局所進行前立腺癌に対するホルモン療法と手術療法の併用療法

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Radical prostatectomy with neoadjuvant hormone therapy for cT3 prostate cancer

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Abstract

The efficacy of neoadjuvant hormone therapy and radical prostatectomy for cT1–2 prostate cancer have been reported to be negative from some randomized prospective studies. On the other hand, radical prostatectomy alone for cT3 prostate cancer is understood as out of indication because of high rate of positive surgical margin and PSA failure. Several investigators have examined the role of neoadjuvant hormone therapy before radical prostatectomy for cT3 prostate cancer to improve outcome.

This document was reviewed the literature whether neoadjuvant hormone therapy is beneficial or not, for organ confined prostate cancer and for locally advanced prostate cancer, and presented our extended resection of prostate with neoadjuvant hormone therapy is improved the results in cT3 prostate cancer.

Key words: radical prostatectomy, neoadjuvant hormone therapy, surgical resection

はじめに

前立腺全摘(radical prostatectomy: RP)に先立ちある程度の期間，術前ホルモン療法(neoadjuvant hormone therapy: NHT)を実施することにより，downstaging(micrometastasisを消滅させることも含む)が起こることで治療成績の向上が期待されたが[1-4]，各種のランダム化試験ではその効果は否定的である[5-7]．

本稿ではまずNHTに関する各種の試験の結果を提示，考察する．最後にまだ経過観察期間が短くpreliminaryな結果ではあるが，NHTを施行した後，より確実な切除を目指した前立腺広汎全摘の成績を供覧して，局所進行前立腺癌に対する前立腺全摘除術の意味を考察したい．

1. 術前内分泌療法の治療成績

既に述べたようにNHTに関するランダム化試験の結果の長期成績ではその効果は否定的である．しかし，結果を解釈するときに注意が必要ではと考えている．一つはNHTの期間に関してである．多くのスタディでは3カ月程度のNHTが施行されている点である[5-7]．もちろんカナダでの3カ月と8カ月のNHTのランダム化試験[8]で8カ月のNHTでは切除断端陰性となりやすい(表1)が，切除断端が陰性となっても最終的にはPSA failureには関与しないのではと考えられている[8]ことも事実であるが，3カ月

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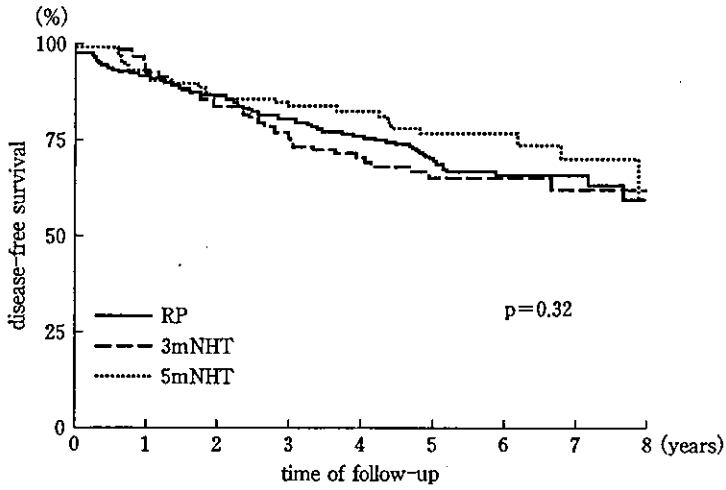
表1 Statistically significant differences found : 3 versus 8 months' neoadjuvant hormone therapy (NHT)[8]

	3 months NHT	8 months NHT	p-value
presurgery PSA nadir level	35%<0.1ng/dl	73%<0.1ng/dl	<0.0001
TRUS prostate volume (mean)	40.5 cm^3 to 25.7 cm^3 (37%)	40.5 cm^3 to 22.8 cm^3 (48%)	0.0001
positive margins after surgery	23%	12%	0.01



	Hazard ratio	95% CI
RP alone	1	
3mNHT	1.01	0.70-1.45
5mNHT	0.60	0.38-0.94

図1 Kaplan-Meier curves for disease-free survival until PSA failure and Hazard ratios[9]
3m: 3 months, 5m: 5 months, NHT: neoadjuvant hormone therapy, RP: radical prostatectomy

程度のNHTでは効果が期待できないとの報告もある[9]. この報告によるとRP単独に対して3カ月のNHTはhazard ratioが1.01, 5カ月のNHTでは0.60となっており(図1), 適切なNHT期間に関するエビデンスは乏しいと思われる.

またそもそもスタディの対象としている病態についても注意が必要ではと考えている. もともと前立腺癌の術前病態は過少評価される傾向があることより, 本来のRPの適応と考えられるT1-2主体のスタディと逆に, T3, T4といった本来monotherapyでは限界があるとされる局所進行前立腺癌を対象にし, その生存率の向上を狙ったスタディかという点である. 多くのランダム化[5-7]あるいはphase IIスタディ[10-12]では前者を対象としている. つまりスタディコンセプトとしてはT2癌の20-30%がpT3であり, NHTを施行することでdownstagingが起こり, pT3前立腺癌がpT2, つまり本来の前立腺全摘の適応となるのではということを期待したスタディである. しかし結果的にNHTによるdownstagingは期待できず, またNHTにより切除断端陽性(positive surgical margin: PSM)が回避

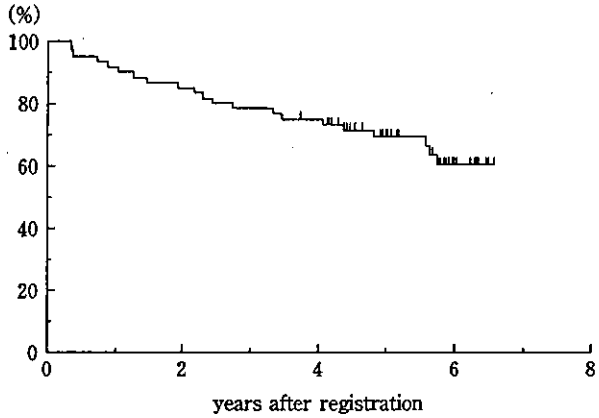


図2 Progression-free survival in SWOG 9109 study[17]

されてもNHTによるartifactであり，PSA failureを回避することはできないとの結論[13-15]となっていると解釈される．実際NHTによりpT0となっていても2割程度に再発を来すことがあり，このデータはRP単独と同様ではと失望させられるという報告[16]もある．更に詳細は不明な文献もあるが，このような疾患を対象として実施された手術は神経温存前立腺全摘が大半であると想定される点である．downstagingが起こらなければ，pT3前立腺癌に対して神経温存手術を行うことはPSMを来す危険性があることは当然である．

以上の結果は，cT1-2前立腺癌に対してdownstagingを狙ってNHTを施行してもPSMをなくすことで治療成績を向上させるという目的は無効であるという解釈となる．

2. 局所進行前立腺癌に対する内分泌療法併用前立腺全摘の成績

本来手術の適応と考えられるcT1-2前立腺癌に対して，治療成績の向上を狙ったNHTの試みはnegativeな結果となったわけであるが，局所進行前立腺癌に対する治療成績を考察するうえで注意を有するのはNHTの後に施行される前立腺癌全摘においてどのような立場で手術がなされたかという点である．cT1-2においては当然，神経温存前立腺全摘，これが標準の手術療法というのがコンセンサスであり，cT3においても，NHTによりdownstagingを来すことにより，このような手術でも対応可能としてスタディがなされたのか，cT3では神経温存を目的とせず，より切除断端を確保すべくwide resectionがなされたか否かという点である．

この点でcT3を対象としたNHTのphase IIスタディとしてSouthwest Oncology Group (SWOG) Study 9109[17]の結果とWalshらが確立した前立腺全摘を施行したGomellaら[18]の結果の比較は興味深い．図2にSWOG studyの結果と図3にGomellaの結果を示した．Gomellaのスタディは症例数が少なく，背景も違うことから単純な比較はもちろんできないのであるが，結果の違いはあまりに大きい．SWOG studyではPSMの率が明らかに他のスタディと比較して低く，その理由として切除断端を広くとる努力がなされたことによるのではと考察している．

このようにNHTを施行した後に実施する前立腺全摘の方法により治療成績が異なる可能性は十分に考えられる．一方ではNHTを施行しなくても神経温存を行わない前立腺全摘によって同様の結果が得られるのではとの仮定もあるが，この点を比較した試験はないように思われる．

3. 術前内分泌療法を併用した広汎前立腺全摘の治療成績

確実な切除断端を追求することは，治療成績

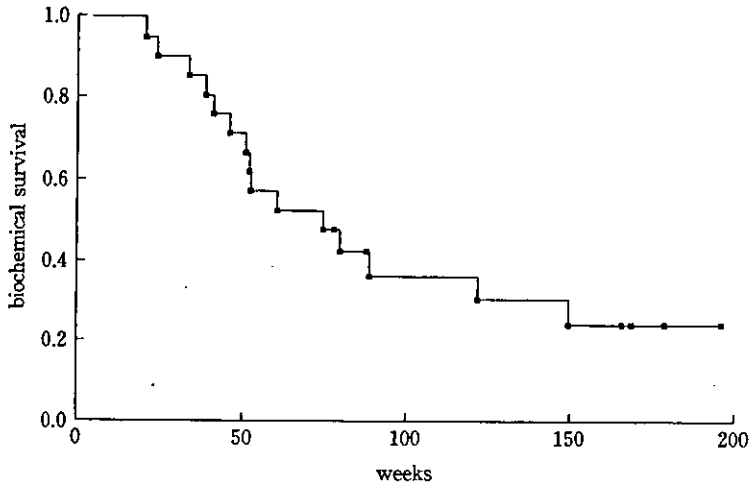


図3 cT3 prostate cancer: freedom from biochemical relapse[18]

の向上につながる可能性があるはずである．もちろん細胞学的な転移があり，局所切除を追求してもその治療成績の向上につながらない病態が存在することは事実であるが，逆に局所施行癌であっても切除が可能な病態もあることも事実であり，著者らは局所の切除をより完全に行うことで局所進行前立腺癌に対して根治の可能性を追求してきた．このアプローチに関するスタディコンセプトを以下に述べる．

これまでのNHTのスタディの結果からdown-stagingはあまり期待せず，したがってNHTの効果を期待して縮小手術を行うのは危険である．NHTを施行することにより前立腺体積が減少することは明らかである．また確実な切除断端を確保することは治療成績の向上につながることも明らかである．したがってNHTはdown-sizingを目的に併用することで相対的に広汎な切除断端を確保することが可能となり，治療成績が期待できるのではと考えた．そもそも日本人の骨盤は狭く，大きな前立腺を摘出する場合には広く切除断端を確保することが困難である．また前立腺全摘において尖部の処理は断端の確保のみではなく，機能温存，出血量のコントロールなどに重要であることはいうまでもない．近年ではPSAにより発見される前立腺癌が増えており，このような病態では前立腺尖部腹側に病巣が多く存在することが認識されている[19]．前立腺尖部と恥骨との間が拡大することにより少しでも距離が確保されることは切除に際して有利に働くはずである．また前立腺尖部が縮小することで相対的に尿道括約筋が長く温存できる可能性が高くなり，術後の尿禁制に対しても有利に作用すると考えられる．

NHTを施行することにより前立腺周囲に線維化が起こることによる手術の困難性が指摘されているが[13,14]，局所進行前立腺癌に対しては神経を温存することはその治療的意義から疑問があり，原則実施していないこと，精嚢は周囲から広汎に切除すること，更には手術に対する慣れもあり，著者らは特に困難を感じることはない．

またこれは我が国の患者の特徴の一つではないかと思われるが，性機能温存に対してそれほどのこだわりがなく，むしろ手術により癌根治を望む症例が多いことも本スタディを可能とした要因である．

以上のコンセプトに基づき，より確実な切除断端を確保する手術法'広汎前立腺全摘：extended radical prostatectomy'[20]を開発し，cT3前立腺癌に対するNHT併用手術療法の治療を行ってきた．

a. 対象と方法

2000年からは術前3カ月以上，1年以内，推奨6カ月の術前ホルモン療法を施行した後，前

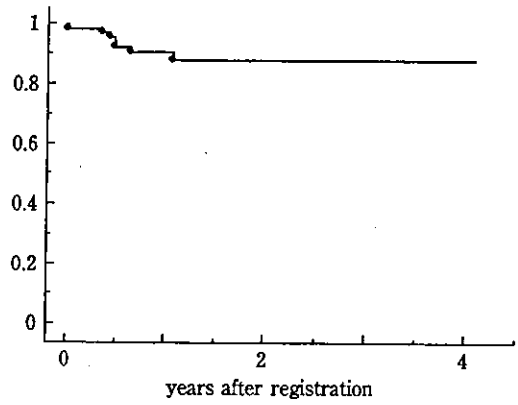


図4 Biochemical-free survival by extended radical prostatectomy in 70 cT3 prostate cancer

立腺全摘を施行し，病理結果のいかんにかかわらず術後は無治療経過観察を行うphase IIスタディを施行している．今回，cT3N0M0前立腺癌に対して上記のプロトコールで治療を行った広汎前立腺全摘症例70例の治療成績を検討した．後述するように本術式の適応拡大を狙った局所の相当な進行癌(TxN0M0)に対してトライアル的に実施(phase II後期)した症例は除いている．広汎前立腺全摘に関する詳細は文献[20]に記載しているが，その概略を述べると，直腸固有筋膜を切開し，直腸筋層を露出し，剥離を進め，腱中心に至ることで前立腺尖部後面の把握を確実にする．神経を含む血管束を可及的末梢で完全切除するとともに，前立腺尖部を直腸の剥離を参考にしながら，前立腺尖部後面と腱中心との間を安全，確実に切断する．中枢に向かい逆行性処理を行い，腹膜翻転部を確認して精嚢基部を露出することなく，また膀胱頸部を大きく切開し，膀胱筋層も含めて前立腺を摘出する手術手技である．

平均年齢は64歳，治療前PSA値は2.4-124 ng/dl，Gleason scoreは6-9であり，平均観察期間は581日(112-1,500日，中央値455日)である．

b. 結果と考察

摘出標本における70例のpT分類はpT0: 1例，pT2a: 2例，pT2b: 25例(pT2: 27例(38.6%))，pT3a: 20例，pT3b: 6例(pT3: 26例(37.1%))，pT4: 16例(22.9%)である．当然，術前診断の正当性が問題になるわけであるが，例えばcT3前立腺癌に対するNHTとして術後pT分類が記載されているEuropean Study Group[21]の結果と比較してみると，NHTの期間に差があり単純な比較はできないのであるが，少なくともpT3以上の病期が50%近くを占めており，特に著者らの臨床診断がoverstagingであるということではないと思われる．著者らの症例の40%近くがpT2と診断された症例が多いことはNHTの期間にも起因しているとも考えられるのであるが，一般的にcT3に対しても15-25%のoverestimationがあるとされており，NHTの効果とステージングエラーの両方を含む症例数としては理解可能な数字ではないかと考える．

図4に全体の成績を示す，PSA failureを0.2 ng/dl以上として検討した結果，88.4%がNEDの状態であり，12%にPSA failureを認めた．摘出標本における病期別の治療成績を図5に示す．興味深いことにpT2a-pT3aでは非常に良好な治療成績であり，局所限局癌と遜色がない．pT2a-pT3a全体で49例中，1例にのみPSA failureを認めている．pT3b 6例中2例，pT4 14例中4例にPSA failureを認め，この群では2例にリンパ節転移を認めている．リンパ節転移陽性例は全例400日以内にPSA failureとなったが，このことは当然のことと考えられた．PSMはNHTを施行しないRP時の大きな予後規定因子

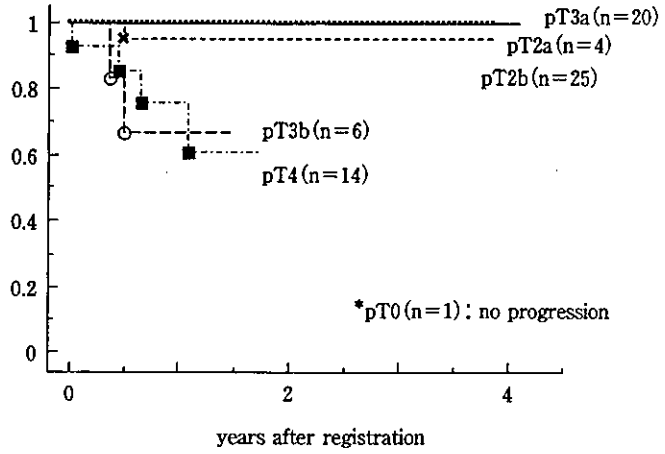


図5 Biochemical-free survival according to the pT stage by extended radical prostatectomy in cT3 prostate cancer

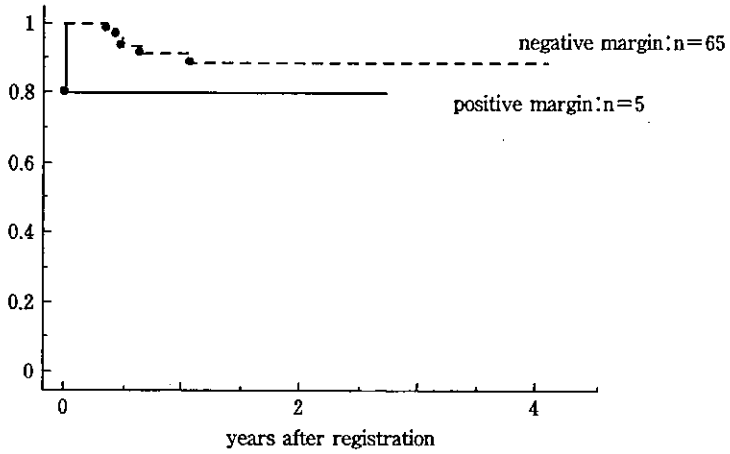


図6 Biochemical-free survival according to the surgical margin by extended radical prostatectomy in cT3 prostate cancer

となるが，NHT下のRPではその判定がartifactなども加わり明確な予後規定因子とならないとの意見[13-15]もあるが，今回の症例群で明らかなPSMは5例に認められ，そのうち1例がPSA failureであった．逆に65例はnegative surgical marginと判断されたがそのうち6例にPSA failureが認められた．図6にその成績を示す．ある程度の傾向は認められるが，negative surgical marginでも10%程度にPSA failureが認められたことになり，pT0となった場合でも再発することが十分あり得る[16]前立腺癌においては病理学的にnegative surgical marginと判断されても決して再発の危険が少ないことを意味するものではないことがやはり伺える．

いずれにしてもこの結果はcT3を対象にしたRPの成績としてはまだ観察期間が短いという問題はあるが文献的にみてもbest resultになるのではないかと考えられる．

我が国の前立腺癌症例においてはPartin nomogram[22]と比較してもnode positiveが低いという印象があり，局所の完全切除を追求することには治療的意義があると考えられる．ただ

し多くの文献でも指摘されているように，cT2前立腺癌にも約3割のunderestimationがあるように，cT3前立腺癌に対しても15-25%のoverestimationがあり得るし，cT3前立腺癌にもunderestimationがあり得る．今回の対象症例も画像などから被膜浸潤ぎりぎり陽性と判定された'早期'のT3症例と，生検などから相当の腫瘍量が想定され，実際，術後pT4と診断された'相当な'T3症例が混在しており，cT3前立腺癌にはcT2前立腺癌以上にその病態はheterogenousな状態と考えられる．cT3前立腺癌で真に局所切除の意味がある症例群の解析が今後の課題と考えており，現在施行している局所の'相当な'進行癌(cTxN0M0癌)に対する広汎前立腺全摘のトライアルスタディの結果を待つ必要がある状況である．

また当初，NHTを併用する意義としてdown-sizingによる相対的な切除断端の確実な確保を目的としていたが，元々前立腺の体積の小さな症例では不要ではという想定もあり，広汎全摘におけるNHTの意義を確認する研究が必要とも考えている．

おわりに

cT3前立腺癌に対するNHTを併用したRPには限界があり，NHTの意義は見いだせないとの意見が多いが，局所をより完全に切除することにより，治療成績の向上が期待できないかとの問題意識から広汎前立腺全摘を開発した．この手術法をもってcT3前立腺癌に対して治療を行ったところ，cT3症例でもpT3aかそれ以下ではorgan confined diseaseと治療成績が全く異ならないことを確認した．我が国の前立腺癌患者では性機能障害よりも手術により根治を望むことが多く，また我が国の前立腺癌のリンパ節転移頻度は低いと想定され，このような環境下では欧米と異なり，cT3という理由で'手術療法ではもはや根治不可'と断定することはできないことを示した．

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Original article

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Phase I and pharmacokinetic study of MCC-465, a doxorubicin (DXR) encapsulated in PEG immunoliposome, in patients with metastatic stomach cancer

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Background: MCC-465 is an immunoliposome-encapsulated doxorubicin (DXR). The liposome is tagged with polyethylene glycol (PEG) and the $F(ab')_2$ fragment of human monoclonal antibody GAH, which positively reacts to >90% of cancerous stomach tissues, but negatively to all normal tissues. In preclinical studies, MCC-465 showed superior cytotoxic activity against several human stomach cancer cells compared with DXR or DXR-incorporated PEG liposomes. The main purpose of this trial was to define the maximum tolerated dose (MTD), dose limiting toxicity (DLT), recommended phase II dose and pharmacokinetics (PK) of MCC-465.

Patients and methods: Patients with metastatic or recurrent stomach cancer were eligible for entry. The initial dose was 6.5 mg/m^2. MCC-465 was administered as a 1-h infusion every 3 weeks and the treatment continued for up to six cycles.

Results: Twenty-three patients received a total of 62 cycles at the 6.5–45.5 mg/m^2 dose level. DLTs were myelosuppression and appetite loss at the 45.5 mg/m^2 dose level. Other toxicities were mild. Neither palmar–plantar erythrodysesthesia nor cardiotoxicity was observed. Acute reactions related to infusion were observed commonly in 16 patients over the entire dose range. While no antitumor response was observed, stable disease (SD) was observed in 10 out of 18 evaluable patients. The pharmacokinetic study showed a similar AUC and C_{max} to Doxil®.

Conclusion: MCC-465 was well tolerated. The recommended dose for a phase II study of MCC-465, for a 3-week schedule, is considered to be 32.5 mg/m^2 in an equivalent amount of DXR.

Key words: doxorubicin, drug delivery system, GAH, immunoliposome, MCC-465, pharmacokinetics

Introduction

There are two main concepts in any drug delivery system, namely active and passive targeting. The former involves monoclonal antibodies to tumor-related molecules that can target the tumor by utilizing a specific binding ability between the antibody and antigen. The latter can be achieved by the so-called enhanced permeability and retention (EPR) effect [1–3]. The EPR effect was named with reference to the pathophysiological characteristics of solid tumor tissue: hypervascularity, incomplete vascular architecture, secretion of vascular permeability factors stimulating extravasation within the cancer, and the absence of effective lymphatic drainage of macromolecules and nanoparticulates. Macromolecules and nanoparticulates have long plasma half-lives because they are too large to pass through the normal vessel walls unless they are trapped by the reticuloendothelial system (RES) in various organs. Such macromolecules can diffuse out of tumor blood vessels, reach the solid tumor tissue effectively and be retained for a long period due to the EPR effect.

PEG-coated liposomes are stable, long-circulating drug carriers useful for delivering doxorubicin (DXR) to the sites of solid tumors. Compared with conventional liposomes, pegylated liposomes are less extensively taken up by the RES and remain in circulation for a long time [4–6]. The long-term circulation and the ability of pegylated liposomes to extravasate through leaky tumor vasculature results in localization of DXR in tumor tissue, probably due to the EPR effect. In a number of animal and human tumors, PEG liposomal DXR produced higher intratumor drug concentrations and better therapeutic responses than equivalent doses of non-pegylated liposome-encapsulated DXR or free DXR [7, 8].

MCC-465 is a newly formulated immunoliposome-encapsulated DXR (Figure 1). This liposome is chemically conjugated to PEG and the $F(ab')_2$ fragment of the human monoclonal antibody,

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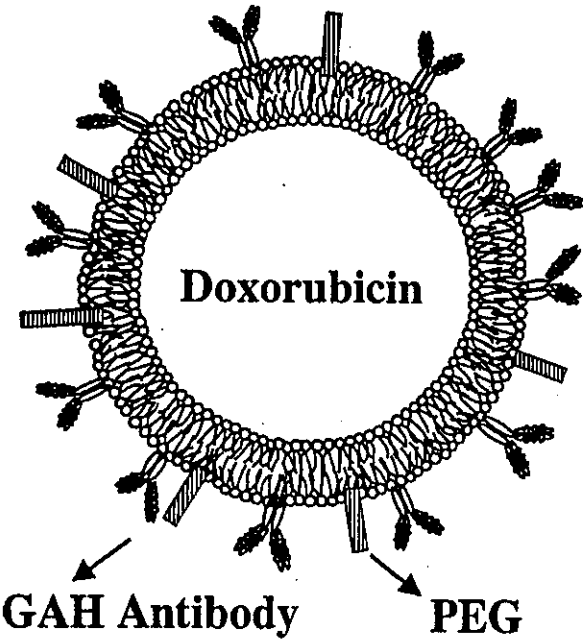

Figure 1. Schematic diagram of MCC-465.

GAH, which recognizes a cell surface molecule on various types of cancer cells [9]. Therefore, this formulation should possess the ability of both active and passive targeting. So far, the antigen recognized by this antibody has not been successfully purified, the reason for which is assumed to be that the antibody may have recognized the epitope as the conformation of the antigen(s). This occurs because of the characteristics of the antibody, which reacts only to viable cells and native protein and not to denatured protein, thus making analysis by conventional methods based on protein chemistry useless.

In reality, at the time of the initiation of this phase I study, it was already clear that the antigen exists on the cell surface, correlates to cytoskeletal protein and positively stains 90% of the various stomach cancer cells and tissues but is always negative in normal cells.

The antitumor activity of MCC-465 against GAH-positive human stomach cancer B37 cells was compared with that of GAH non-conjugated PEG liposomal DXR *in vitro*. The result clearly showed that MCC-465 was much more effective against B37 cells than GAH non-conjugated PEG liposomal DXR [9]. In nude mice, MCC-465 exhibited higher antitumor activity against several GAH-positive stomach cancers transplanted in the renal capsules of mice in comparison with free DXR or GAH non-conjugated PEG liposomal DXR [9]. Using a fluorescence-labeled liposome, it was revealed that GAH-tagged liposomes were extensively internalized, but GAH non-tagged liposomes were not [9]. Taking all the data together, we concluded that the GAH-conjugated immunoliposome was highly potent as a drug-targeting device, especially for human stomach cancer. Therefore, this phase I study was confined to patients with advanced gastric cancer.

Patients and methods

Eligibility criteria

Patients with cytologically or histologically confirmed advanced or recurrent gastric cancer refractory to conventional therapy were eligible for entry in this study. Patients with any serious infection, including hepatitis B and C viruses, and HIV, uncontrollable hypertension, symptomatic brain metastasis, allergy to anthracycline-type drugs, pre-existing cardiac disease including congestive heart failure, arrhythmia requiring treatment and myocardial infarction, vascular disordesr including a history of pulmonary embolism, deep venous thrombosis, and peripheral artery occlusive disease, were excluded. Patients were also excluded if they were pregnant or lactating, or showing gastrointestinal bleeding. In addition, any patient who the principal investigator or investigator considered ineligible was excluded. Eligibility criteria also included the following: (i) World Health Organization performance status ≤2; (ii) age ≥20 and <75 years; (iii) normal hematological (white blood cell count ≥4000/μl, platelet count ≥100 000/μl), hepatic [total bilirubin level ≤1.5 mg/dl; aspartate aminotransferase and alanine aminotransferase ≤2.5× the upper limit of normal (ULN), unless the elevation was a result of hepatic metastasis, in which case elevations ≤3× ULN were permitted], renal (serum creatinine within normal range), cardiac (classification of New York Heart Association ≤I) and pulmonary (PaO_2 ≥60 mmHg) function; (iv) no chemotherapy within 4 weeks (6 weeks for nitrosourea and mitomycin C) before administration of MCC-465; (v) a lifetime cumulative dose of DXR <100 mg/m^2 or that of epirubicin and pirarubicin <200 mg/m^2; (vi) no radiotherapy within 4 weeks before treatment; (vii) life expectancy >3 months; and (viii) full recovery from toxicity caused by any other test drug previously administered. The Institutional Review Boards for each hospital approved the protocol and informed consent brochures. Written informed consent was obtained from all patients.

Study drug and drug administration

MCC-465 was constructed from PEG (molecular weight 5 kDa on average), lipids, doxorubicin hydrochloride and F(ab')$_2$/GAH. Lipids were consisted from dipalmitoylphosphatidylcholine, cholesterol and maleimidated cipalmitoylphosphatidyletahnolamine. The ratio of the conjugated F(ab')$_2$/GAH, PEG, DXR and lipids was 1:4:5:50 (w/w/w/w), respectively. The mean size of MCC-465 was 143 nm [9].

MCC-465, which was manufactured under the good manufacturing practice regulations of the Ministry of Health, Labour and Welfare of Japan, was supplied by Mitsubishi Pharma Corporation (Osaka, Japan) in glass vials. Each vial contained lyophilized PEG immunoliposomes containing a total of 10 mg of doxorubicin hydrochloride. Appropriate amounts of MCC-465 dissolved in cold sterile saline for injection were diluted and adjusted with sterile saline up to 250 ml (or 500 ml when patients received >70 mg DXR equivalent dose/body). MCC-465 solution was infused intravenously for 60 min, or 120 min when the diluted volume was 500 ml, by an electric-driven pump with a fine filter F162 (Forte Grow Medical Co., Tochigi, Japan).

Dosage and dose escalation

The starting dose of MCC-465 was 6.5 mg/m^2, which is equivalent to one-tenth of the LD_{10} in rats. MCC-465 was administered once every 3 weeks and the treatment was continued up to six cycles unless any severe adverse event or disease progression was observed. Dose escalation proceeded according to a accelerated titration method described previously [10]. Toxicity was graded from 1 to 4 using the criteria of the Japan Clinical Oncology Group. If grade ≥2 toxicity occurred during the first 21 days, the dose of the next level should be increased according to the modified Fibonacci method and toxicity was to be confirmed in at least three patients. Intrapatient dose escalation was not permitted. At the level at which dose-limiting toxicity (DLT) was observed, toxicity was confirmed in up to six patients. The maximum tolerated dose (MTD) was then defined as one level below that level at which three out of six patients

Table 1. HPLC conditions

	HPLC condition 1	HPLC condition 2	HPLC condition 3
Column	CAPCELLPAK C18 UG120 (Shiseido)		
Column size (mm)	2.0 × 250	4.6 × 250	2.0 × 250
Column temperature	50°C		
Mobile phase	0.05 M phosphoric acid:acetonitrile:THF:PIC B-7 = 75:23:2:1.5 v/v	0.05 M phosphoric acid:acetonitrile (containing 7.5% THF):PIC B-7 = 75:23:2:1.5 v/v	0.05 M phosphoric acid:acetonitrile:THF:PIC B-7 = 70:28:2:2.5 v/v
Flow rate (ml/min)	0.2	1.2	0.18
Fluorescent detector wavelength (nm)	Ex = 475; Em = 554		

HPLC, high-performance liquid chromatography; THF, tetrahydrofuran; PIC B-7, ion-pair reagent (heptane sulfonic acid); Ex, excitatory; Em, emission.

experienced a DLT [11]. The recommended dose for a phase II trial was defined by the Efficacy and Safety Assessment Committee from the results of the safety and efficacy of this trial. DLT was defined as: (i) neutrophil count of <500/μl for >5 days or associated neutropenic fever of >38.5°C with infection; (ii) platelet count of <25 000/μl; and (iii) non-hematological toxicities except for nausea, vomiting and alopecia.

Pretreatment assessment and follow-up

At enrollment, patients were evaluated by a complete history and physical examination, performance status, complete blood cell count (CBC), blood chemistry, urinalysis, electrocardiogram (ECG), computed tomography or upper gastrointestinal series. Other exams were performed only in the presence of a clinical indication. Patients were monitored by physical examination every day up to the second administration, and at days 1, 2 and 3 and weekly thereafter by CBC and blood chemistry. ECG was recorded before and during treatment. Ultrasonic cardiography was repeated before every other administration. Human antihuman antibody (HAHA) was evaluated before every cycle. Tumor markers were also measured at the same time as HAHA.

Tumor response was evaluated according to the criteria of the Japan Society of Clinical Oncology. Complete response (CR) was defined as the disappearance of cancerous lesions and partial response (PR) required a >50% reduction in the sum of the bidimensional length of tumors on two points separated by at least 4 weeks. Stable disease (SD) was defined as a <50% reduction or <25% growth of lesions for at least 4 weeks. Progressive disease (PD) was defined as >25% of tumor growth, appearance of new malignant lesions or unequivocal worsening of other clinical evidence of malignancy. The Clinical Trial Coordinating Committee and the Efficacy and Safety Assessment Committee were organized to bridge between the three institutions at which the study was performed.

Sampling and storage

The measures recorded at the first cycle were as follows: (i) plasma concentrations of DXR (total DXR concentration); (ii) concentrations of DXR after gel filtration (liposomal-encapsulated DXR); (iii) concentration of DXR after ultrafiltration (free DXR); (iv) plasma concentrations of DXR metabolites; (v) urinary concentrations of DXR; and (vi) urinary concentrations of DXR metabolites. After the second cycle, only total DXR was measured. All the samples were chilled on ice during preparation and prepared using the appropriate method described below. Urine samples were stored in a refrigerator from the day before the drug administration to day 4. Prepared plasma and urinary samples were stored at –20°C except for encapsulated DXR samples, which were stored at –80°C.

Assay conditions

For the measurement of total DXR and metabolites [doxorubicinol (Dxol) and 7-deoxydoxorubicinol aglycon (7H-Dxol)] in plasma, 200 μl of boric acid buffer (pH 9.8) and 3 ml of chloroform/methanol (80:20 v/v) were added to 200 μl of human plasma and mixed. The separated organic phase was evaporated to dryness under a nitrogen stream. Residue was re-dissolved with the mobile phase and injected into a high-performance liquid chromatograph (HPLC) under the conditions described in 'HPLC conditions 1' in Table 1. For the measurement of encapsulated DXR by HPLC, plasma from each patient (60 μl) and the marker solution (60 μl) containing sufficient empty MCC-465 (no DXR), which is used as the marker for UV detection, were mixed. Then, 100 μl of the mixture was loaded onto a gel filtration column. The peak fraction of encapsulated DXR through gel filtration was collected, and 100 μl of the fractionated sample was mixed with 230 μl of methanol containing 0.15% trifluoroacetic acid. The mixture was injected into the HPLC under the condition described in 'HPLC conditions 2' in Table 1. For the free DXR measurement, 1 ml of freshly prepared plasma at each point was centrifuged immediately in Centrifree (Amicon Co., Ltd) at 2000 *g* at room temperature for 10 min. One hundred microliters of the collected sample were mixed with 250 μl of the mobile phase (described in Table 1) containing the internal standard. DXR and the metabolites in urine were measured using the same method as for plasma, except the urine volume was 400 μl.

Pharmacokinetic analysis

Pharmacokinetic (PK) parameters of total DXR, free DXR and metabolites in human plasma were calculated by a non-compartmental model using WinNonlin, version 2.1 (Pharsight Corporation). The parameters calculated were as follows: peak plasma concentration (C_{max}); time to reach the peak plasma concentration (T_{max}); half-life of terminal phase ($T_{1/2\lambda z}$); area under the concentration–time curve (AUC); total clearance (CL); volume of distribution at steady state (V_{dss}); and mean residence time (MRT). The total urinary excretion rates for DXR and metabolites were calculated for each subject, and mean urinary excretion rates of DXR and metabolites were calculated at each level. Microsoft Excel 97 was used for data management.

Results

Enrollment and dosing

Twenty-three patients were enrolled in this study. The details of each patient's background are shown in Table 2. Dose escalation was from 6.5 up to 45.5 mg/m^2. In total, 62 cycles of administra-

Table 2. Patient characteristics

	Dose (mg/m²)					
	Level 1	Level 2	Level 3	Level 4	Level 5	
	6.5	13.0	21.0	32.5	45.5	Total
No. patients (male:female)	7 (6:1)	3 (3:0)	3 (3:0)	3 (2:1)	7 (5:2)	23 (19:4)
Age (years)						
Median	58	57	54	68	65	58
Range	40–68	57–58	30–60	56–72	52–69	30–72
Performance status						
0	1	2	1	1	3	8
1	5	1	2	2	4	14
2	1	0	0	0	0	1
Original lesion						
Yes	3	1	1	0	2	7
No	4	2	2	3	5	16
Metastatic lesion						
Lung	1	0	1	1	1	4
Liver	3	1	0	2	5	11
Adrenal gland	1	0	0	0	1	2
Bone	0	0	2	0	1	3
Peritonea	3	1	0	0	0	4
Esophagus	0	0	1	0	0	1
Lymph node	3	2	3	1	6	15
Prior therapy						
1	5	1	1	2	2	11
2	1	2	0	1	5	9
≥3	1	0	2	0	0	3

tion were performed. Sixteen patients received more than two cycles of administration. The maximum was six cycles at level 4 (32.5 mg/m²) to one patient, and the average number of cycles over all levels was 2.7 (Table 2). The first patient, 1-101, developed obstructed jaundice caused by tumor progression. The Efficacy and Safety Assessment Committee suggested that the patient should not be a subject for PK and efficacy analysis, but was eligible for safety analysis, and recommended the enrollment of an additional patient at the first dose level. The second patient, 1-102, experienced grade 3 hypertension with grade 2 fever and shivering during infusion. At this time, administration was stopped immediately and restarted cautiously after the patient had recovered from all the symptoms induced by the medication, along with an anti-hypertensive drug. These symptoms were considered as an infusion-related reaction (IRR). The dose escalation method was switched to the modified Fibonacci method thereafter, and five more patients were enrolled at the first level.

IRR and other non-hematological toxicities

All the patients who received administration were assessed for safety. Major non-hematological toxicities are summarized in Table 3. IRRs were observed in 16 patients at the first exposure to the drug. Early reactions by infusion occurred 5–20 min after the start of the infusion, and chest discomfort, lumbago, back pain, itching and urticaria were observed as symptoms. All the symptoms disappeared shortly after chlorpheniramine maleate treatment or with no treatment. Late reactions to infusion developed usually at the end of infusion of MCC-465, and were characterized by chills and shivering. All the symptoms disappeared quickly, with no treatment. However, one patient at level 1 experienced grade 3 hypertension accompanying chills and shivering. Some patients developed grade 1 or 2 fever 30 or 60 min after the termination of the infusion; however, fever was transient in all cases. Patients who experienced such IRRs could be treated with MCC-465 repeatedly without pre-medications, and revealed no severe reactions thereafter. Skin toxicities such as mild rash, alopecia, erythema, pruritis and urticaria were observed in five patients. Palmar–plantar erythrodysesthesia (PPE) was not observed in this study. Grade 1 or 2 stomatitis was observed in four patients; grade 1–2 nausea and vomiting were observed in 11 patients; and grade 3 appetite loss was observed in one patient at level 5, which was defined as a DLT. There was no remarkable evidence of liver

Table 3. Non-hematological toxicity

	6.5 mg/m²		13.0 mg/m²		21.0 mg/m²		32.5 mg/m²		45.5 mg/m²		Total No. patients
	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4	
General disorders											
Pyrexia	3		1		2		2		6		14
Rigors	2		1		2		2		2		9
Malaise							2		5		7
Shivering	2		1		1		1		1		6
Performance status decreased	1								3	2	6
Chest discomfort					1				1		2
Feeling hot									2		2
Gastrointestinal disorders											
Nausea	2		2				1		2		7
Anorexia			1		1				3	2	7
Vomiting					1		1		4		6
Stomatitis	1		1				2				4
Diarrhea	1								2		3
Skin and subcutaneous tissue disorders											
Urticaria NOS	1						1				2
Depilation									2		2
Others											
Blood pressure increased	1	1	1		1				1		5
Flushing	1						1		1		3
Back pain							1		1		2
Taste disturbance			1						1		2
Heart rate increased	1		1								2

NOS, not otherwise specified.

Table 4. Hematological toxicity

Dose (mg/m²) (No. patients)	21.0 (3)				32.5 (3)				45.5 (7)			
Grade	1	2	3	4	1	2	3	4	1	2	3	4
Leukopenia	1	0	0	0	1	0	1	0	1	2	3	1
Neutropenia	1	0	0	0	1	0	1	0	0	1	2	3
Thrombocytopenia	0	0	0	0	0	0	0	0	1	1	0	0

function or kidney function abnormalities related to the treatment. No pain or local toxicity in the area of injection was observed.

No HAHA was detected throughout all treatment cycles.

Hematological toxicity

No significant myelosuppression was observed up to dose level 3. Grade 4 leucopenia was observed in one patient at level 5 (45.5 mg/m²). Grade 4 neutropenia was observed in three patients at the same level (Table 4). One of these three patients experienced grade 4 neutropenia that lasted for 5 days, which was defined as a DLT. Leucopenia and neutropenia started from day 4–8, and the median time to the nadir was 15 days.

MTD and DLT

In total, three DLTs were observed through this trial, including grade 3 hypertension at level 1, grade 4 neutropenia lasting for

Table 5. Mean PK parameters at each level in the phase I clinical study

	n	Dose (mg/m²)	C_{max} (μg/ml)	T_{max} (h)	$AUC_{0-\infty}$ (μg·h/ml)	CL (ml/h/kg)	V_{dss} (×10²) (ml/kg)	MRT (h)	$T_{1/2\lambda z}$ (h)
Total and free DXR									
Level 1			2.49 ± 1.45	1.01 ± 0.01	31.5 ± 23.7	(4.45 ± 1.78)[a] 20.4 ± 25.4	(0.413 ± 0.114)[a] 2.61 ± 3.43	10.9 ± 2.53	9.09 ± 4.30
Level 2	3	13.0	7.18 ± 0.847	1.28 ± 0.13	120 ± 44.6	3.36 ± 1.13	0.325 ± 0.0499	10.3 ± 3.11	7.66 ± 1.68
Level 3	3	21.0	11.4 ± 1.42	1.06 ± 0.10	177 ± 27.5	3.39 ± 0.509	0.282 ± 0.0190	8.39 ± 0.761	21.7 ± 12.0
Level 4	3	32.5	15.1 ± 1.47	1.39 ± 0.54	310 ± 139	4.00 ± 1.97	0.511 ± 0.140	14.5 ± 6.42	33.2 ± 17.7
Level 5	3[b]	45.5	30.3 ± 4.05	1.00 ± 0	637 ± 257	2.53 ± 1.16	0.345 ± 0.0257	16.2 ± 8.14	69.3 ± 58.9
	3[c]	45.5	21.3 ± 1.99	2.67 ± 0.58	397 ± 118	3.28 ± 1.07	0.325 ± 0.0170	10.6 ± 3.42	40.7 ± 26.8
Free DXR in level 4	3	32.5	0.325 ± 0.0990	0.67 ± 0.29	3.07	504	210	73.5	54.5
Free DXR in level 5	3[b]	45.5	0.251 ± 0.0874	0.50 ± 0	2.35	690	279	48.6	36.8
	3[c]	45.5	0.131 ± 0.0795	2.00 ± 0	3.02 ± 1.57	473 ± 197	234 ± 26.3	57.1 ± 28.2	41.2 ± 21.6

	n	Dose (mg/m²)	C_{max} (μg/ml)	T_{max} (h)	AUC_{0-t} (μg·h/ml)	$AUC_{0-\infty}$ (μg·h/ml)	CL (×10²) (ml/h/kg)	V_{dss} (×10³) (ml/kg)	MRT (h)	$T_{1/2\lambda z}$ (h)
Metabolites										
Dxol in level 5	3[b]	45.5	0.0099 ± 0.0026	19.67 ± 9.24	1.13 ± 0.274	1.52 ± 0.155	9.22 ± 0.730	145 ± 28.0	156 ± 18.7	109 ± 25.8
	3[c]	45.5	0.0074 ± 0.0068	50.00 ± 24.00	1.10 ± 1.17	1.30 ± 1.21	15.2 ± 9.39	216 ± 134	142 ± 1.73	90.4 ± 4.65
7H-Dxol in level 5	3[b]	45.5	0.0036 ± 0.0013	1.33 ± 0.577	0.104 ± 0.0956	0.204	108	354	44.8	29.0
	3[c]	45.5	0.0079 ± 0.0071	2.67 ± 0.577	0.160 ± 0.143	0.272	44.3	126	27.4	19.2

PK parameters are shown as mean ± SD, but in case of $n = 2$, PK parameters are shown as means.

[a] $n = 4$ (exclusion of patients 1-105 and 1-107).

[b] 60 min infusion.

[c] 120 min infusion.

C_{max}, peak plasma concentration; T_{max}, time to reach the peak plasma concentration; $T_{1/2\lambda z}$, half-life of terminal phase; AUC, area under the concentration–time curve; CL, total clearance; V_{dss}, volume of distribution at steady state; MRT, mean residence time; DXR, doxorubicin; PK, pharmacokinetic; SD, standard deviation.

Table 6. The efficacy and number of doses at each dose level

Level	Dose (mg/m^2)	No. patients	NE	Excluded	PD	SD	No. cycles administered	
							Range	Average
1	6.5	7	2	1	3	1	1–2	1.4
2	13	3			1	2	2–5	4
3	21	3			1	2	2–5	4
4	32.5	3			1	2	3–6	4
5	45.5	7	1	1	2	3	1–4	2.3

NE, not evaluable; PD, progressive disease; SD, stable disease.

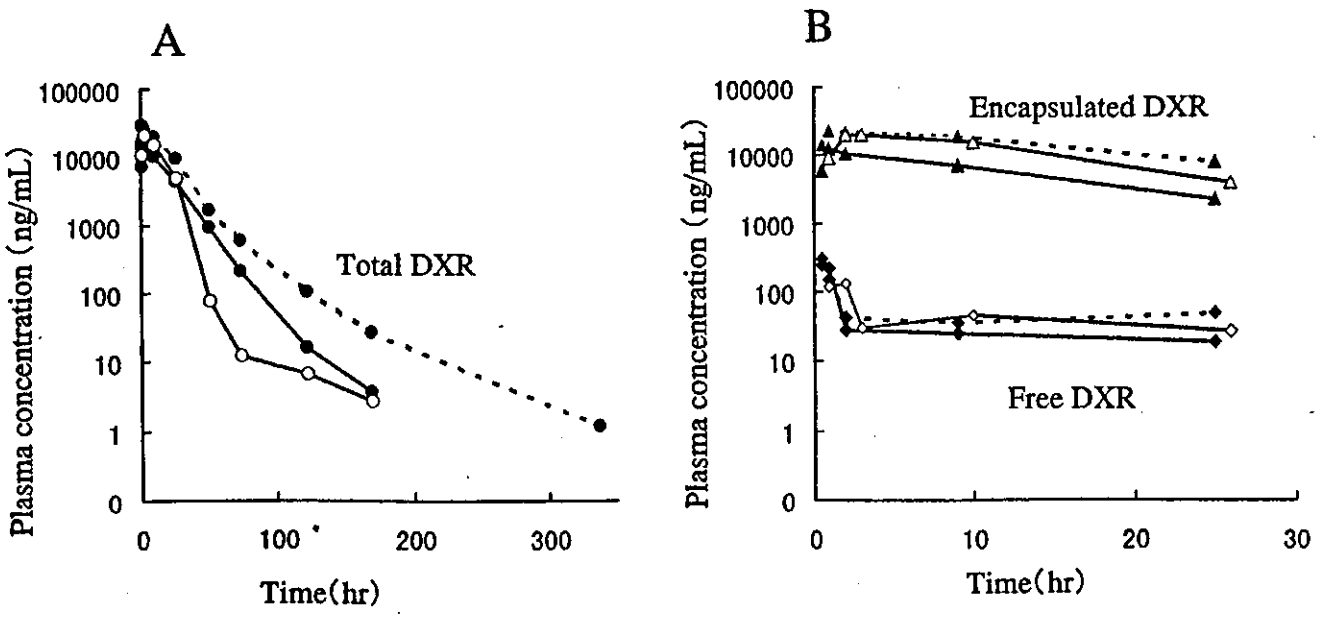

Figure 2. Mean plasma concentrations of doxorubicin (DXR) in the phase I clinical study (level 4 and level 5 of 60 or 120 min infusion). (A) Total DXR in plasma: filled circles, solid line, level 4; filled circles, dotted line, level 5 (60 min); open circles, level 5 (120 min). (B) Free (open or filled diamonds) and encapsulated (open or filled triangles) DXR in plasma: filled triangles or diamonds, solid line, level 4; filled triangles or diamonds, dotted line, level 5 (60 min); open triangles or diamonds, level 5 (120 min). Time in the figures was expressed as scheduled time. $n = 3$, except level 5 (*$n = 2$, patient 1-122 excluded).

>5 days at level 5, and grade 3 appetite loss at level 5. At level 5, two out of six patients experienced grade 4 neutropenia, in addition to two patients with DLT. Since it was suggested that >50% of the patients would develop DLT at the next level (level 6), the dose escalation was stopped at level 5. The MTD was considered to be 45.5 mg/m^2 (level 5).

Antitumor activity

Although the antitumor activity was not the primary end point, 18 out of 23 patients were evaluable. No responses were observed definitely in these evaluable 18 patients. However, 10 patients had SD (median duration was 92.5 days, range 48–135) (Table 6). Seven of these 10 received more than four cycles of treatment within at least 16 weeks, while these patients have received multiple (once as many as 5 cycles; median 2 cycles) prior chemotherapy cycles (Tables 2–5).

Pharmacokinetics

PKs were evaluated in 21 patients. The mean plasma concentrations of total DXR, free DXR and encapsulated DXR at levels 4 and 5 (60 and 120 min infusion, respectively) are shown in Figure 2. Most of the total DXR existed in the circulating blood in an encapsulated form, because the plasma concentration of encapsulated DXR was very close to that of total DXR. The peak plasma concentration of total DXR at each level reached C_{max} at the end of the infusion or 1 h after the infusion. While the plasma concentration profiles of total DXR showed a biphasic elimination pattern at levels 1–3, a monophasic elimination pattern was observed at

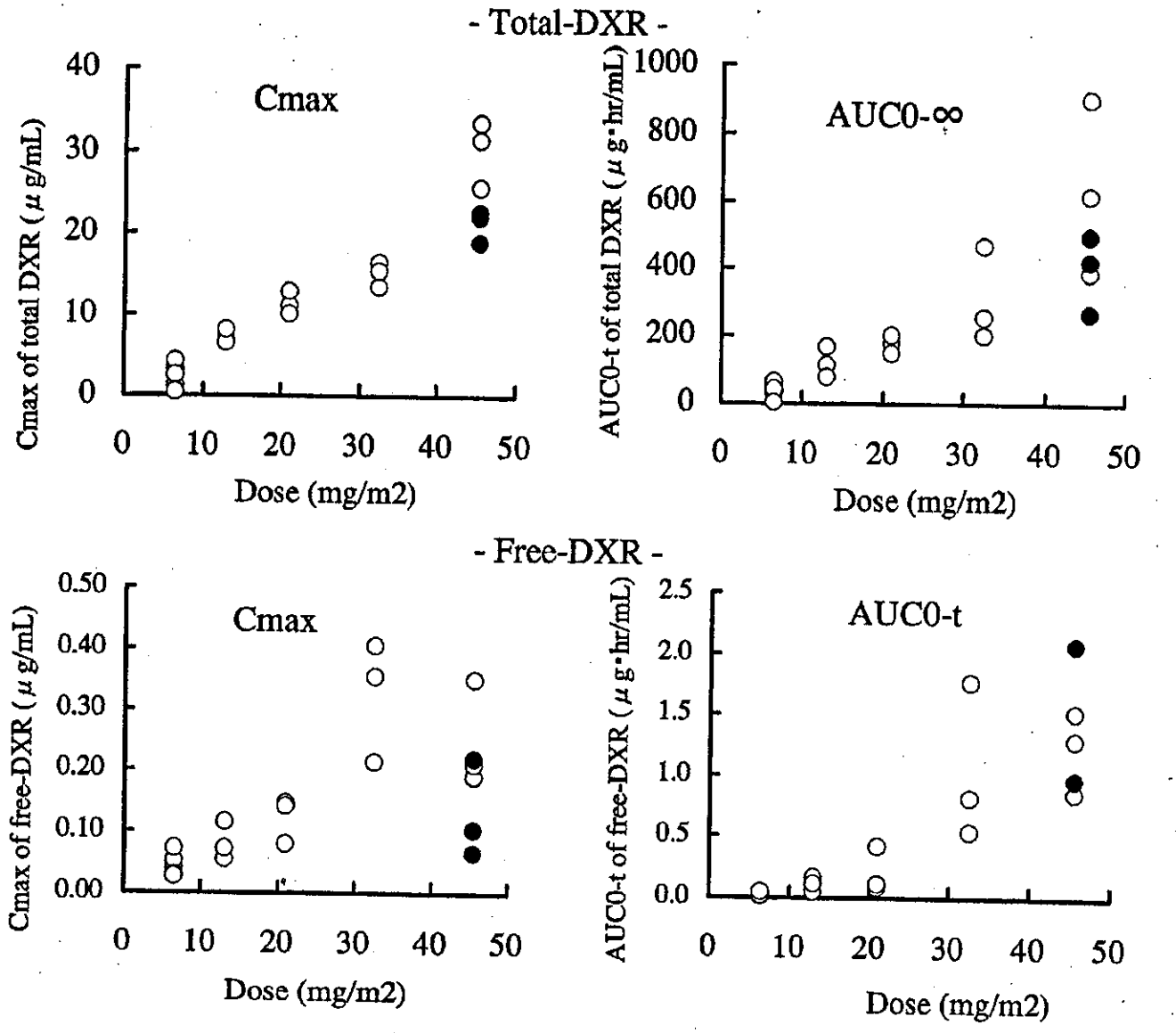

Figure 3. Relationship between dose and C_{max} or AUC. Open circles, 60 min infusion; filled circles, 120 min infusion.

level 4 and 5, where one subject at each level (one at level 4 and one at level 5) showed a monophasic elimination pattern with a long half-life. The mean pharmacokinetic parameters of total DXR, free DXR and metabolites in plasma at levels 1–5 are shown in Table 5. The CL, V_{dss} and MRT of total DXR were almost the same for all the dose levels. The $T_{1/2\lambda z}$ of total DXR was prolonged by the dose escalation because the concentration of the terminal phase was detected as the dose increased. There was no difference in terms of total DXR concentration during repeated administrations (data not shown). It was suggested that the PK profile of total DXR in plasma was not affected by repeated administration of MCC-465 every 3 weeks. Plasma concentration profiles of free DXR showed slow elimination at levels 4 and 5. The CL and V_{dss} of free DXR were almost identical among the dose levels tested. The plasma concentration of Dxol (60 min infusion) reached C_{max} at 19.67–25.26 h (mean value at each level), and was eliminated more slowly than DXR. The plasma concentration of 7H-Dxol was detected at level 4 and 5, but was close to the lower limit of quantification.

The C_{max} and AUC of total DXR and free DXR increased with dose escalation (Figure 3).

Discussion

Goren et al. [12] prepared immunoliposomes conjugated with whole monoclonal antibody against Her-2 and studied the tumor targeting efficacy of the immunoliposomes in comparison with plain liposomes. They suggested that the antitumor efficacy of the drug-containing liposomes depended on the drug delivery to the tumor, and that the rate-limiting factor of liposome accumulation in tumors was the liposome extravasation process, irrespective of

liposome affinity to, or targeting of, tumor cells. In addition, it was reported that immunoliposomes conjugated with whole IgG had a shorter plasma half-life because the immunoliposomes were entrapped by the RES. In order to overcome this problem, Maruyama et al. [13] prepared an Fc-removed antibody for immunoliposomes and succeeded in reducing the RES entrapment of the immunoliposomes. Taking these reports into consideration, $F(ab')_2$ of GAH was conjugated to MCC-465.

The present clinical data indicate that the PK parameters of MCC-465 differ from those of free doxorubicin, but were very similar to those of Doxil in humans [6]. These data show the stability of MCC-465 in the blood circulation. They also indicate that the conjugation of the $F(ab')_2$ of GAH does not interfere with the stealth effect of the PEG liposomes.

The DLTs of MCC-465 were neutropenia and appetite loss. Unlike the findings from previous reports of phase I trials for a similar drug, Doxil, we did not experience any severe skin toxicity such as PPE or mucositis. The reason for the difference in toxicity between MCC-465 and Doxil is not known; however, it is speculated that the accumulation of MCC-465 in the skin is different from Doxil, or that Caucasian patients are more prone to skin damage by such stealth liposomal DXR in terms of skin toxicity compared with Japanese patients.

IRR was the most common adverse effect. Sixteen out of 23 treated patients showed a variety of symptoms. The major symptoms were fever, rigors, shivering, flushing, chest discomfort, back pain, red eye, itching, vomiting, feeling hot and numbness, which developed at the beginning of the infusion, while fever, rigors, shivering and hypertension were observed at the end of the infusion. Some patients showed two or more symptoms at the same time, but these symptoms were mild, and they disappeared during the infusion or within a few hours. Eight out of nine patients who had chill or shivering also experienced fever. Among these patients, four also had hypertension, and one developed grade 3 hypertension. Chlorpheniramine maleate was administered to four patients for shivering, itching or flushing, and an antihypertensive drug was administered to one patient. The other patients had no medication for IRR.

Other non-hematological toxicities were mild, and no malfunctioning was observed in the organs such as the liver and kidney. Although cardiac safety was not addressed specifically, since the maximum cumulative dose of doxorubicin in this phase I study was 195 mg/m^2, no patients presented with cardiac toxicity symptoms or loss of function in terms of the left ventricular ejection fraction.

In this study, we needed a 3-week interval schedule in order to determine the safety, tolerability and PKs. No objective tumor response was seen. It is, however, worth noting that 10 SD patients were observed among our 18 evaluable patients, who belonged to a population with a very low probability of response because of extensive prior chemotherapy for gastric cancer.

Our study shows that the MTD of MCC-465 using the 3-week schedule (45.5 mg/m^2) is slightly lower than the MTD of Doxil (50 mg/m^2). This result may be due in part to the unstable condition of heavily treated patients with advanced gastric cancer. These data warrant further investigation of MCC-465, and the recommended dose for a phase II study with a 3-week administration protocol is considered to be 32.5 mg/m^2.

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Phase I/II study of S-1 combined with cisplatin in patients with advanced gastric cancer

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A dose-escalation study of cisplatin (CDDP) combined with S-1, a new oral dihydropyrimidine dehydrogenase inhibitory fluoropyrimidine, was performed to determine the maximum-tolerated dose (MTD), recommended dose (RD), dose-limiting toxicities (DLTs), and objective response rate (RR) in advanced gastric cancer (AGC). S-1 was given orally at 40 mg m^{-2} b.i.d. for 21 consecutive days following a 2-week rest. CDDP was planned to be given intravenously on day 8, at a dose of 60, 70, or 80 mg m^{-2} depending on the DLT. Treatment was repeated every 5 weeks, unless disease progression was observed. In the phase I portion, the MTD of CDDP was presumed to be 70 mg m^{-2}, because 33.3% of patients (2/6) developed DLTs, mainly neutropenia. Therefore, the RD of CDDP was estimated as 60 mg m^{-2}. In the phase II portion, 19 patients including six patients of the RD phase I portion were evaluated. The median administered courses was four (range: 1–8). The incidences of severe (grades 3–4) haematological and nonhaematological toxicities were 15.8 and 26.3%, respectively, but all were manageable. The RR was 74% (14/19, 95% confidence interval: 54.9–90.6%), and the median survival day was 383. This regimen is considered to be active against AGC with acceptable toxicity.

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The significant survival benefit of 5-fluorouracil (5-FU)-based chemotherapy for unresectable advanced gastric cancer (AGC) compared with best supportive care is reported (Murad *et al*, 1993; Glimelius *et al*, 1994; Pyrhonen *et al*, 1995). To improve the objective response rate (RR) and survival for AGC, many combination regimens based on 5-FU and its derivatives have been studied clinically. However, the median survival times (MST) with these combination chemotherapies were only 5.7–10.5 months (Wils *et al*, 1991; Kelsen *et al*, 1992; Kim *et al*, 1993; Vanhoefer *et al*, 2000). Although some combination chemotherapies showing superior results in AGC have been reported (Wils *et al*, 1991; Webb *et al*, 1997), there is no regimen accepted worldwide as the standard treatment (Ohtsu *et al*, 2003). Therefore, we need to develop new agents and combination chemotherapy regimens to achieve greater survival benefit in AGC.

As administered 5-FU is rapidly degraded by dihydropyrimidine dehydrogenase (DPD), DPD seems to be a typical prognosis factor against 5-FU-based chemotherapy (Takabayashi *et al*, 2000). Therefore, a new oral drug that inhibits DPD namely, DPD inhibitory fluoropyrimidine (DIF), was invented (Diasio, 1999). S-1 is a new oral DIF, and consists of tegafur (FT), 5-chloro-2,4-dihydroxypyridine (CDHP), and potassium oxonate (Oxo) at a molar ratio of 1 : 0.4 : 1. It achieved high efficacy without increasing gastrointestinal (GI) toxicity, based on biochemical modulation theory (Shirasaka *et al*, 1996).

In two late phase II clinical studies for AGC in Japan, the combined RR of the two studies was 44.6%, with a very low (2.0%) incidence of grade 3 diarrhoea (Sakata *et al*, 1998; Koizumi *et al*, 2000). S-1 was approved in Japan for AGC under an accelerated approval regulation system in 1999, and for head and neck cancer in 2001, and clinical trials against colorectal (Ohtsu *et al*, 2000), breast (Sano *et al*, 2000), and lung cancer (Kawahara *et al*, 2001) are now ongoing and high responses have been reported. The phase II studies of S-1 against gastric (Chollet *et al*, 2003) and colorectal cancer (den Brande *et al*, 2003) in Europe by EORTC-Early Clinical Study Group also revealed high efficacy. Therefore, S-1 can be anticipated to be one of the key drugs for AGC.

Several combination regimens show high RR; however, toxic effects limited the survival benefit (Kelsen *et al*, 1992; Kim *et al*, 1993; Vanhoefer *et al*, 2000; Ohtsu *et al*, 2003). Therefore, new chemotherapy regimens to achieve survival benefit with low toxicities are needed. Combinations of 5-FU and cisplatin (CDDP) were synergistic in preclinical (Scanlon *et al*, 1986; Yamada *et al*, 1990; Shirasaka *et al*, 1993) and clinical studies (Rougier *et al*, 1994) on AGC with acceptable toxicity. Based on these studies, we conducted a phase I/II study of S-1 in combination with CDDP.

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PATIENTS AND METHODS

Patients

Prior to entry, tumour size was determined by chest or GI X-ray, endoscopic examination of the upper GI tract, computed tomographic (CT) scan of the abdomen, barium enema, and bone scintigram. A complete blood cell count, liver and renal function test, and urinalysis were executed within 7 days before entry.

The eligibility criteria were as follows: aged 20–74 years; histologically proven unresectable locally advanced or metastatic gastric adenocarcinoma; no prior chemotherapy except adjuvant chemotherapy more than 30 days prior to entry; adequate organ function, defined as haemoglobin $>8.0\,g\,dl^{-1}$, leucocyte count $>4000-12000\,mm^{-3}$, platelet count $>100000\,mm^{-3}$, serum bilirubin level $<1.5\,mg\,dl^{-1}$, serum transaminase (aspartate aminotransferase and alanine aminotransferase) $<100\,U\,l^{-1}$, alkaline phosphatase (ALP) < twice the upper limit of the normal range (ULN) of each hospital, serum creatinine level less than the ULN of each hospital, creatinine clearance $>50\,ml\,min^{-1}$; Eastern Cooperative Oncology performance status (PS) 0–2; expected survival period more than 3 months; and written informed consent from the patients. Patients with symptomatic brain metastases were not eligible.

This study was approved by the ethics committees in each institution.

Treatment and dose escalation schedule

S-1 was given orally at a dose that did not exceed $40\,mg\,m^{-2}$ based on the patient's body surface area (BSA): BSA $<1.25\,m^2$, 40 mg; $1.25-1.5\,m^2$, 50 mg, and BSA $>1.5\,m^2$, 60 mg, for 21 consecutive days (b.i.d.) and CDDP was diluted in 400 ml physiological saline, and administered as a 120-min i.v. infusion on day 8. The starting dose of CDDP was $60\,mg\,m^{-2}$ (level 1), which was planned to be increased in $10\,mg\,m^{-2}$ increments to $80\,mg\,m^{-2}$ unless maximum-tolerated dose (MTD) was achieved. The starting dose of CDDP corresponded to 66.7–85.7% of the recommended dose (RD) for gastric cancer in Japan. No intrapatient dose escalation was allowed. At least three patients were treated at each dose level. If one of three patients at a given dose developed any dose-limiting toxicity (DLT), other three or more patients were to be entered at the same dose. Before proceeding to the next dose level, all previously treated patients had received at least one course.

This treatment course was repeated every 5 weeks with an allowance for a delay in treatment if toxicity was observed.

To avoid CDDP-induced renal damage, patients were hydrated on day 8 with 1500 ml 5% glucose, and furosemide was given 30 min prior to the start of CDDP infusion, and 4000 ml 5% glucose was continued for another 48 h.

The next course was started only for the patient whose organ biological parameter had been maintained as eligibility criteria, except the leucocyte count ($>3000\,mm^{-3}$) and no disease progression observed. Prophylactic administration of antiemetic medication ($5\text{-}HT_3$ antagonist plus corticosteroid) at standard doses was routinely used when CDDP was administered to prevent nausea and vomiting. The treatment was repeated unless disease progression or severe toxicity was observed. S-1 was provided by Taiho Pharmaceutical Co., Ltd (Tokyo, Japan).

Evaluation

A complete blood cell count, liver and renal function test, and urinalysis were assessed at least once a week during the first course, and every other week afterwards. Before each course, additional examinations were performed to evaluate sites.

The National Cancer Institute common toxicity criteria version 2.0 was applied to evaluate the toxicity of this therapy. DLTs were defined as grade 4 neutropenia lasting more than 3 days, any febrile grade 3 or 4 (severe) haematological toxicity, or grade 3 nonhaematological toxicity (except nausea and vomiting). It was also categorised as DLT when the second course treatment was not resumed within 18 days after the first course. The MTD was defined as the dose at which 33% or more patients experienced DLTs during the first course.

Tumour responses were evaluated according to the classification of the Japanese Research Society for Gastric Cancer based on its volume, which was estimated by X-ray imaging or CT scan (Nishi *et al*, 1995). A complete response (CR) was defined as the disappearance of all evidence of cancer for at least 4 weeks, and a partial response (PR) was defined as less than complete, but more than 50% reduction of tumour volume for at least 4 weeks without any evidence of new lesions or progression, respectively. No change was defined as less than a 50% reduction or less than a 25% increase without any new lesions. Progressive disease (PD) was defined as a more than 25% increase in a solitary lesion or the appearance of new lesions. Tumour responses of the primary site were evaluated by the roentgenographic and endoscopic evaluation criteria proposed by the Japanese Research Society for Gastric Cancer. The survival period was calculated from the start of treatment to death or the latest followed-up day. The time to remission was defined as the period from the start of treatment to the onset of PR. The duration of PR was defined as the period from the onset of PR to the first day when progression was noted. The eligibility and suitability for assessment and the objective response to the treatment were reviewed extramurally.

Pharmacokinetics

In the phase I portion, a pharmacokinetic (PK) study was conducted for FT, 5-FU, CDHP, and Oxo on days 6 and 8 during the first course to evaluate if any metabolic interactions between the component of S-1 and CDDP were seen in this study. Whole blood samples were taken before and 1, 2, 4, and 8 h after S-1 administration on days 6 and 8 during the first course.

Statistics

The PK parameters were compared between patients treated with S-1 alone on day 6, and combined with CDDP on day 8 by paired *t*-test.

RESULTS

Between April 1999 and July 2000, 25 patients were entered from three participating centres. The first 12 patients were entered into the phase I portion and the next 13 patients were entered into the phase II portion to confirm the toxicities and efficacy at the RD. All patients were eligible for toxicity evaluation in any course and objective response evaluations (Table 1). Six patients had undergone gastrectomy and one had also received adjuvant chemotherapy after gastrectomy. Although all patients had metastatic lesion, one patient whose lymph node metastasis lesion was too small to evaluate was evaluated only for primary gastric lesion. Histological evaluation revealed eight patients to be intestinal type and 17 patients to be diffuse type. A total of 109 courses were given: 14 patients (74%) received four or more courses, and seven patients (37%) received six to eight courses at level 1 (CDDP: $60\,mg\,m^{-2}$), three patients (50%) received four or more courses, and one patient (17%) received six courses at level 2 (CDDP: $70\,mg\,m^{-2}$). The median number of courses and duration of therapy per patient was four (range: 1–8). The median number of course per patient was four (range: 1–8) at level 1, and four (range: 1–6) at level 2, respectively. The median duration of therapy per patient was 140 days (range: 21–280) at level 1, and 100 days (range: 18–187) at

Table 1 Patient characteristics

	Phase I portion		Phase II portion
CDDP (mg m^{-2})	**60**	**70**	**60**
No. of patients	**6**	**6**	**19**
Age (years)			
Median	60.5	63.5	60
Range	39–71	31–72	39–72
<65	4	5	13
≥65	2	1	6
Sex			
Female	1	2	2
Male	5	4	17
Performance status			
0	4	5	14
1	2	1	4
2	0	0	1
Pathology			
Intestinal	2	2	6
Diffuse	4	4	13
Gastrectomy	0	1	5
Adjuvant chemotherapy	0	0	1

CDDP = cisplatin.

Table 2 Toxicity incidence

(a)	Phase I portion				(b) Phase II portion	
Course	**First course**				**All courses**	
CDDP (mg m^{-2})	**60**		**70**		**60**	
No. of patients	**6**		**6**		**19**	
Toxicity/grade	**All events**	**Grade 3/4**	**All events**	**Grade 3/4**	**All events**	**Grade 3/4**
Haematological						
Leucopenia	2	0	6	2	15	1
Neutropenia	4	1	6	3	13	3
Anaemia	2	0	4	2	10	3
Thrombocytopenia	1	0	3	1	10	0
Nonhaematological						
Anorexia	5	0	6	1	18	5
Nausea	2	0	3	0	13	3
Vomiting	0	0	2	0	7	2
Diarrhoea	0	0	1	0	6	1

Grade is based on the National Cancer Institute common toxicity criteria, version 2.0.
CDDP = cisplatin.

level 2, respectively. Seven patients were treated with S-1 alone after this combination therapy whose number of course by S-1 alone was four (range: 1–5). Two patients received reduced CDDP during the second course at each level. One patient received both reduced S-1 and CDDP doses at level 2.

The median number of days until the start of the second course after completion of scheduled S-1 in the first course was 14 (range: 7–21 days) among 18 patients who were treated with two courses or more. Seven out of the 18 patients required more than 14 days interval to start the second course.

Determination of MTD

In the phase I portion at level 1, one patient developed grade 3 neutropenia during the first course and required 20 days to start the second course, but the other two patients in the same cohort showed no DLT. An additional three patients were enrolled for safety evaluation, but overall only one of the total of six patients developed a DLT at 60 mg m^{-2} of CDDP. As dose level 2, two of six patients exhibited DLTs in the first course, one of whom had grade 4 neutropenia, and the other had grade 4 anorexia concomitant with grade 3 leucopenia, colitis, and febrile neutropenia. The frequency of severe haematological toxicities increased according to the increment of the CDDP dose (Table 2a). Based on these results, dose level 2 was declared as the MTD, and level 1 was declared as the RD in the following phase II portion. Thus no case was treated with the originally scheduled 80 mg m^{-2} CDDP. The phase II portion was continued with treatment of 60 mg m^{-2} CDDP on day 8, and 40 mg m^{-2} S-1 from days 1 to 21 every 5 weeks, followed by a 2-week rest.

Safety

In the phase II portion, the most frequently observed severe (grades 3 and 4) haematological toxicity was neutropenia (three cases, 16%). Frequently observed nonhaematological toxicities (all events) included anorexia (18 cases, 95%), nausea (13 cases, 68%), and vomiting (seven cases, 37%) even though prophylactic antiemetic medications were given after CDDP infusions. In addition, the overall incidence of diarrhoea was 32% (six out of 19); however, grade 3 diarrhoea was observed only in one out of 19 (5.3%), and recovered within 2 days (Table 2b).

The median number of days at which grade 3 neutropenia occurred was 29 days (range: 26–69 days) at level 1, whereas the median neutrophil nadir was on day 25 (range: 21–28 days) at level 2, with no differences between dose levels.

During this study, two patients received granulocyte colony-stimulating factor because of neutropenia. Incidences of the worst grade toxicities in patients treated with the RD were grade 1 (one case, 5.3%), grade 2 (nine cases, 47.4%), grade 3 (six cases, 31.6%), and grade 4 (two cases, 10.5%), respectively. Neither treatment-related death nor delayed severe toxicity was observed.

Efficacy

A total of 19 patients were evaluated to determine the RR at the RD. Of these, six patients were treated with the RD of 60 mg m^{-2} CDDP in the phase I portion and 13 patients were treated with the same CDDP dose in the phase II portion. The RR at the RD in the phase II portion was 73.7% (14/19, 95% confidence interval (CI): 48.8–90.9%). The RR of all 25 eligible patients was 76% (19/25, 95% CI: 54.9–90.6%); four patients showed stable disease as their best response, two patients had PD. The median time to progression was 179 days (range: 24–384) in the phase II portion (Table 3). The median time to PR and the median overall durations of response in 19 responders were 29 (range: 24–64) and 162 days (range: 63–244), respectively. Two responders treated at level 1 were able to adapt gastrectomy after four courses of this combination therapy.

Subgroup analysis by tumour lesion and pathological type for the 25 patients showed that the RR was 67% (4/6) for liver metastasis, 76% (16/21) for lymph node metastasis, and 74% (14/19) for primary lesions, and the RR according to pathological type was 75% (6/8) for the intestinal type, and 76% (13/17) for the diffuse type.

The MST of all eligible patients was 383 days (95% CI: 256–569) and 1- and 2-year survival rates were 52 and 20%, respectively. The median follow-up time for survival analysis was 789 days.