 10 m*M* caffeine after 6 Gy. The bars show the standard errors (SE) of the mean.

subculture, it is likely that most DSBs were repaired using the NHEJ repair pathway. The radiosensitizing effect of caffeine on G_0 human fibroblast cells is clearly demonstrated by the colony formation study (Fig. 1). To assess the effect of caffeine on the fidelity of G_0 repair, we scored color junctions and deletions using the FISH technique. Figure 3 shows that in the presence of 10 m*M* caffeine around twice the number of color junctions are induced compared with samples allowed to repair without caffeine, while 10 m*M* caffeine does not affect the frequency of deletions, which include both terminal deletions (unrejoined breaks) and interstitial deletions. These results may indicate that the efficiency of chromosome break rejoining through NHEJ may be independent of caffeine; however, the fidelity of the rejoining process is impaired. These results appear to be different from the previously published finding that caffeine-induced radiosensitization is independent of NHEJ but is mediated through affecting HR (*47–49*). This difference may be attributed to comparison of results obtained under different experimental conditions; i.e., most previous experiments were performed using exponentially growing phase cells, not confluent G_0 cells. In a population of exponentially growing cells exposed to radiation, an accumulation of G_2-phase cells occurs as a result of the G_2 block. Since HR plays an important role in repairing breaks in G_2 phase, the effect of caffeine on the HR pathway could be enhanced and that of NHEJ would be minimized when exponentially growing cells are used. By using synchronized normal human fibroblast cells in G_0, we could clearly demonstrate that caffeine affects the fidelity of repair in G_0 normal human fibroblast cells.

In conclusion, we have shown that the presence of caffeine can result in a high frequency of misrejoining in irradiated G_0 human fibroblast cells. Caffeine may not influence the efficiency of joining breaks through NHEJ, but it can influence the fidelity of repair through NHEJ. Although caffeine is known to be an efficient inhibitor of ATM, caffeine is a relatively non-selective agent and has many effects in cells. For example, caffeine inhibits alkaline phosphatase activity (*50*) and phosphodiesterase activity (*51, 52*). It should therefore be noted that a high induction of misrejoining in G_0 cells after treatment of caffeine would not be attributed solely to the inhibition of ATM. Inclusion of more data from additional cell lines of different radiosensitivity and study using molecular techniques is necessary to confirm these results. Further studies using additional normal and AT heterozygous fibroblast cells are under way to investigate the mechanism of radiosensitization of caffeine in cells exposed to low- or high-LET radiations.

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CASE REPORT

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Weekly cisplatin administration concurrent with radiation therapy for locoregionally advanced nasopharyngeal carcinoma

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Abstract Radiation therapy (RT) with concurrent and adjuvant chemotherapy has been a widely accepted treatment for patients with locoregionally advanced nasopharyngeal carcinoma (NPC). We administered 40 mg/m^2 cisplatin (CDDP) weekly, concurrently with RT, to six consecutive patients with locoregionally advanced NPC to evaluate its toxicity and efficacy. The median number of courses of CDDP administration was 4.5 and the median radiation dose was 69.7 Gy. Grade 3 leukopenia was observed in three patients. All but one patient experienced grade 3 or 4 skin reactions, pharyngitis, or dysphagia. All but one patient achieved a complete response, and the remaining patient received radical neck dissection for persistent cervical lymphadenopathies, which contained no cancer cells. All six patients were disease-free at last contact, with a median follow up of 23.5 months. This regimen is well tolerated in patients with locoregionally advanced NPC.

Key words NPC · Concurrent chemoradiotherapy · Weekly CDDP · Intergroup Study 0099

Introduction

Concurrent chemoradiotherapy is the mainstay of treatment for various malignancies.[1–4] Platinum agents, including cisplatin (CDDP), with or without other agents, are used commonly in this setting; however, an optimal CDDP administration schedule remains to be determined. In patients with nasopharyngeal cancer (NPC), the Intergroup Study 0099 (IGS) has demonstrated significant results by administering 100 mg/m^2 CDDP concurrent with radiation therapy (RT) at 3-week intervals.[5,6] After the publication of the IGS, we attempted to adopt an identical combined modality treatment for patients with locoregionally advanced NPC, between March 2001 and June 2002. Although we treated only three patients according to the this regimen, we failed to demonstrate its feasibility and efficacy, because of its severe acute adverse events, poor compliance, and unsatisfactory outcome.[7]

In contrast to the IGS, a Hong Kong group conducted a phase III randomized trial comparing radical RT with concurrent weekly CDDP and RT.[8] They demonstrated significant improvement of progression-free survival in patients with advanced stages, with 40 mg/m^2 weekly CDDP administration. We report the feasibility and efficacy of this weekly chemotherapy CDDP schedule, given concurrently with RT.

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Case report

From July 2002, we have treated six consecutive patients with biopsy-proven stage IIB to IVB NPC. All six patients met the inclusion criteria of Chan et al.,[8] and underwent a complete history, physical examination, complete blood counts, screening blood tests of hepatic and renal function, and 3 consecutive days of 24-h creatinine clearance. The disease evaluation included a chest radiograph; bone scintigraphy; computed tomography (CT) of the head and neck, chest, and abdomen; magnetic resonance imaging (MRI) of the nasopharynx and base of skull; and fiberoptic endoscopy and biopsy of the nasopharynx. The patients were staged according to the 1997 International Union Against Cancer (UICC)-TNM staging system. The patients' characteristics are shown in Table 1. Informed consent was provided according to the Declaration of Helsinki.

The patients received 40 mg/m^2 CDDP weekly during RT, starting on the first day of RT. All patients received adequate hydration and a serotonin antagonist against emesis during the CDDP administration. Chemotherapy was

delayed until bone marrow suppression recovered, and was suspended if serum creatinine was greater than 1.5 mg/dl, and/or creatinine clearance fell to less than 50 ml/min. No patients were scheduled to receive adjuvant and/or neoadjuvant chemotherapy.

With regard to RT, CT-based treatment planning was used to assess the extent of the primary tumor and the neck nodes. The nasopharynx and the upper neck were treated with two opposed lateral fields. A separate anterior supraclavicular field was used to irradiate the lower neck and supraclavicular fossa. The patients were treated with a combination of 4- and 10-MV photons to achieve dose homogeneity. An electron field of appropriate energy was also applied to treat posterior neck nodes after sparing the spinal cord. The fractional daily dose was 2 Gy, with a planned total dose of 66 Gy.

The response assessment included physical examination, fiberoptic endoscopy, and CT and/or MRI of the nasopharynx and neck, and responses were classified according to the *New guidelines to evaluate the response to treatment in solid tumors*.[9] Acute toxicities were graded according to the National Cancer Institute common toxicity criteria.

All six patients received RT without treatment breaks, with a median dose of 69.7 Gy (range, 64 to 70 Gy). Chemotherapy was delivered in three to six weekly courses, with a median course number of 4.5. The reasons for suspension of CDDP administration included renal toxicity in two patients, and grade 3 leukopenia, pharyngitis, and patient refusal in 1 patient each. The acute toxicity profiles are shown in Table 2. Although three patients developed grade 3 leukopenia, the other hematological toxicities were well tolerated. However, all but one patient experienced grade 3 or 4 skin reactions, pharyngitis, or dysphagia. Body weight loss ranged from 4% to 20.7%, with a median of 14.5%. At the end of the treatment, five patients achieved a complete response, and the remaining patient obtained a partial response; this patient received radical neck dissection for persistent lymphadenopathies; however, histopathological examination revealed no cancer cells in the surgical specimens. All six patients were alive without disease at last contact, with a median follow up of 23.5 months (range, 13 to 27 months).

Table 1. Patient characteristics

	Age (years)	Sex	TNM	Histology
Case 1	49	Male	T4N2M0	WHO III
Case 2	20	Male	T1N3bM0	WHO III
Case 3	59	Male	T4N0M0	WHO III
Case 4	62	Female	T3N2M0	WHO II
Case 5	65	Female	T3N1M0	WHO III
Case 6	74	Male	T2bN1M0	WHO II

Discussion

Concurrent chemoradiotherapy with adjuvant chemotherapy has become standard practice following the publication of excellent results by the IGS.[5,6] Subsequently, several phase II or III studies have demonstrated encouraging results with regard to concurrent chemoradiotherapy with or without adjuvant or neoadjuvant chemotherapy.[8,10–15] In the IGS, patients in the experimental arm received 100 mg/m^2 CDDP as a single agent at 3-week intervals, concurrently with radical RT. In four other studies, patients were also administered 100 mg/m^2 CDDP as a single agent, at 3- or 5-week intervals, concurrently with RT;[10–13] in two of these studies, the CDDP dose was divided equally and given on 4 or 5 consecutive days.[12,13] In our previous study, three patients were to receive the IGS regimen; however, they were not able to complete their planned chemotherapy because of its severe acute adverse events.[7]

In contrast, Chan et al.[8] examined the efficacy of weekly administration of 40 mg/m^2 CDDP concurrently with RT (66 Gy/6.5 week), compared with RT alone, in a randomized phase III trial. They demonstrated that progression-free survival was significantly prolonged in patients with advanced stage disease; however, in the overall comparison, progression-free survival was not different among the treatment arms. This CDDP administration schedule has been shown to have acceptable toxicities, with an encouraging outcome for cervical cancer,[2] and, in light of our previous experiences employing the IGS protocol, we incorporated weekly CDDP administration into the present study. Although, in comparison to our previous study, hematological toxicities were more frequent in the present one, nonhematological toxicities were comparable to those in the previous study,[7] and we obtained encouraging results in the present study.

Table 2. Acute adverse events: maximum grade observed for each patient

	Case 1 Grade	Case 2 Grade	Case 3 Grade	Case 4 Grade	Case 5 Grade	Case 6 Grade
Leukopenia	2	3	1	3	3	1
Anemia	1	1	1	2	3	1
Thrombocytopenia	1	1	1	1	0	0
Weight loss	2 (−13.5%)	2 (−18.8%)	2 (−10.7%)	2 (−15.6%)	0 (−4%)	3 (−20.7%)
Dermatitis	2	2	3	3	2	2
Dysphagia	3	3	2	2	2	3
Pharyngitis	3	4	2	3	2	3