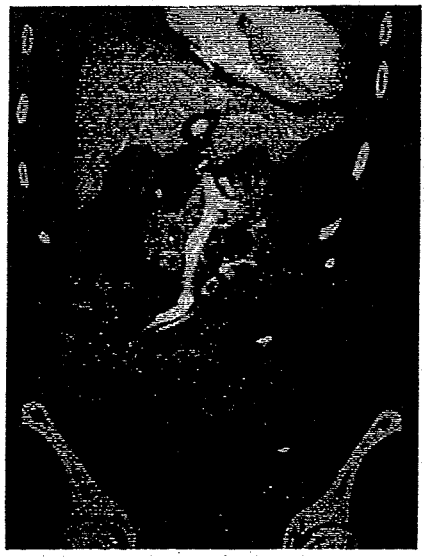

図2　腹腔内投与直後の腹部造影CT
腸間膜間を含めた腹腔内全体にわたる薬液の分布を認める．

科では1,000ccの生理食塩水を用いて溶解している．症例により腹腔内投与後にCTを撮影し腹腔内の薬液分布を観察しているが，必要十分な量だと考えている(図2)．薬液の排出は行わない．

Huber針を用いて自然滴下による投与を行うが，一般に使用するHuber針が21Gと細く，また腹圧が加わるため，クレンメを全開にしても1,000ccの腹腔内投与に2時間程度かかることが多い．19GのHuber針も発売されているが，患者の疼痛ならびに表皮へのダメージが強く推奨されない．しかし注入にあまりにも長時間(3時間以上)必用な場合は，なんらかの異常が生じている可能性があり原因の究明が必要である．

抗癌剤の投与が終了すれば100cc生理食塩水でポートならびにカテーテル内を洗浄し治療を終了する．また，投与中ならびに投与後は，薬液を腹腔内全体に行き渡らすため，患者に適宜，左右の側臥位をとるように指導する．

ポートならびに カテーテルに関連した合併症

GOG 172ではポートを用いた腹腔内化学療法が119症例に行われた．この119症例中ポートならびにカテーテルに関連した合併症として，カテーテル感染21例(18%)，カテーテルの閉塞10例(8%)，カテーテルのリーク3例(3%)，アクセスに関する問題5例(4%)，膣からの薬液の流出1例(0.8%)が発症したと報告されている．またポートならびにカテーテルに関連する可能性がある合併症として，カテーテルに関連しない感染7例(6%)，腹痛4例(3%)，患者の拒否19例(16%)，腸関連の合併症4例(3%)の発症を報告している[3]．

このようにポート特有の合併症が存在することを念頭に置いた治療方針の選択が必要である．

当科での後治療として用いた 腹腔内化学療法の使用経験

当科ではS-1の経口投与とリザーバーシステム

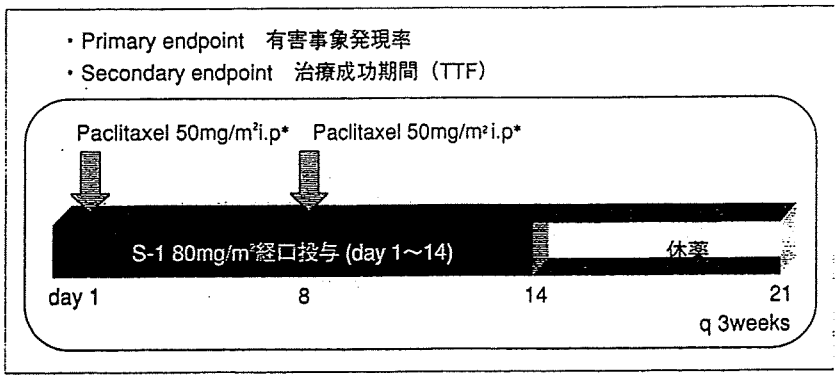

図3　腹膜転移(P1)を伴う胃癌症例に対するweekly paclitaxel腹腔内投与とS-1経口投与による安全性の検討
＊生理食塩液1,000mlに溶解．

表1 Feasibility study選択基準

選択基準 ・組織診にて胃癌の確定診断の得られた症例のうち，Staging Laparosopyにて腹膜転移陽性(P1)が認められた症例． ・ECOGのPerformance Status(PS)が0-2の症例． ・年齢20歳以上75歳以下の症例． ・右の主要臓器機能が十分保たれている症例． ・重篤な合併症，活動性の重複癌のない症例． ・文書による同意の得られている症例．	白血球数	3,000/mm³以上
	好中球数	1,500/mm³以上
	血小板数	75,000/mm³以上
	ヘモグロビン	8.0g/dl以上
	GOT, GPT	施設の正常値上限の2倍以下（ただし，肝転移を伴う場合は4倍以下とする）
	総ビリルビン	1.5mg/dl以下
	血清クレアチニン	1.5mg/dl以下
	心電図	正常

表2 Feasibility studyの対象症例

	患者背景		Front-line	2nd-line	3rd-line	4th-line
1	54歳 女性	4型 TG後 T2(ss), N1, ly0, v0	S-1＋ Paclitaxel	Paclitaxel	S-1	*
2	68歳 男性	4型 TG後 T3, N1, ly3v1CY1	S-1＋ Paclitaxel	*		
3	61歳 女性	4型, P1	S-1＋ Paclitaxel	*		
4	66歳 男性	4型, P1	S-1＋ Paclitaxel	*		
5	58歳 男性	4型 DG後 T2(ss), N1, ly2,v2	S-1	Paclitaxel	*	

* Feasibility study

表3 血液毒性

有害事象	Grade 1	Grade 2	Grade 3	Grade 4	≧Grade3 (%)
白血球減少	1	1	1	0	20%
好中球減少	1	2	1	0	20%
Hb減少	1	3	1	0	20%
血小板減少	1	0	0	0	0%

NCI-CTC Version 3.0)

を用いたpaclitaxelの同時併用化学療法のdose finding studyを行っている．化学療法施行中に癌性腹水が出現した再発・切除不能進行胃癌患者に対する本studyの安全性と有用性，ならびに手技を報告する．

レジメンは図3に示す．S-1は通常量である80mg/m²を14日間連続で経口投与し7日間休薬する．Paclitaxelの腹腔内投与はday 1とday 8に上記方法にて投与する．Primary endpointを有害事象発現率としSecondary endpointを治療成功期間(TTF)とした．症例選択基準の抜粋は表1に示す．現在までに5例が登録された．登録された5症例の患者背景は表2に示す．2nd lineとして3例，3rd line, 4th lineとしてそれぞれ1例である．また全例にpaclitaxelを用いた全身化学療法が行われていた．当科ではポートの留置に前もって，全身麻酔下に診断的腹腔鏡を行っている．診断的腹腔鏡の結果P1, CY1が確認できた症例を本studyに登録している．有害事象として血液毒性はGrade 3以上の白血球減少，好中球減少，Hb減少がそれぞれ1例ずつ認められた(表3)．またその他の臨床検査値異常はGrade

表4　その他の臨床検査値異常

有害事象	Grade 1	Grade 2	Grade 3	Grade 4	≧Grade3 (%)
GOT	2	0	0	0	0 %
GPT	2	0	0	0	0 %
T.Bil	2	0	0	0	0 %
クレアチニン	2	1	0	0	0 %

(NCI-CTC Version 3.0)

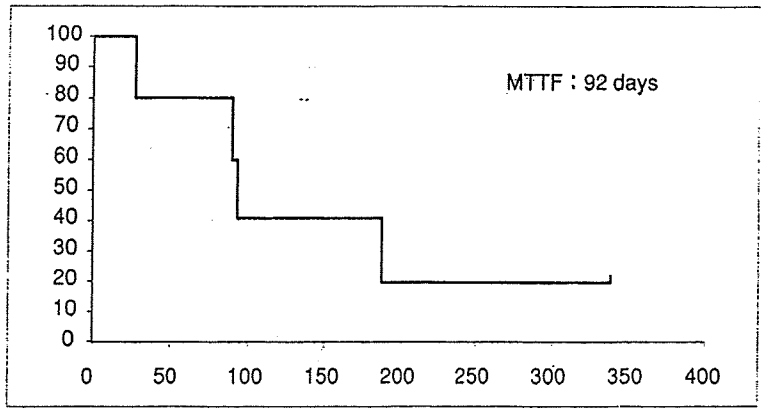

図4　治療成功期間

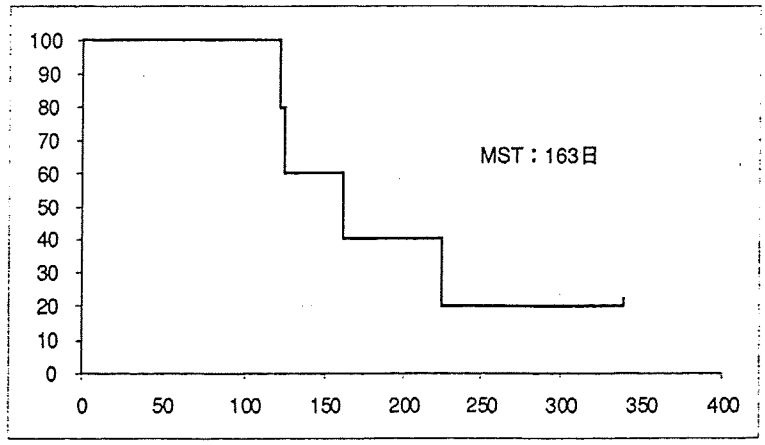

図5　ポート挿入後の生存曲線

3以上のものは認められていない(表4)．腹腔内投与特有の有害事象とされる腹痛も現在のところ経験していない．しかし感染が生じたためポートの抜去が必要であった症例，ならびにポートが反転した症例を経験した．ポートが反転した症例は用手的に整復した後に腹腔内投与を行っている．現在のところsecondary endpointである治療成功期間は中央値で92日(図4)，ポートを挿入した後の生存期間は中央値で163日である(図5)．今のところ全例に腹水の減少を診ることができ(図6)，在宅での治療が可能となった．

おわりに

がん治療の目的は，人の体に巣くった癌細胞に介入して，その人の死期を再び未確定の彼方へ追いやり，死を忘却させる方法を成就させることであるとされている．高度進行胃癌患者に対してこの目的を達成するためには，確実な手

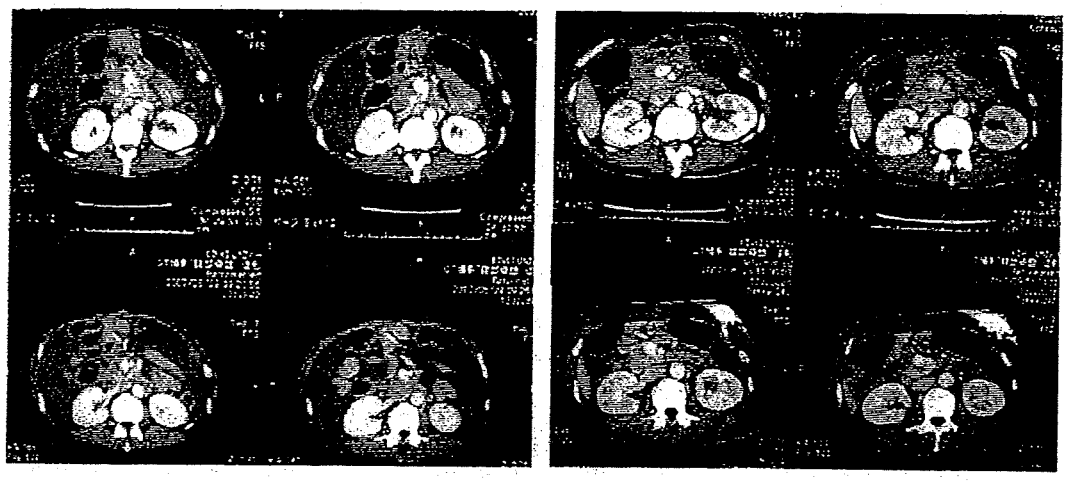

図6 治療効果
2クール施行．著明な腹水の減少を認める．

術手技に加え，分子標的薬剤に代表されるようなより強力な薬剤の出現が望まれる．しかし今目の前にいる患者には，確実な手術手技に加えて，使用可能な薬剤を，腹腔内投与などその特性を生かした投与法を利用して治療する集学的治療が必要ではないかと考えている．もちろん質の高いエビデンスを作る努力も忘れてはならない．

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*　　*　　*

Ⅲ．複合領域

ワークショップ1　進行消化器癌の集学的治療（肝癌を除く）

腹膜播種陽性胃癌症例に対する集学的治療

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「消化器病学の進歩―原点から未来への情報発信」
第94回日本消化器病学会総会記念誌
別冊
2009年7月1日　発行

日本消化器病学会

腹膜播種陽性胃癌症例に対する集学的治療

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Ⅰ．はじめに

腹膜播種を伴う胃癌は急速に進展することが知られており，そのため腹水の貯留，腸管閉塞，尿管閉塞による水腎症などを引き起こすことが多い．いったんこのような病状が生じると全身状態は急速に悪化し，患者の予後は極めて不良となる．現行の「胃癌取扱い規約」（第13版）では，腹膜播種陽性症例（P1）はもちろんのこと，腹膜播種が成立する前段階と考えられている腹腔内に癌細胞が散布された状態（腹腔細胞診陽性：CY1）ですらStage Ⅳと位置づけている[1]．

しかし腹腔を，諸臓器を包み込む1枚の膜として捉えた場合，局所療法が有用である可能性が示唆される．また，近年上市された新規抗癌剤を有効に使用することにより，これらの病態に対して積極的な集学的治療が行える可能性が表れてきた．

本稿ではわれわれの施行している腹膜播種陽性症例に対する簡便かつ外来通院で治療継続が可能なPaclitaxel腹腔内投与と逐次S-1＋Weekly Paclitaxel併用療法（Hybrid Chemotherapy：HC）を中心とした集学的治療法の現在までの治療成績について述べる．

Ⅱ．ターゲットである腹膜播種巣の存在部位

癌細胞が胃の漿膜を超えて浸潤，もしくは脈管系を介して腹腔内に遊離すると，この癌細胞は中皮細胞が表面を覆う腹膜上層だけではなく，癌細胞の腹腔内遊離により肥厚したsub-methotelial layerに浸潤し病巣を形成する[2]．つまり腹膜播種を制御するためには，①腹腔内に遊離する癌細胞，②中皮細胞近傍に存在する癌細胞，③sub-methotelial layer深層に存在する癌細胞，これら三つの領域を標的としなければならない．

この3領域に存在する癌細胞を効率的に制御するために，われわれは抗癌剤の腹腔内投与に逐次全身化学療法を組み合わせた治療方法：HCを臨床研究として施行している．HCの理論的根拠などは他稿を参照いただきたい[3, 4]．

Ⅲ．実際の治療方法

近畿大学医学部外科では，大型3型胃癌や4型胃癌など臨床的に腹膜播種を高率に疑う胃癌症例に対しては，基本的に開腹手術に先立ち，まず診断的腹腔鏡を施行している．その結果，肉眼的に腹膜播種を確認した症例を本治療法の対象としている．

レジメンは，まず，腹腔内化学療法として診断的腹腔鏡施行時にpaclitaxel 80mg/m^{2}を生理食塩水1,000mlに溶解し腹腔内に投与する．腹腔内に投与した薬液のドレナージは行わない．引き続き施行する全身化学療法は，腹腔内投与後14日以内に施行することを原則とした．レジメンは大阪消化管がん化学療法研究会（OGSG）0105のレジメンに則り[5]，paclitaxel 50mg/m^{2}をDay 1と8に投与，S-1は通常量（800mg/m^2）をDay 1から14まで経口投与，これを3週ごとに繰り返し，少なくとも3サイクル施行することとした（図1）．

Ⅳ．治療成績

現在まで46例に本治療法を施行した．その内訳は男性：29例，女性：17例で年齢の中央値は

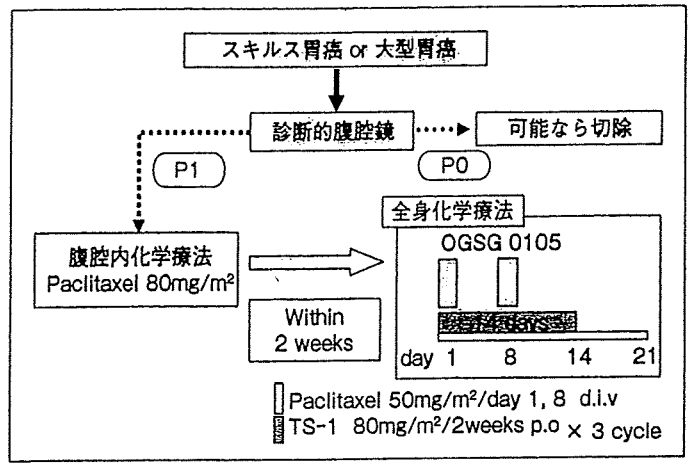

図1 近畿大学外科における腹膜播種を疑う症例に対する臨床試験

表1 患者背景

症例数	46例
年齢中央値(range)	63.5(22～75)
年齢平均	60.3
性別 男/女	29/17
PS 0/1	43/3
肉眼型 スキルス/その他	30/16

表2 Hematologic toxicity(n=46)

有害事象	Grade				
	1	2	3	4	≧3(%)
白血球減少	9	8	7	0	15
好中球減少	7	12	7	5	26
Hb 減少	16	18	6	1	15
血小板減少	15	0	1	0	2

(NCI-CTC 2.0 Grade)

表3 臨床検査値異常(n=46)

有害事象	Grade				
	1	2	3	4	≧3(%)
GOT	15	3	0	1	2
GPT	18	2	2	1	6
T.Bil	10	7	0	1	2
クレアチニン	4	1	1	0	2

(NCI-CTC 2.0 Grade)

63.5歳(22～75)，PSは0：43例，1：3例であった(表1)．本治療法による有害事象は，血液毒性としてGrade 3以上の好中球減少が12例(26%)(表2)，また，Grade 4の臨床検査値異常が1例(2%)発現したが，重篤な症状発現には至らなかった(表3)．全症例の現在までの生存期間中央値は475日で，1年生存率は65.2%，2年生存率は34.5%，3年生存率は17.8%であった．

また，2005年から，腹膜播種巣へのHCの抗腫瘍効果が望まれる症例には，更なる治療成績の向上を目指し，胃切除の付加を前提とした2nd look laparoscopyを行った．

V．腹膜播種巣に対する治療効果の判定

これらの症例に胃切除を付加するにはCY0，P0が必要不可欠な条件と考える．しかし術前のCTなどの臨床所見では腹膜播種の確診を得ることができない症例が本臨床試験の対象症例である．当然，HC後の腹膜播種巣に対する治療効果も臨床所見では判断しがたい．

したがって現在われわれは原発巣もしくは転移リンパ節に対する抗腫瘍効果をもって，腹膜播種巣に対する治療効果を推測している．つまり原発巣や転移リンパ節への抗腫瘍効果が得られた症例は，腹膜播種巣に対する抗腫瘍効果も得られているであろうと考え，2nd look laparoscopyを施行している．

しかし，腹膜播種巣は，腹腔内化学療法により経静脈的投与された抗癌剤よりAUCで比較すると約1,000倍の薬剤と直接接触する[4]．つまり原発巣とは全く異なった薬物動態下に存在する．

この状況下におかれる腹膜播種巣に対する治療効果と，経静脈的経路で薬剤がdeliveryされる原発巣や転移リンパ節巣に対する治療効果の違いは

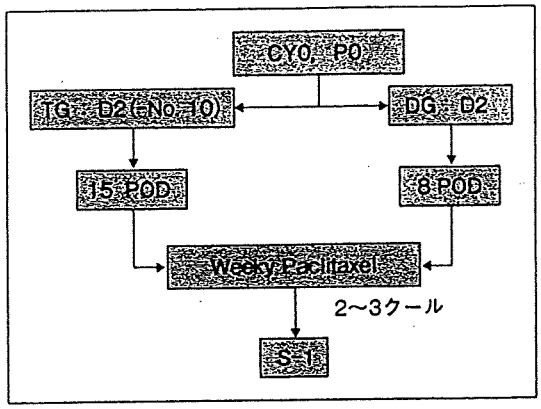

図2 2nd look laparoscopy 以降の治療方針

今後の検討課題である．

Ⅵ. 2nd look laparoscopy による評価と胃切除術

再度行う腹腔鏡検査では腹腔洗浄細胞診ならびに腹膜生検を行う．特に肉眼的には播種を疑う病巣でも病理組織学的には瘢痕組織であることが多く，播種を疑う病巣の生検は必須と考えている．両者とも陰性の場合にのみ D2 郭清を伴う胃切除術を施行する．

この化学療法は National Cancer Institut（NCI）の Dictionary Cancer Terms で言う Induction therapy であり，胃切除は additional therapy としての役割を持つ．当然術後早期の化学療法追加が必要であるため，胃全摘の場合，当科では基本的に脾臓を温存している．この場合，リンパ節郭清の範囲は正確には D2－No.10 となる．

Ⅶ. 術後化学療法

化学療法後に徹底したリンパ節郭清を伴った胃切除を施行した患者の術後化学療法に対する compliance 不良であると予想される．その欠点を克服するために，比較的 compliance が良好である paclitaxel の分割投与（weekly paclitaxel）から化学療法を開始している（図2）．また，これらの症例は PTX の responder であり，理にかなった術後化学療法と考えている．

また，体内の癌細胞が最も少ない状況で化学療法を施行することが最善と考えられるため，幽門側胃切除では 7POD を，胃全摘術では 14POD を目処に術後化学療法を開始している．

Ⅷ. 化学療法による down staging

この治療方法を用いて現在までに 13 症例に胃切除を行った．これらの症例では腹膜浅層はもちろん，深層に存在する播種にも瘢痕化が認められた（図3）．化学療法の効果を，HC 前の臨床もしくは手術所見（HC 前）と，手術後の病理所見（HC 後）で比較検討した．胃壁深達度では HC 前には全例が T3 であったのが，HC 後には 12 例が T2 以下であった．しかしリンパ節転移について検討してみると，HC 前には N2 と判断した症例が 4 例であったが，HC 後 down staging が認められた症例は 1 例のみであった．また，HC 前に 9 例を N1 と判断したが，HC 後の判定では N2：2 例，N1：1 例，N0：6 症例であった．加えてリンパ節周囲の脂肪織に癌細胞の遺残が認められる症例も 3 例経験した．これら 3 症例のうち 2 例は HC 前に N2 と判断した症例であった．

これは二つの問題点を提示していると考えられる．一つは CT などによる術前のリンパ節転移の診断精度にかかわる問題点，他は N2 症例に対する HC の治療効果に関する問題である．播種性転移とリンパ行性転移，この両者とも高度な進展様式を示す胃癌患者に対しては，他の治療 modality が必要なのかもしれない．

Ⅸ. おわりに

腹膜播種の予後は数か月と不良で，その残された数か月さえ，ほとんどの症例が腹水貯留などのために quality of life は著しく損なわれる．また，腹膜播種を伴う胃癌の組織型は分化が不良な場合がほとんどで，多くの抗癌剤に対して抵抗を示す．

本稿ではこのような理由で従来対象となり難かった腹膜播種に対する治療の可能性を示唆した．今後，分子標的治療薬を含めた腹膜播種に対し更に有効な抗癌剤の開発が望まれる．

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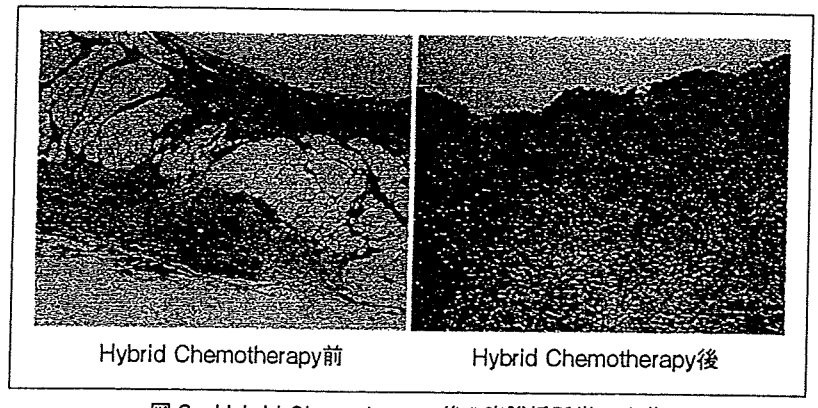

図3　Hybrid Chemotherapy後の腹膜播種巣の変化

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J Gastrointest Surg (2009) 13:1577–1582
DOI 10.1007/s11605-009-0955-y

ORIGINAL ARTICLE

Immunohistochemical Expression of Osteopontin in Gastric Cancer

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Received: 14 April 2009 / Accepted: 12 June 2009 / Published online: 7 July 2009

Abstract
Background/Aims Osteopontin (OPN) is significantly overexpressed in a variety of malignancies. However, little is known concerning the significance of OPN expression in human cancers. Thus, the aim of this study was to determine the relationship between the degree of OPN expression, the proliferative activity of cancer cells, and the clinicopathological findings for surgically resected gastric cancer.
Methodology We evaluated the immunohistochemical expression of OPN in 85 specimens of cancer. Additionally, we investigated a cancer cell proliferative index using an anti-MIB-1 antibody and terminal deoxynucleotidyl transferase-mediated dUTP biotin nick end labeling staining. Levels of OPN expression in gastric cancers were classified into three groups. To compare the relationship between OPN expression and clinicopathological findings, the features of cancer lesions were classified using the TNM Classification of Malignant Tumors, 6th Edition.
Results Immunohistochemical examination of OPN expression in gastric cancer revealed diffuse granular staining in the cytoplasm. High OPN expression was observed in 37 of 85 carcinomas. Strong OPN expression was significantly associated with a low apoptotic index, a high proliferative index, depth of invasion, lymphatic invasion, and venous invasion. Pathologically, intestinal type carcinoma showed strong expression of OPN.
Conclusions These data suggested that OPN may play an important role in the invasiveness and the progressive nature of gastric cancer.

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Keywords Osteopontin · Gastric cancer · Apoptotic index · Proliferating index

Introduction

Advanced gastric cancer remains one of the most common neoplasms in Japan and has a poor prognosis. Even when curative surgery is performed, a considerable number of patients will die from cancer, with recurrence such as distant metastasis, lymph nodes metastasis, and carcinomatous peritonitis. Osteopontin (OPN) is a non-collagenous acidic bone matrix glycoprotein, which is sialated and phosphorylated, and has a cell-binding peptide sequence of glycine-arginine-glycine-aspartate-serine. OPN has been demonstrated in a limited number of organs such as bone, kidney, lung, breast, smooth muscle, and stomach.[1,2] OPN is also a cytokine that is

associated with a rapid T-cell-dependent response to bacterial infection.[3]

With respect to cancer metastasis, it has been suggested that OPN exhibits both cell attachment and cell signaling functions through integrin-mediated signal transduction,[4] although the function of OPN in tumor cells remains poorly understood. A correlation between OPN expression and clinicopathological findings has been previously shown in gastric cancer.[5,6] In vitro, Song et al. showed that the anti-apoptotic activity of OPN in gastric cancer cells was mediated, in part, through the PI3-K/Akt pathway via alpha v beta 3 integrins,[7] while Zhao et al. reported that OPN may facilitate tumorigenesis and metastasis through prevention of tumor cell apoptosis.[8] However, the exact role of OPN in regulating proliferative activity in gastric cancer is not fully understood.

Thus, the aim of the present study was to determine whether the expression of OPN in gastric cancer prevents apoptosis and correlates with clinicopathological characteristics.

Materials and Methods

Specimens were obtained from 85 patients (59 males and 26 females, mean age 60.9 years, 29 to 87 years old) with gastric cancer resected between 1998 and 2005 in our department at the time of operation. Freshly obtained cancerous and non-cancerous tissues were fixed with 4% paraformaldehyde in 0.1 M PBS at 4°C overnight, dehydrated in graded alcohols, and then embedded in paraffin. Next, 4-μm thick serial sections were processed for immunohistochemistry, in addition to routine hematoxylin and eosin staining. The depth of tumor invasion, lymphatic invasion, venous invasion, lymph node metastasis, and stage were determined according to the TNM Classification of Malignant Tumors, 6th Edition criteria. Gastric cancer was also classified as intestinal or diffuse type using the Laurens system.

Immunohistochemistry

Monoclonal antibodies against human OPN antibody and Ki-67 antigen (MIB-1; DAKO Corporation, Carpinteria, CA, USA) were evaluated. The OPN antibody was developed by M. Solursh and A. Franzen and was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by the University of Iowa (Department of Biological Sciences, Iowa City, IA, USA). Sections were deparaffinized in xylene, dehydrated through graded ethanols, and treated with 3% H_2O_2 in methanol for 30 min at room temperature to eliminate endogenous peroxidase activity. After blocking nonspecific binding with 10% normal goat serum in PBS for 30 min at room temperature, reaction with the primary antibodies (OPN 1:100; MIB-1 1:50) was carried out at 4°C overnight. The sections were then incubated with EnVision™ (DAKO Corporation) for 60 min, in place of biotinylated goat anti-rabbit IgG secondary antibody and the streptavidin-peroxidase conjugate.[9] Color development was performed by incubation with 0.5% 3,3-diaminobenzidine solution containing 0.01% H_2O_2 in 0.05 M Tris-HCl buffer (pH 7.2) for two to 10 min as required for optimal staining. Sections were assessed and photographed under a light microscope. Control staining was performed with normal rabbit serum without the appropriate primary antibody.

Terminal Deoxynucleotidyl Transferase-Mediated dUTP Biotin Nick End Labeling Staining

To evaluate the incidence of apoptotic cells in gastric cancer, we used the terminal deoxynucleotidyl transferase-mediated dUTP biotin nick end labeling technique[10] using the TaKaRa In Situ Apoptosis Detection Kit (TaKaRa, Shiga, Japan). In brief, the deparaffinized and rehydrated 4-μm thick sections were digested with proteinase K (20 μg/ml; Sigma–Aldrich, St. Louis, MO, USA) for 20 min at room temperature. The slides were then washed in distilled water and immersed in 2% H_2O_2 in distilled water for 10 min to block endogenous peroxidase activity. The sections were washed in PBS (pH 7.4) and then incubated in equilibration buffer for 10 min at room temperature. The control sections were prepared in parallel with substitution of distilled water instead of TdT enzyme.

Immunohistochemical Evaluation

OPN immunoreactivity was evaluated in three areas of each slide for correlation and confirmation of the tissue diagnosis. The number of tumor cells with cytoplasmic staining of OPN was counted, and OPN expression was classified as follows: weak or focal expression (±), moderate expression with focal strong expression (1+), and strong expression (2+). OPN expression was evaluated by two of the authors without any prior knowledge of the patient's clinical information. If different grades were assigned, final agreement was obtained after careful review of the images on the same digital monitor screen.

Evaluation of Apoptotic and Proliferating Cells

For quantitation of apoptotic and proliferating cells, more than several hundred cancer cells from all patients were

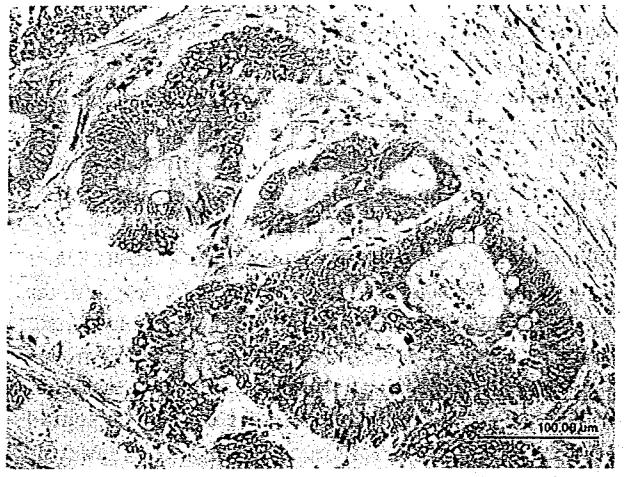


Figure 1 Immunoreactivity of Osteopontin in cancerous tissue. Fine and rough granular immunoreactivity was observed in the cytoplasm of cancer cells. ×40 magnification.

counted under a light microscope (×40 objective) within the arbitrary area. The ratio (%) of apoptotic- or proliferating-positive cells per 1,000 cancer cells were calculated and were termed the apoptotic index (AI) and the MIB-1 index (MI), respectively.[11]

Statistical Analyses

Statistical analyses were performed using Stat View® (SAS Institute Inc., Cary, NC, USA). The χ^2 test was used to analyze the association between OPN expression and clinicopathologic features of gastric cancers. The relationship between OPN expression and the MI or the AI was evaluated by the student t test. A difference of $P<0.05$ was considered significant.

Table 1 Association between Osteopontin Expression and Clinicopathological Characteristics of Gastric Cancer

Variables	OPN ±	OPN 1+	OPN 2+	*P* value
Depth of invasion				
Tis	13 (15)	10 (12)	6 (7)	<0.05
T1	3 (4)	6 (7)	4 (5)	
T2	5 (6)	1 (1)	15 (18)	
T3	2 (2)	6 (7)	7 (8)	
T4	1 (1)	1 (1)	5 (6)	
Lymph node metastasis				
N0	18 (21)	16 (19)	13 (15)	<0.05
N1	3 (4)	3 (4)	13 (15)	
N2	0 (0)	2 (2)	6 (7)	
N3	3 (4)	3 (4)	5 (6)	
Histological type				
Intestinal type	7 (8)	10 (12)	24 (28)	<0.05
Diffuse type	17 (20)	14 (16)	13 (15)	
Lymphatic invasion				
L0	20 (24)	15 (18)	8 (9)	<0.05
L1	4 (5)	9 (11)	29 (34)	
Venous invasion				
V1	21 (25)	23 (27)	23 (27)	<0.05
V2	3 (4)	1 (1)	3 (4)	
TNM stage				
0	13 (15)	10 (12)	6 (7)	<0.05
1A	2 (2)	4 (5)	2 (2)	
1B	3 (4)	3 (4)	6 (7)	
2	1 (1)	1 (1)	5 (6)	
3A	0 (0)	0 (0)	5 (6)	
3B	0 (0)	0 (0)	0 (0)	
4	5 (6)	6 (7)	13 (15)	

Figures in parentheses are percentage

OPN osteopontin

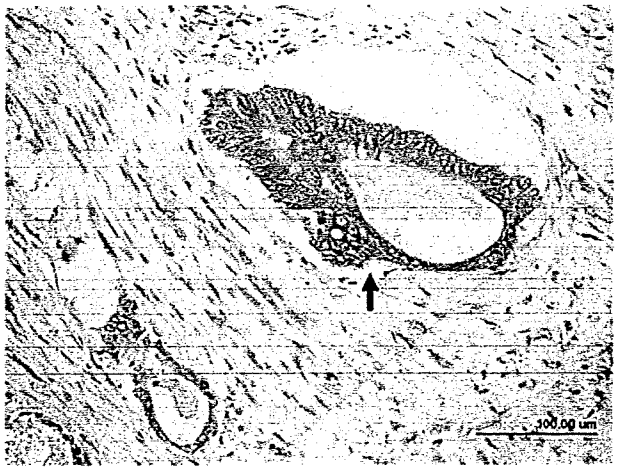


Figure 2 Intense immunoreactivity of OPN was seen in lymphatic invading cancer cells. ×40 magnification.

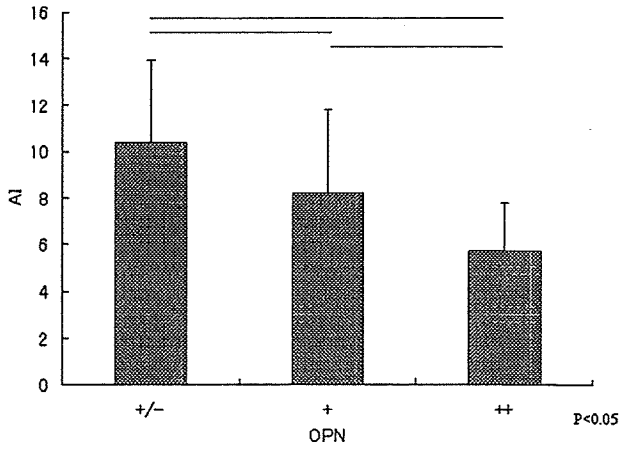


Figure 3 Osteopontin (*OPN*) expression in gastric cancer and apoptotic index (*AI*).

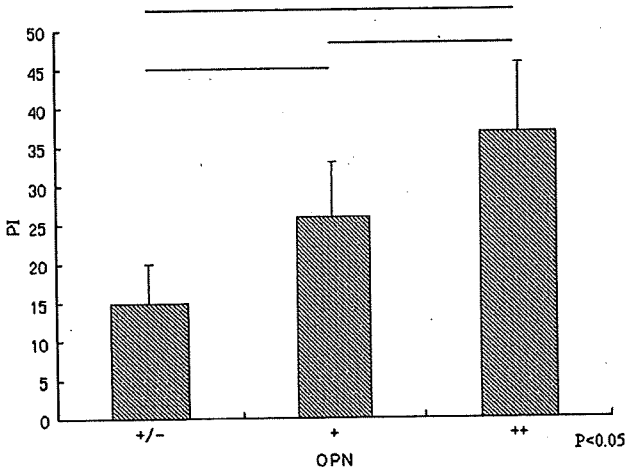


Figure 4 Osteopontin (*OPN*) expression in gastric cancer and proliferating index (*PI*).

Results

Specificity of Immunohistochemical Staining

The negative control sections incubated with the normal mouse immunoglobulin G showed no reaction products. Both smooth muscle cells and macrophages expressed intense OPN immunoreactivity.

Immunoreactivity of OPN in Normal Gastric Mucosa

In the area of the fundic gland, OPN immunoreactivity in normal gastric mucosa was mainly in the chief cells, with expression in some mucous neck cells. In the area of the pyloric gland, OPN immunoreactivity was observed in some mucous neck cells and pyloric gland cells.

Immunoreactivity of OPN in Gastric Cancer Tissue

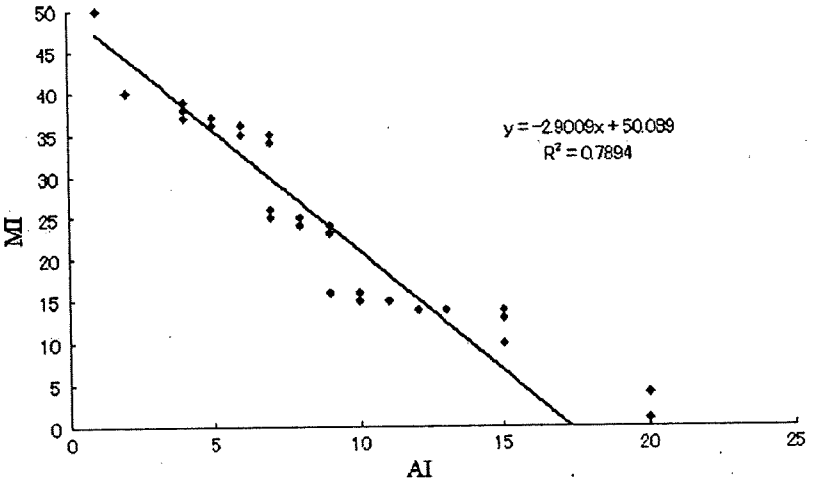
OPN immunoreactivity in gastric cancer showed fine and rough granular immunoprecipitates in the cell cytoplasm (Fig. 1). Cancer cells invading in the lymphatic vessel revealed strong immunoreactivity (Fig. 2). A few inflammatory cells, mainly macrophages, also showed OPN immunoreactivity; in particular, macrophages in the tumor stroma expressed intense OPN immunoreactivity. At the area of the tumor invasion, both cancer cells and macrophages showed strong OPN immunoreactivity. However, the degree of macrophage infiltration in the cancerous tissue exhibited no relationship with the clinicopathological findings of the cancer.

There was an obvious correlation between the degree of OPN expression in cancer cells and the depth of invasion, lymph node metastasis, histological type, lymphatic invasion, venous invasion, and conclusive stage grouping (Table 1).

Apoptotic and MIB-1 Indices

The AI of cancer cells in the ± group, the 1+ group, and the 2+ group were 10.4±3.5, 8.2±3.6, and 5.7±2.1, respectively, with a significant difference between the ± group versus the 1+ group ($P<0.05$) and the 1+ group versus the 2+ group ($P<0.05$; Fig. 3). The MI of cancer cells in the ± group, the 1+ group, and the 2+ group were 14.9±5.1, 25.9±7.1, and 36.8±8.9, respectively, with a significant difference between the ± group versus the 1+ group ($P<0.05$) and the 1+ group versus the 2+ group ($P<0.05$; Fig. 4). Finally, in gastric carcinoma, there was a significant negative correlation of AI with MI (Fig. 5; $y=-2.9009x+50.089$; $R^2=0.7894$; $P<0.05$).

Figure 5 There was a significant negative correlation of *AI* with *MI* in gastric carcinoma ($y=-2.9009x+50.089$; $R^2=0.7894$; $P<0.05$).



Discussion

OPN is a calcium-binding phosphoprotein believed to play an important role in several different and apparently distinct cellular processes. Recently, expression of OPN has been linked to tumorigenesis[12] and metastasis[13] in several experimental animal models and human studies. In a previous study of OPN expression in human cancer tissue, including the colon, stomach, and duodenum, both cancer cells and macrophages were reported to show OPN immunoreactivity, while only macrophages exhibited OPN mRNA signals.[1] These findings suggest that OPN secreted by macrophages might bind cancer cells to each other via the alpha v beta 3 integrin. Moreover, the presence of OPN mRNA in macrophages was only observed at the front of tumor invasion, suggesting that OPN from macrophages affects cell adhesion, tumor cell invasion, and metastasis.[14] However, there are only a few reports describing the exact relationship between OPN expression and the clinicopathological features of gastric cancers. In one study, OPN protein expression was shown to be significantly associated with age, tumor depth, histological grade, and hematogenous metastasis, but there was no correlation with the development of lymph node metastasis.[5]. However, a correlation between OPN expression and depth of invasion, lymph node metastasis, and distant metastasis was reported for gastric cancer.[6]

In the present study, we examined the expression of OPN in 85 resected carcinomas of the stomach using immunohistochemical staining and compared the degree of OPN expression with the pathological features, AI, and MI of gastric cancers. Intense OPN immunoreactivity was detected in 37 of 85 cases (43.5%). The level of OPN immunoreactivity correlated with depth of invasion, lymphatic invasion, venous invasion, lymph node metastasis, and conclusive stage grouping. Gastric cancer cells that invaded lymphatic vessels showed intense OPN immunoreactivity and had a low apoptotic index and high proliferating index. There are contradictory data on the relationship between AI and MI. Kupnicka et al.[15] and Ikeguchi et al.[16] demonstrated a significant correlation between AI and MI in gastric carcinoma, while Lu et al.[17] and Shinohara et al.[18] found no significant correlations.

OPN can bind both extracellular matrix components such as collagen[19] and cell surface receptors. The prominent OPN–cell surface receptor interaction studied is that of arg-gyl-asp, as OPN receptors are alpha v beta 3, alpha v beta 1, and alpha v beta 5 integrins.[20,21] Certain variants of the hyaluronic receptor CD44 have also been shown to be receptors for OPN.[22] The signaling pathway for proliferation and apoptosis involves an early interaction of OPN with specific cell surface receptors.[21,23] Lin et al. demonstrated that in a synergistic reaction with GM-CSF, OPN stimulates growth of both the proB cell line Ba/F3 and IL-3-dependent mouse bone marrow cells via an interaction with CD44.[24] In endothelial cells, the interaction of surface-bound OPN with the alpha v beta 3 integrins has been shown to activate the NF-κB pathway and to inhibit apoptosis in these cells.[25] Potentially, in gastric carcinoma some surface receptors including CD44, alpha v beta 3 integrins, or other receptors are involved in cell proliferation or apoptotic reactions. Thus, these data suggest that OPN secreted from gastric cancer cells may play an important role in metastasis. In support of this, in the present study, expression of OPN was significantly associated with low AI and high MI in gastric carcinoma. Additionally, there was a significance negative correlation between AI and MI.

Recent studies have shown that OPN is a potential target for anticancer therapy.[26] The expression of OPN can be inhibited at both the transcription and the RNA message levels, while OPN protein can be blocked with antibodies or synthetic peptides. Furthermore, OPN receptors can be targeted; CD44 has been widely applied as a cytotoxic and immunological therapeutic target, while integrin alpha v beta 3 is being investigated as a therapeutic target using small molecular inhibitors as drug candidates.[27] The results of the present study provide further support for the targeting of OPN as a potential therapeutic strategy for prevention of cancer through induction of apoptosis and inhibition of cell proliferation. Moreover, it was also concluded that investigating the expression of OPN from preoperative tumor biopsy specimens obtained by endoscopy could lead to a way to tailor therapy.

Conclusion

We investigated OPN immunoreactivity in gastric cancer cells. The expression of OPN was correlated with depth of invasion, lymphatic invasion, lymph node metastasis, and conclusive stage. Because expression of OPN can reduce apoptosis and increase proliferation, OPN inhibitors may be a useful strategy for increasing apoptosis and inhibiting proliferation of gastric cancer cells.

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Langenbecks Arch Surg (2009) 394:647–653
DOI 10.1007/s00423-008-0311-9

ORIGINAL ARTICLE

Analysis of the prognostic factors and evaluation of surgical treatment for synchronous liver metastases from gastric cancer

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Received: 28 September 2007 / Accepted: 31 January 2008 / Published online: 15 March 2008

Abstract
Background and aims Whether or not a synchronous resection of liver metastases from gastric cancer provides a survival benefit has been a key issue. We identify the significant prognostic factors and clarify the beneficial effect on the survival of liver surgical treatment.
Materials and methods We reviewed 72 patients who underwent a gastrectomy for gastric cancer with synchronous liver metastases and classified the liver metastases into three grades, such as H1: metastases were limited to one of the lobes, H2: there were a few scattered metastases in both lobes, and H3: there were numerous scattered metastases.
Results H1, 2 metastases, and an absence of peritoneal dissemination (P0) were significantly independent prognostic factors for liver metastases of gastric cancer. In addition, the cumulative 1 and 5-year survival rates of liver surgical treatment (hepatic resection and/or microwave coagulation therapy) were 80.0% and 60.0%, whereas the survival rates for non-hepatic surgical treatment were 36.4% and 0% in 26 patients with H1, 2, and P0. In those patients, the radical operation, the solitary metastatic liver tumor, and no-distant lymph node metastases were independent prognostic determinants of survival.
Conclusion The radical operation including the surgical treatment for metastatic liver tumors should be performed to improve the prognosis in gastric cancer patients with synchronous H1, 2, and P0.

Keywords Gastric cancer · Synchronous liver metastases · Prognostic factors for survival · Hepatic surgical treatment

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Introduction

Gastric cancer is the second most common cancer worldwide and has a substantial mortality for distant metastases in the liver, peritoneum, or extensive lymph nodes despite technical advances in surgery and the use of adjuvant therapy [1]. Of all patients with gastric cancer, 2–9% have synchronous liver metastases that are a frequent and crucial problem [2–5] because patients with metachronous metastases have a longer survival (5-year survival, 29%) than those with a synchronous disease (5-year survival, 6%) [6], and a synchronous resection of metastatic liver tumors does not contribute to a survival benefit [7]. In fact, a lot of studies have reported that the effect of hepatic resection for gastric liver metastases on survival was dubious [8–11], whereas some reports have demonstrated that only a hepatic resection for liver metastases with gastrectomy was able to obtain a long-term survival when both the primary tumor and metastatic lesions were potentially respectable [7, 12–14]. It is, thus, a key question whether or not a synchronous resection of liver metastases provides a survival benefit. The reason for this is that patients with liver metastases from gastric cancer often have other simultaneous or future

incurable factors, such as peritoneal dissemination, widespread lymph node metastases, and direct invasion to adjacent organs. [7, 13, 14]. In addition, the clinicopathological characteristics related to the prognosis of gastric cancer with synchronous liver metastases have not been comprehensively identified. Therefore, the surgical indications for synchronous liver metastases from gastric cancer are very important and must be carefully determined.

In this study, we retrospectively reviewed 72 patients who underwent gastrectomy for gastric cancer with synchronous liver metastases during the last 15 years and identified which population of the patients obtained a clinical benefit from multimodality treatment for synchronous metastases.

Materials and methods

At the Second Department of Surgery of Wakayama Medical University Hospital, 1,602 gastric cancer patients were surgically treated between January 1991 and December 2005. Of these patients, 81 patients (5.1%) had synchronous liver metastases, which were found with routine abdominal computed tomography before gastrectomy. Among these 81 patients, we retrospectively reviewed the records of 72 patients (88.9%) who underwent a gastrectomy for primary gastric carcinoma. The group consisted of 58 men and 14 women ranging from 25 to 85 years of age (median 67.0 years). None of the patients died of postoperative complications, and the follow-up and outcome of all of the patients were completed by clinical visits, telephone interviews, or correspondence until December 2006.

The classifications of the degree of liver metastases (H1: metastases were limited to one of the lobes, H2: there were a few scattered metastases in both lobes, and H3: there were numerous scattered metastases in both lobes), which were determined from the first English edition of the Japanese Classification of Gastric Carcinoma [15], were used for a prognostic estimation in the gastric cancer patients with synchronous liver metastases. It is thought that the radical operation would be possible against H1 and H2 metastases, and we defined H2 metastases as the number of metastases which was less than five in this study. The following clinicopathological risk factors were also examined for prognostic influence: age, gender, histological differentiation, tumor size, tumor depth of invasion, lymphatic invasion, venous invasion, lymph node metastases, the absence (P0) or presence (P1) of peritoneal dissemination based on gross intraoperative finding and peritoneal cytology, serum carcinoembryonic antigen (CEA), and serum carbohydrate antigen 19-9 (CA-19-9) level before operation. The pathological diagnosis and classification of the resected specimens were performed according to the General Rules for Gastric Cancer Study and Pathology in Japan [16].

Overall survival was analyzed from the date of surgical treatment to the date of death or the last follow-up and was estimated according to the Kaplan–Meier method and compared using the log-rank test. A multivariate analysis was performed to identify the significant contributors that were independently associated with the prognosis among the factors that were found to be significant in the univariate analysis using the Cox proportional hazards model. Statistical significance was defined as a p value of less than 0.05. All statistical analyses were performed with the Statview software program (Version 5.0; Abacus Concepts Inc., Berkeley, CA, USA).

Table 1 Clinicopathological characteristics of gastric cancer patients with synchronous liver metastases (n=72)

Characteristics		Total (n=72) (%)	H1, 2 (n=34)	H3 (n=38)
Age (years)	<65	29 (40.3)	15	14
	≥65	43 (59.7)	19	24
Gender	Male	58 (80.6)	27	31
	Female	14 (19.4)	7	7
Histologic differentiation	Diffe.	46 (63.9)	34	23
	Undiffe.	26 (36.1)	11	15
Tumor size (cm)	<5	15 (20.8)	7	8
	≥5	52 (72.2)	24	28
	Unknown	5 (7.0)	3	2
Tumor depth of inversion	T1,2	27 (37.5)	14	13
	T3,4	45 (62.5)	20	25
Lymphatic invasion	ly0,1	13 (18.1)	10	3
	ly2,3	56 (77.8)	21	35
	Unknown	3 (4.1)	3	0
Venous invasion	V0,1	22 (30.6)	15	7
	V2,3	46 (63.9)	16	30
	Unknown	4 (5.5)	3	0
Lymph node metastases	N0,1	22 (30.6)	14	8
	N2,3	46 (63.9)	19	27
	Unknown	4 (5.5)	1	3
Peritoneal dissemination	P0	50 (69.4)	26	24
	P1	22 (30.6)	8	14
CEA level (ng/ml)	<5	23 (33.0)	12	11
	≥5	46 (63.9)	21	25
	Unknown	3 (4.1)	1	2
CA19-9 level (ng/ml)	<37	34 (47.2)	19	15
	≥37	32 (44.4)	11	21
	Unknown	6 (8.4)	4	2

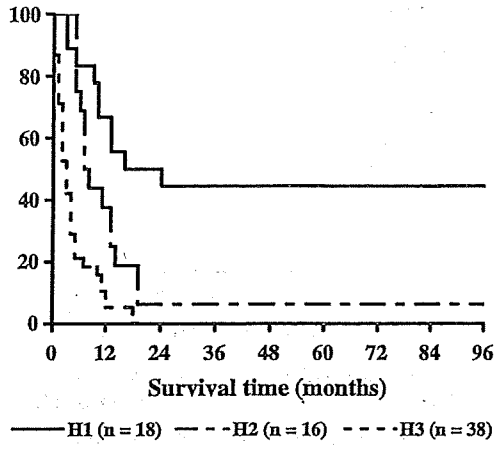

Fig. 1 The overall survival curve for 72 gastric cancer patients with H1, H2, and H3 metastases. Comparison of actuarial survival rates (Kaplan–Meier) for H1 group (n=18) vs. H2 (n=16): P=0.0120 (log-rank test); H1 group vs. H3 group (n=38): p<0.0001; H2 group vs. H3 group: p=0.0005

Results

Clinicopathological data in 72 gastric cancer patients with synchronous liver metastases

The clinicopathological characteristics of 72 gastric cancer patients with synchronous liver metastases are summarized in Table 1. Of the patients, 34 (47.2%) had H1 or H2 metastases, whereas 38 (52.8%) patients had H3 metastases. Tumor size, tumor depth of invasion, extent of lymph node metastases, and lymphatic and venous invasion of the primary gastric cancer were high grade in more than 60% of all patients. Twenty-two patients (30.6%) were positive for peritoneal dissemination, and 63.9% and 47.2% of the patients had abnormally elevated CEA and CA19-9 levels, respectively. In addition, the median survivals of the H1, H2, and H3 groups were 16.6, 10.2, and 4.4 months, and the difference in these groups' curves was statistically significant (p<0.02), as shown in Fig. 1.

Univariate and multivariate analyses of prognostic factors

The univariate analysis was performed for all the clinicopathological factors in Table 1 to further elucidate the prognostic factors. As shown in Table 2, degree of liver metastases (H1, H2/H3), tumor depth of invasion, lymphatic invasion of primary gastric cancer, lymph node metastases, absence (P0) or presence (P1) of peritoneal dissemination, and CA19-9 level were found to be univariately related to patient survival (p<0.05). Next, a multivariable analysis was performed to determine the independent prognostic factors among those six factors that were found significant on the univariate analysis, as shown in Table 2. It was demonstrated that the degree of liver metastases (p<0.0001) and the presence of peritoneal dissemination (p=0.0033) were significantly high risk factors for liver metastases of gastric cancer.

Treatment methods of liver metastases in patients with H1, H2, and P0

The treatment methods for the 26 patients with H1, 2, and P0 are summarized in Table 3. Twelve patients underwent a hepatic resection in the same time of gastrectomy, and a radical operation was performed in 11 of these patients. Of these 12 patients, five patients received hepatic artery infusion (HAI) chemotherapy after the operation, and two patients with H2 metastases were additionally treated with microwave coagulation therapy (MCT) [17, 18]. Three patients received MCT+HAI, and of these three, two patients also received a radical operation.

The overall survival curves of the liver surgery (hepatic resection and/or MCT) group (n=15) and the non-liver surgery group (n=11) are shown in Fig. 2. The cumulative 1, 2, and 5-year survival rates of the liver operation group were 80.0%, 60.0%, and 60.0%, whereas the 1-year survival rate of the non-hepatic treatment group was only 36.4%, and the patients in this group did not survive for more than 2 years. The difference in these survival curves

Table 2 Univariable and multivariate analyses of the risk factors for a prolonged overall survival

Risk factors		Univariable analyses		Multivariable analyses	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Tumor depth	T1,2/T3,4	1.710 (1.008–2.900)	0.0466	1.149 (0.547–2.413)	0.7137
Lymphatic invasion	ly0,1/ly2,3	3.654 (1.644–8.124)	0.0015	2.036 (0.731–5.677)	0.1739
Lymph node metastases	N0,1/N2,3	2.228 (1.240–4.002)	0.0074	1.385 (0.701–2.739)	0.3489
Liver metastases	H1,2/H3	4.102 (2.386–7.053)	<0.0001	3.819 (2.004–7.278)	<0.0001
Peritoneal dissemination	P0/P1	3.121 (1.777–5.482)	<0.0001	3.070 (1.454–6.479)	0.0033
CA19-9 level	<37/≥37 ng/ml	1.718 (1.018–2.898)	0.0426	0.845 (0.415–1.723)	0.6436

Table 3 Therapeutic methods of patients with H1, 2, and without peritoneal dissemination

Liver treatment	Total	Liver metastases		Radical operation	
		H1	H2	R 0	R 1
Hepatectomy	12	9	3	11	1
Lobectomy	4	4	0		
Segmentectomy	1	1	0		
Partial resection	7	4	3		
Combination of HAI	5	4	1		
Hepatectomy + MCT	2	0	2		
MCT+HAI	3	1	2	2	1
HAI alone	5	2	3	0	5
No treatment	6	1	5	0	6

MCT microwave coagulation therapy, *HAI* hepatic artery infusion chemotherapy

was statistically significant (p=0.001). In addition, these 26 patients were divided to four groups: liver surgery + HAI group (n=8), liver surgery alone group (n=7), HAI alone group (n=5), and non-hepatic treatment group (n=6), and the overall survival curves are shown in Fig. 3. The cumulative 1, 2, and 5-year survival rates of the liver surgery + HAI group were 87.5%, 75.0%, and 75.0%, whereas those of the liver surgery alone group were 57.1%, 42.9%, and 42.9%, respectively. The difference between these two groups was not statistically significant (p= 0.2255). The 1-year survival rates of the HAI alone and non-hepatic treatment groups were 60.0% and 16.7%, and the HAI alone group had a tendency toward better survival

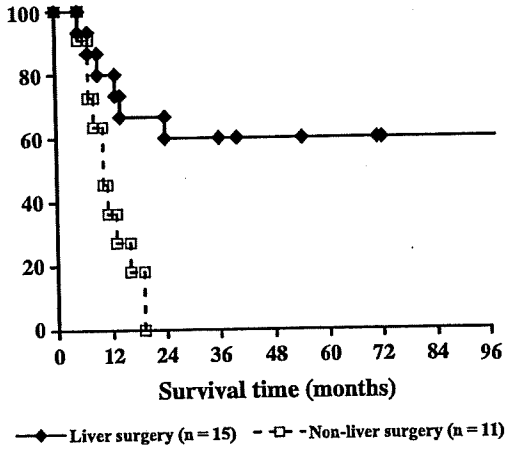

Fig. 2 The overall survival for the 26 patients with H1 or H2 metastases without peritoneal dissemination. A comparison of the actuarial survival rates (Kaplan–Meier) for liver surgery including hepatic resection and/or microwave coagulation therapy (*MCT*; n=15) vs. non-liver surgery at the operation (n=11): p=0.001 (log-rank test)

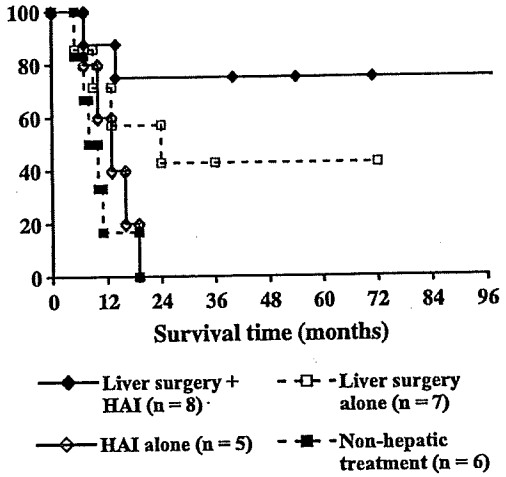

Fig. 3 The overall survival for the 28 patients with H1 or H2 metastases without peritoneal dissemination. Comparison of actuarial survival rates (Kaplan–Meier) for hepatic resection and/or microwave coagulation therapy (Liver surgery) + hepatic artery infusion chemotherapy after the operation (*HAI*; n=8) vs. liver surgery alone (n=7): P=0.2255 (log-rank test); liver surgery + HAI vs. HAI alone (n=5): p=0.0113; liver operation + HAI vs. non-hepatic treatment (n=6): p= 0.0009; liver surgery alone vs. HAI alone: p=0.1317; liver surgery alone vs. non-hepatic treatment: p=0.0380; HAI alone vs. non-hepatic treatment: p=0.4309

than the non-hepatic treatment group; however, the difference between those two groups was not statistically significant (p=0.4309). These results suggested that only liver surgery, but not HAI, could significantly prolong the survival period of patients with H1, 2, and P0.

Analysis of risk factors for prolonged overall survival in patients with H1, 2, and P0

To examine the risk factors for prolonged overall survival in patients with H1, 2, and P0, univariate and multivariate analyses using the Cox proportional hazards model were performed as shown in Table 4. The radical operation (p= 0.0133), the solitary metastatic liver tumor (p=0.0224), and N0, 1 of lymph node metastases (p=0.0260) were independent prognostic determinants of survival.

Characteristics of patients who survived more than 5 years

Furthermore, we reviewed the data on five patients who survived more than 5 years after operation and are alive at present as shown in Table 5. In all of the patients, a radical operation for primary gastric cancer and liver metastases had been performed, and the maximum size of the liver metastases was less than 3 cm. Interestingly, two of the five patients received only MCT and not hepatic resection for liver metastases, and HAI had not been performed after the radical operation in one of the five patients.

Table 4 Univariable and multivariate analyses of the risk factors for a prolonged overall survival in patients with H1, 2, and without peritoneal dissemination

Characteristics		Number	Univariable analyses		Multivariable analyses	
			HR (95% CI)	*P* value	HR (95% CI)	*P* value
Radical operation	R0	13	9.693 (2.685–34.989)	0.0005	33.339 (2.073–536.269)	0.0133
	R1	13				
Treatment method						
Hepatic resection	(−)	14	3.304 (1.141–9.569)	0.0276	0.375 (0.047–2.982)	0.354
	(+)	12				
MCT	(−)	21	0.839 (0.240–2.925)	0.7825		
	(+)	5				
HAI	(−)	13	1.916 (0.727–5.052)	0.1885	0.576 (0.185–1.795)	0.3414
	(+)	13				
Systemic chemotherapy	(−)	6	1.422 (0.500–4.044)	0.5092		
	(+)	20				
Liver metastases						
Number of liver tumor	Solitary	11	4.364 (1.390–13.700)	0.0116	7.218 (1.323–39.370)	0.0224
	Multiple	15				
Size of liver tumor (cm)	<3	11	6.696 (1.878–23.870)	0.0034	1.884 (0.378–9.403)	0.4398
	≥3	15				
Gastric carcinomas						
Histologic differentiation	Diffe.	20	0	0.4823		
	Undiffe.	5				
	Unknown	1				
Tumor size (cm)	<5	6	1.358 (0.433–4.260)	0.5999		
	≥5	18				
	Unknown	2				
Tumor depth of inversion	T1,2	13	1.759 (0.665–4.651)	0.2548		
	T3,4	13				
Lymphatic invasion	ly0,1	10	2.801 (0.887–8.846)	0.0792	2.010 (0.676–5.971)	0.209
	ly2,3	14				
	Unknown	2				
Venous invasion	v0,1	12	0.649 (0.233–1.807)	0.4084		
	v2,3	12				
	Unknown	2				
Lymph node metastases	N0,1	12	2.296 (0.825–6.392)	0.1115	8.159 (2.076–32.076)	0.026
	N2,3	12				
	Unknown	1				

Discussion

The clinicopathological factors of primary gastric cancer may influence survival in gastric cancer patients with liver metastases. It was previously reported that the pathological factors associated with the primary tumor, such as serosal invasion and lymphatic and venous invasion, are significant prognostic factors [14, 19]. However, the impact of these factors was not significant in this study, although tumor depth of invasion (≤T2) and lymphatic invasion (≤ly1) were picked up for the predictor of survival by a univariate analysis. Most authors have reported that these are not predictive factors for the prognosis of patients with liver metastases [3, 5, 6, 13]. Therefore, the clinicopathological factors of the primary tumor may be not directly related to the prognosis and the surgical indications of a hepatic resection. We have demonstrated that the degree of liver metastases (H1, 2) and the absence of peritoneal dissemination (P0) were significant prognostic factors for survival after surgery in patients with liver metastases according to a multivariate analysis. These results emphasize that the indication of the surgical treatment for synchronous liver metastases from gastric cancer is H1, 2 metastases, and P0, and of course, the curative operation for primary and metastases tumors should be treated. We have also found that in those patients, the number of liver metastases (solitary versus multiple) and lymph node metastases (N0, 1 versus N2, 3) were independent prognostic factors of