厚生労働科学研究費補助金

がん臨床研究事業

浸潤性膀胱がんの予後改善をめざした集学的治療の研究

平成17年度　総括研究報告書

主任研究者　塚本　泰司

平成18（2006）年3月

目　次

Ⅰ．総括研究報告書

厚生労働科学研究費補助金（がん臨床研究事業）
総括研究報告書

浸潤性膀胱がんの予後改善をめざした集学的治療の研究

主任研究者　塚本　泰司　札幌医科大学医学部教授

研究要旨

前年度に引き続き、浸潤性膀胱癌の標準治療である根治的膀胱摘除＋リンパ節郭清の補助療法としての術前化学療法（MVAC 療法）の臨床的な位置づけを確立するための臨床研究を遂行した。さらに、術前化学療法、根治的膀胱摘除にともなう QOL の変化の調査および本疾患の臨床経過を予測しうるような指標の特定を試みた。2006 年 3 月現在の登録症例の合計は計 58 例となっている。これまでのところ、プロトコール治療による重篤な有害事象あるいは未知の有害事象は発生していない。本年度はさらに、①骨盤リンパ節郭清の意義の検討、②浸潤性膀胱癌に対する術前あるいは術後の化学療法の検討、③予後予測因子の臨床病理学的検討、さら④根治的膀胱摘除（尿路変向を含む）にかかわる術後早期合併症の検討、⑤高齢者における根治的膀胱摘除の検討を行い以下の結果を得た。①骨盤リンパ節郭清の意義の検討：根治的膀胱摘除に伴う骨盤リンパ節郭清では摘出リンパ節の個数が多変量解析でも独立して予後に影響を与える要因となった。②浸潤性膀胱癌に対する術前あるいは術後の化学療法の検討：浸潤性膀胱癌の一部の症例では、cis-platin を中心とした術前あるいは術後化学療法が明らかに生存率向上に寄与した。③予後予測因子の臨床病理学的検討：リンパ節転移を予測しうるマーカーが見出された。④根治的膀胱摘除にかかわる術後早期合併症の検討：根治的膀胱摘除（尿路変向）の早期合併症は 30%程度に認められたが、軽度あるいは臨床的に対処可能なものが多く、手術による治療効果を考慮すると十分許容可能なものと思われた。術前化学療法の有無は術後早期合併症の頻度に影響しなかった。⑤高齢者における根治的膀胱摘除の検討：75 歳以上の症例と 74 歳未満の根治的膀胱摘除（尿路変向を含む）における術後早期合併症を比較すると、重大な合併症はむしろ前者に少ない結果であった。

分担研究者氏名・所属機関名及び所属機関における職名

高橋　敦・札幌医科大学医学部講師、篠原信雄・北海道大学大学院医学研究科講師、羽渕友則・秋田大学医学部教授、冨田善彦・山形大学医学部教授、小松原秀一・新潟県立がんセンター新潟病院臨床部長、赤座英之・筑波大学医学部教授、西沢　理・信州大学医学部教授、藤元博行・国立がんセンター中央病院医長、庭川　要・静岡がんセンター部長、杉村芳樹・三重大学医学部教授、小野佳成・名古屋大学大学院医学研究科助教授、小野　豊・大阪府立成人病センター診療主任、小川　修・京都大学大

学院医学研究科教授、平尾佳彦・奈良医科大学教授、林　正・日赤和歌山医療センター部長、公文裕巳・岡山大学大学院医歯学総合研究科教授、筧　善行・香川大学医学部教授、荒井陽一・東北大学大学院医学系研究科教授、上田昭一・熊本大学医学部教授、中川昌之・鹿児島大学医学部教授

A. 研究目的

浸潤性膀胱癌に対するこれまでの標準治療（根治的膀胱摘除＋リンパ節郭清）による治療は限界に来ている。特に膀胱外に進展した癌およびリンパ節転移に対する効果は不良である。この現状を打開する可能性があるのは術前化学療法の併用である。膀胱癌に対する MVAC 化学療法が保険適用となった現在、術前化学療法の有効性をエビデンス　レベルで確認することは、今後の進行性膀胱癌の治療の進歩に不可欠なプロセスである。

そこで、浸潤性膀胱癌に対する標準治療（根治的膀胱摘除＋リンパ節郭清）に術前化学療法（MVAC 療法）を追加施行することで、これまでの標準治療の治療成績を向上させることができるかを検討する（図１）。また、これらの治療による臨床経過を予測することが可能な臨床病理学的因子を特定する。この特定は、膀胱温存が可能な浸潤性膀胱癌症例の特徴を見出すことにつながる。さらに、これらの治療にともなう QOL の変化を調査する。

さらに、本臨床研究の付随研究として参加各施設で以下の独自の臨床研究を行うこととした。

B. 研究方法

研究①　浸潤性膀胱がんに対する多施設共同無作為化臨床試験

臨床試験のプロトコールは以下の通りである。MVAC 療法は Day 1: MTX 30mg/m^2 、Day 2:VBL 3mg/m^2、 ADM 30mg/m^2、CDDP: 70mg/m^2、Day 15、22: MTX 30mg/m^2、VBL 3mg/m^2 のスケジュールで施行する。

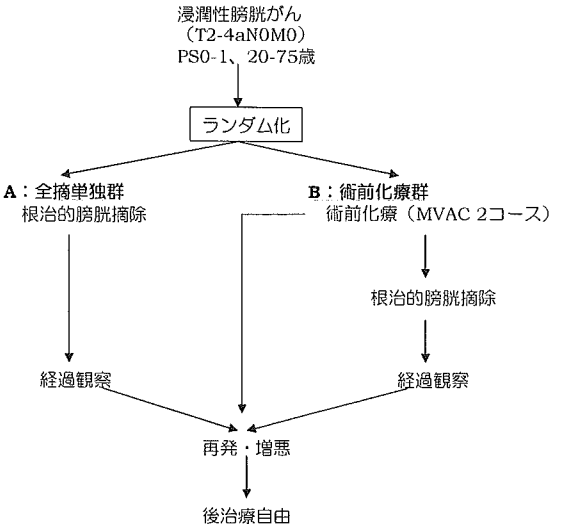

図１　臨床試験の治療プロトコール

上記の臨床試験の適格条件を満たし、本人の文書による同意が得られた症例を無作為に割り付けた（JCOG データーセンター）。

さらに、骨盤リンパ節郭清、周術期化学療法の効果、リンパ節転移予測因子などの検討、根治的膀胱摘除の術後早期合併症の検討、高齢者における根治的膀胱摘除の検討、も合わせて行った。

（倫理面への配慮）

本臨床試験では以下のような倫理面への配慮を行っている。

1) 本試験に参加可能な症例の各種条件（年齢、臨床病期、各種臓器機能など）を設定し、臨床試験プロトコールに明示している。
2) 本試験はヘルシンキ宣言を遵守し、試験参加の利益、不利益を「説明文書」に記載することで、参加者が容易に理解可能となるようにしている。
3) 本試験への参加はあくまでも本人の自由意志であることを明示し、文書で参加の意思を確認するようにしている。
4) 本臨床試験での治療費は通常の保険診療で行われることを明示している。

5) 本臨床試験に参加する施設での倫委員会あるいはそれに準ずる委員会の承認を求めている。
6) 既にJCOG臨床試験員会あるいは各施設の倫理員会で承認された臨床試験であることを明示している。

研究②　本年度の付随研究

1) 骨盤リンパ節郭清の意義の検討
2) 浸潤性膀胱癌に対する術前あるいは術後の化学療法の検討
3) 予後予測因子の臨床病理学的検討
4) 根治的膀胱摘除（尿路変向を含む）にかかわる術後早期合併症の検討
5) 高齢者における根治的膀胱摘除の検討。

C. 研究結果

1）臨床試験

この臨床試験開始後、MVAC療法の保険適用の承認、臨床の状況に合わせた適格規準の見直し、などによりプロトコールの一部改正を行った（2004年3月）。このプロトコール改定により、症例の組み入れがより容易になった。2003年12月末までの登録症例は計5例であったが、2004年11月末まででは計21例、その後、本年は2005年11月末までの1年間で29例の登録があり、2006年3月現在の登録症例の合計は計58例となっている。これまでのところ、プロトコール治療による重篤な有害事象あるいは未知の有害事象は発生していない。

2）付随研究

A）臨床的研究

① 骨盤リンパ節郭清の意義に関する検討

浸潤性膀胱癌における根治的膀胱摘除＋骨盤リンパ節郭清の治療においてリンパ節郭清が予後にどのような影響を与えるのかを1施設において検討した。根治的膀胱摘除＋骨盤リンパ節郭清を行った146例を対象とした。リンパ節の郭清範囲は外腸骨リンパ節、閉鎖リンパ節、内腸骨リンパ節とした。25例（17%）にリンパ節転移が認められた。摘出リンパ節数は、リンパ節転移陽性群：13.9個、陰性群：14.2個であった。リンパ節転移陰性群では、摘出リンパ節数と予後とのには関係が認められなかったが、陽性群では摘出リンパ節個数が13個以上と未満との間に有意の差があった（図2）。多変量解析でも摘出リンパ節個数はリンパ節転移陽性症例では有意な予後因子であった（表1）。

② 周術期化学療法の効果

浸潤性膀胱癌341例にcisplatinを主体とする周術期化学療法（術前：174例、術後：114例、術前および術後：53例）を行った。術前化学療法はT3N0症例で有意に生存率を向上させた。一方、術後化学療法はpT2N0あるいはpT3N0症例での生存率を向上させた。浸潤性膀胱癌の一部の症例では、周術期化学療法が有効である可能性が示唆された。

③ リンパ節転移予測因子などの検討

骨盤リンパ節転移予測因子としてのuroplakin II（UP II）の意義に関して検討した。UP II mRNAは転移が病理学的に認められたリンパ節組織の94%にその発現が陽性であった。一方、cytokeratin 20 mRNAの発現は57%に検出されたのみであった。転移予測に関する因子測するマーカーになる可能性が示唆された。

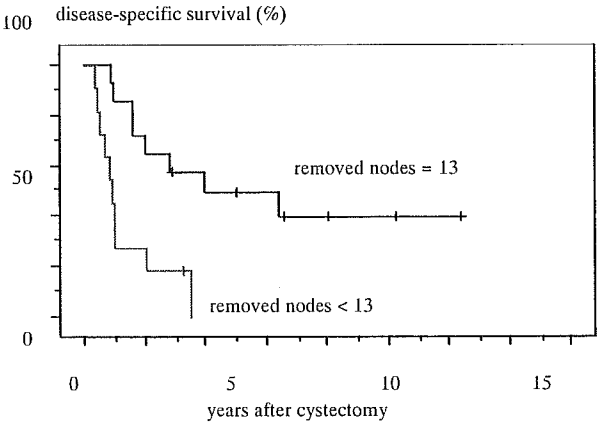

図2　リンパ節転移陽性症例における摘出リンパ節個数と疾患特異的生存率

表1　リンパ節陽性症例における予後因子の検討：多変量解析による

因子	多変量解析 p値	リスク比	95%CI
原発巣組織像 pure UC vs. others	0.6897	1.279	0.382 -4.279
grade 1/2 vs. 3	0.4450	1.936	0.355 -10.550
pT　T2以下 vs. >T2)	0.0132	8.205	1.553 -43.343
摘出リンパ節個数 13以下 vs. >13	0.0008	9.363	2.526 -34.704
陽性リンパ節数 <4 vs. 4以上	0.0115	4.944	1.431 -17.085
術前/術後化学療法 あり vs. なし	0.6164	1.344	0.423 -4.274

④ 根治的膀胱摘除の術後早期合併症の検討

根治的膀胱摘除（尿路変向を含む）は浸潤性膀胱癌の標準的治療法として確立されているが、その手術侵襲は低くはない。また、術前の化学療法が術後早期の合併症に与える影響も十分には評価されていない。そこで、術後早期（術後30日以内）合併症と術前化学療法がそれに与える影響に関して検討した。1994年1月から204年9月までの162例の根治的膀胱摘除を検討した。全体で50例（30.9%）に合併症が見止られたMajor complicationは10例（6.2%）に認められた。5例は敗血症性ショック、4例は術後肺炎であった。死亡例は1例（0.6%）のみであった。Minor complicationとして多かったのはイレウス（18例、11.1%）であったが、いずれの症例も一時的なものであった。次いで、創部感染症（17例、10.5%）であった。術前化学療法の術後合併症への影響を検討するために、術前化学療法を施行した群と施行しなかった群とで術後合併症の頻度を検討したが差はなかった。

⑤ 高齢者における根治的膀胱摘除の検討

今回の臨床試験のプロトコールでは75歳以下の症例を対象としているが、実際の臨床では75歳以上の症例においても根治的膀胱摘除を余儀なくされる場合が少なくない。これらの高齢者での問題点の把握は今回の臨床試験の遂行にも有用と思われたので、75歳以上の高齢者における根治的膀胱摘除の問題点を検討した。1994年から2005年に根治的膀胱摘除を行った症例（75歳未満：170例-非高齢者群、75歳以上：26例－高齢者群）を対象とした。Major complication、minor complicationの発生頻度は両群では差がなかった。むしろ、高齢者群でいずれの合併症も頻度が低い傾向があった。高齢者群で根治的膀胱摘除を行った症例と非手術/無治療とした症例を比較すると前者のASA（アメリカ麻酔学会）スコア、PSが明らかに良好であった。

D．考案

1）臨床試験

この臨床試験における2006年3月現在の登録症例の合計は計58例となっている。予定に反して登録症例数が少なく、これが本臨床試験の大きな問題点となっている。この問題の解決のためにこれまで種々の改善を行ってきたが、今後以下のような改善策を計画し、実際に実施している。

① 現在症例登録のある施設から臨床試験のコアになる医師を7－8人選び、幹事会を設け本臨床試験の実施を推進する。これらの医師が登録のない施設への働きかけ（施設訪問・講習会、など）を行う。

② 研究代表者が泌尿器科関連学会（泌尿器科学会総会、東部・中部・西部総会）の開催に合わせて当該地域の試験参加施設医師を集めて説明会を開催する。

③ 同意取得率を向上させるため統一した説明パンフレット、DVDなどを準備する。特に、浸潤性膀胱癌の症例を紹介してくれる地域の泌尿器科医に対する本臨床試験をさらにアピールするために、臨床試験の意義、目的、適格規準などを明記した印刷物を配布する。

④ 現在、月1回症例登録状況をメールで配信しているが、そのほかに浸潤性膀

膀胱癌の診断・治療に関する新しい情報をメールで提供する。

2）骨盤リンパ節郭清の意義に関する検討

浸潤性膀胱癌に対する治療で骨盤リンパ節郭清がどのような位置を占めるのかはこれまで検討でも十分明らかになってはいない。病期診断としての意義はあきらかではあるが、治療的な意義については否定的な意見が多かった。また、リンパ節郭清の範囲に関しても一致した見解はない。通常、内・外腸骨および閉鎖リンパ節が所属リンパ節と定義されているので、この領域を郭清しているが、この範囲を拡大したリンパ節郭清に治療上の意義があるとする報告もある。今回の検討で行われたリンパ節郭清の範囲は通常の骨盤リンパ節郭清の範囲であるが、それでも摘出リンパ節個数がその後の臨床経過に影響を与えるとする結果であった。最近、大動脈分岐部以下のリンパ節郭清に治療上の効果があるとの報告もあり、リンパ節郭清の範囲、治療上の意義に関して再検討する必要があろう。

3）周術期化学療法の効果

浸潤性膀胱癌にたいする術前あるいは術後の化学療法が臨床経過にどのような影響を与えるかは、確立されていない。本臨床研究の目的もこの点にある。最近のメタアナリシスの結果はcisplatinを主体とする術前多剤併用化学療法の効果を支持している。一方、cisplatinを主体とする術後多剤併用化学療法に関しては、術前化学療法ほどその効果が明らかではない。その理由の1つは、無作為化試験が少ないことも関係している。cisplatinを主体とする術前多剤併用化学療法がどのような症例でより効果的なのかに関しては、不明な点が少なくない。今回のretrospectiveな検討では、周術期化学療法の効果を支持し、術前化学療法は臨床病期T3症例に、術後化学療法はpT2/3の予後を改善することが示されたが、これまでの報告の結果を裏付けるものと考えられた。

4）リンパ節転移予測因子などの検討

リンパ節転移を病理学的に正確に証明することはルーチンの作業としては困難であるが、今回の方法ではその陽性率から臨床的に応用可能と思われた。今後、この結果をもとに多数例での検討が望まれる。これまで見逃されていた微小なリンパ節転移の可能性が明らかになれば先の述べたリンパ節郭清の範囲あるいは意義に関して関連が出てくると思われる。

5）根治的膀胱摘除の術後早期合併症の検討

根治的膀胱摘除（尿路変向を含む）は浸潤性膀胱癌の標準的治療法として確立されていて、多くの施設で行われているが、どの程度の合併症の頻度があるのかは意外に報告されていない。一般に、術後早期合併症の頻度は12-57%と報告されているが、合併症の定義によりその頻度が大きく異なるようである。今回は、CTCAE ver.3（JCOG日本語訳）を用いたが、軽症の合併症も含めると30%であった。しかし、大部分の合併症は一過性のもので対処可能であることから、根治的膀胱摘除のbenefitを考慮すると十分許容できるものではないかと考えられた。また、術前化学療法は早期合併症の頻度に影響を与えなかったが、出血量、同種血輸血量は多かった。この点に関する今後の改善策が必要と思われた。

6）高齢者における根治的膀胱摘除の検討

今回の検討の対象にした高齢者根治的膀胱摘除症例26例は、明らかに選択された症例であった。換言すれば、適切な症例選択を行えば75歳以上の高齢者でも根治的膀胱摘除が十分可能であることを示す。適切な症例選択にはASA（アメリカ麻酔学会）スコア、PSが参考にあると思われる。

E. 結論

1) 前年度に引き続き、浸潤性膀胱癌の標準

治療である根治的膀胱摘除＋リンパ節郭清の補助療法としての術前化学療法（MVAC 療法）の臨床的な位置づけを確立するための臨床研究を遂行した。2006 年 3 月現在の登録症例の合計は計 58 例となっている。これまでのところ、プロトコール治療による重篤な有害事象あるいは未知の有害事象は発生していない。

2) 根治的膀胱摘除に伴う骨盤リンパ節郭清では摘出リンパ節の個数が多変量解析でも独立して予後に影響を与える要因となった。

3) 浸潤性膀胱癌の一部の症例では、cis-platin を中心とした術前あるいは術後化学療法が明らかに生存率向上に寄与した。

4) リンパ節転移を予測しうるマーカーが見出された。

5) 根治的膀胱摘除（尿路変向）の早期合併症は 30%程度に認められたが、軽度あるいは臨床的に対処可能なものが多く、手術による治療効果を考慮すると十分許容可能なものと思われた。術前化学療法の有無は術後早期合併症の頻度に影響しなかった。

6) 75 歳以上の症例と 74 歳未満の根治的膀胱摘除（尿路変向を含む）における術後早期合併症を比較すると、重大な合併症はむしろ前者に少ない結果であった。

F. 健康危険情報

なし

G. 研究発表

1. 論文発表

1) Matsui Y, Nishiyama H, Ogawa O, et al. The current status of perioperative chemotherapy for invasive bladder cancer : a multiinstitutional retrospective study in Japan. Int J Clin Oncol, 2005; 10: 133-138.

2) Wu X, Kakehi Y, et al. Uroplakin II as a promising marker for molecular diagnosis of nodal metastases from bladder cancer: comparison with cytokeratin 20. J Urol, 2005; 174: 2138-2143.

3) Kawamoto K, Enokida H, Nakagawa M, et al. p16 (INK4a) and p14 (ARF) methylation as a potential biomarker for human bladder cancer. Biochem Biophys Res Commun 2006; 339: 790-796.

4) 福田歷視、光森健二、羽渕友則、他. HD-MVAC 療法が奏効した尿路上皮癌の 2 例. 泌尿紀要、2005; 51: 113-116.

5) Kitamura H, Tsukamoto T. Early bladder cancer: concept, diagnosis, and management. Int J Clin Oncol, 2006; 11: 28-37.

6) Tanaka T, Kitamura H, Takahashi A, Masumori N, Itoh N, Tsukamoto T. Long-term functional outcome and late complications of Studer's ileal neobladder. Jpn J Clin Oncol, 2005; 35: 391-394.

7) 舛森直哉、市原浩司、廣部恵、高橋敦、塚本泰司. 高齢者の浸潤性膀胱癌の治療：根治性との QOL のバランスをどうとるか. Urology View, 2006; 4: 36-42.

8) Honma I, Masumori N, Tsukamoto T, et al. Removal of more lymph nodes may provide a better outcome as well as more accurate pathology in patients with bladder cancer-An analysis of the role of pelvic lymph node dissection-, Urology, 2006, in press.

H. 知的財産権の出願・登録状況

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

Ⅱ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書 籍

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

雑 誌

発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
Matsui Y, Nishiyama H, Ogawa O, et al.	The current status of perioperativer chemotherapy for invasive bladder cancer: a multiinstitutional retrospective study in Japan.	Int. J. Clin. Oncolo.	10	133-138	2005
Wu X, Kakehi Y, et al	Uroplakin II as a promising marker for molecular diagnosisd of nodal metastasis from bladder cancer: comparison with cytokeratin 20.	J. Urol.	174	2138-2143	2005
Kawamoto K, Enokida H, Nakagawa M, et al.	P16 (INK4a) and p14 (ARF) methylation as a potential biomarker for human bladder cancer.	Biochem. Biophys. Res. Commun.	339	790-796	2006
福田歷視、光森健二、羽渕友則ほか	HD-MVAC 療法が奏効した尿路上皮癌の 2 例.	泌尿紀要	51	113-116	2005
Kitamura H, Tsukamoto T	Early bladder cancer: concept, diagnosis, and management	Int. J. Clin. Oncol.	11	28-37	2006
Tanaka T, Kitamura H, Takahashi A, Masumori N, Itoh N, Tsukamoto T	Long-term functional outcome and late complications of Studer's ileal neobladder	Jpn. J. Clin. Oncol.	35	391-394	2005
舛森直哉、市原浩司、廣部恵、高橋敦、塚本泰司	高齢者の浸潤性膀胱癌の治療：根治製との QOL のバランスをどうとるか	Urology View	4	36-42	2006
Honma I, Masumori N, Tsukamoto T, et al.	Removal of more lymph nodes may provide a better outcome as well as more accurate pathology in patients with bladder cancer -An analysis of the role of pelvic lymph node dissection-	Urology		in press	2006

Ⅲ. 研究成果の刊行物・別刷

Int J Clin Oncol (2005) 10:133–138
DOI 10.1007/s10147-004-0474-5

ORIGINAL ARTICLE

Yoshiyuki Matsui · Hiroyuki Nishiyama · Jun Watanabe
Satoshi Teramukai · Yoshinari Ono · Shinichi Ohshima
Kiyohide Fujimoto · Yoshihiko Hirao
Masanori Fukushima · Osamu Ogawa

The current status of perioperative chemotherapy for invasive bladder cancer: a multiinstitutional retrospective study in Japan

Received: October 4, 2004 / Accepted: December 20, 2004

Abstract
Background. We conducted a multiinstitutional analysis to clarify the clinical significance of perioperative chemotherapy, in invasive bladder cancers in Japan, and to identify the patient subpopulations who could benefit from perioperative chemotherapy.
Methods. A total of 913 consecutive patients aged less than 80 years who underwent radical cystectomy for invasive bladder cancer from 1990 to 2000 at 32 Japanese hospitals were retrospectively analyzed. Median follow-up was 3.8 years (range, 0.1 to 11.8 years).
Results. In total, 341 patients (37.3%) were treated with perioperative chemotherapy, including neoadjuvant chemotherapy (n = 174), adjuvant chemotherapy (n = 114), or a combination of both chemotherapies (n = 53). With cisplatin-based combination chemotherapy, the MVAC (methotrexate, vinblastine, doxorubicin, and cisplatin) regimen was the one most frequently used for perioperative chemotherapy, but the average number of cycles was distinctly less than that in reported randomized trials. MEC (methotrexate, epirubicin, and cisplatin) chemotherapy had efficacy similar to that of the MVAC regimen. On analysis of patients stratified by stage, the overall survival of patients with adjuvant chemotherapy was significantly better than that of those without adjuvant chemotherapy, in patients with pT2b, pN0 or pT3, pN0 (P = 0.016 or 0.020, respectively), but adjuvant chemotherapy had no, or the opposite, effect on patients with pT2a, pN0, pT4, pN0, or pTany, pN+. On the other hand, neoadjuvant chemotherapy provided a statistically significant survival benefit only for patients with clinical T3N0 (P = 0.015). Of note, in the high-risk subgroup, the overall survival rate for patients with complete response (CR) after neoadjuvant chemotherapy was significantly better than that of patients with partial response (PR) or no change (NC)/progressive disease (PD) (P = 0.043).
Conclusion. In Japan, cisplatin-based combination chemotherapy has been the main modality adopted perioperatively for high-risk patients with radical cystectomy. This study's clinical results indicated that perioperative chemotherapy may improve survival in patients with T3N0 or pT2b/pT3, pN0 bladder cancer.

Key words Bladder cancer · Radical cystectomy · Adjuvant chemotherapy · Neoadjuvant chemotherapy · Overall survival

Introduction

Bladder cancer is one of the most common genitourinary malignancies, in which 20% to 40% of patients present with or develop invasive disease.[1] Invasive bladder cancer is very aggressive, and about half of the patients die of the disease within 5 years, even following radical cystectomy with lymph node dissection.[2,3] This high mortality rate has been considered to be due to micrometastasis that is present at the time of radical cystectomy. To improve the prognosis of invasive bladder cancer, perioperative systemic chemotherapy has been adopted empirically, in combination with radical cystectomy, because bladder cancer is sensitive to chemotherapy, as shown by an overall response rate of 12%–73%.[4,5] Several clinical trials have been performed since around 1985 to assess the efficacy of perioperative chemotherapy with definitive local therapy.[6–11] Most of the trials, however, failed to elicit conclusive results regarding the clinical significance of perioperative systemic

Y. Matsui · H. Nishiyama · J. Watanabe · O. Ogawa (✉)
Department of Urology, Kyoto University, Graduate School of Medicine, 54 Shogoin Kawaharacho, Sakyo-ku, Kyoto 606-8507 Japan
Tel. +81-75-751-3325; Fax +81-75-761-3441
e-mail: ogawao@kuhp.kyoto-u.ac.jp

S. Teramukai · M. Fukushima
Translational Research Center, Kyoto University Hospital, Kyoto, Japan

Y. Ono · S. Ohshima
Department of Urology, Nagoya University, School of Medicine, Nagoya, Japan

K. Fujimoto · Y. Hirao
Department of Urology, Nara Medical University, Nara, Japan

chemotherapy in the management of invasive bladder cancer. This may have been due to the suboptimal design of these clinical trials, including less effective chemotherapy regimens, sample sizes too small for important changes in survival to be detected or ruled out, inadequate selection of patients, and premature closure of the trials. We conducted a multiinstitutional retrospective study to clarify the current status of perioperative chemotherapy in invasive bladder cancers in Japan, and to identify the subgroups of patients who might benefit from perioperative chemotherapy.

Patients and methods

We have established a database at 32 Japanese institutions, including three university hospitals (Kyoto University Hospital, Nagoya University Hospital, and Nara Medical University Hospital), containing detailed and comprehensive clinical and pathologic information about all patients who have undergone cystectomy from January 1990 to December 2000.[12] The intent of the present analysis was to focus on a relatively homogeneous cohort of patients with invasive transitional cell carcinomas (TCCs) of the bladder who underwent a complete resection of all grossly evident tumors at the time of cystectomy. Although the records of 1131 consecutive patients were identified in the database, excluded from analysis were 89 patients with non-TCC bladder cancers, 115 patients who underwent simple cystectomy without bilateral iliac lymph node dissection, and 14 patients over 80 years old (because systemic chemotherapy is generally not indicated for these patients. These patients will be the subject of a separate report. These exclusions may create some biases; however, the analysis of patients with a completed cystectomy with curative intent remains a critical topic. The remaining 913 patients underwent radical cystectomy for primary TCC of the bladder with intent to cure, and were the focus of this analysis. For entry to this study, informed consent was not obtained from each patient, because the analysis was conducted retrospectively.

The clinical data that were collected from the medical records included age, sex, past history, histologic grading according to the World Health Organization system, clinical and pathological staging according to the TNM classification,[13] and the presence of and regimen of perioperative therapy. The follow-up procedures varied slightly among the institutions considered; however, some general statements should be made because of the long study period. Patients were followed every 3–6 months during the first 2–3 years; the visits were every 6 months or annually thereafter. The follow up at each visit included a physical examination (general and local). This prompted, when needed, and according to the clinical suspicion of persisting/relapsing disease, the use of the same specific diagnostic tests as those performed during the baseline workup (e.g., ultrasonography, chest X-ray, computed tomography [CT], and bone scan).

Table 1. Clinical characteristics of the 913 study patients

Characteristics	Number of cases
Total number of patients	913
Sex	
Male:Female	760:153
Clinical tumor stage	
T1 or less:T2:T3:T4:Tx	243:263:297:54:56
N0:N(+):Nx	855:25:33
Pathological tumor stage	
pT1 or less:pT2a:pT2b:pT3:pT4	369:171:120:185:68
pN0:pN+	773:140
Regimen of perioperative chemotherapy	
Neoadjuvant chemotherapy	227
MVAC:MEC:CISCA:PAM:other	99:35:35:3:55
Adjuvant chemotherapy	167
MVAC:MEC:CISCA:PAM:other	73:26:23:20:25

MVAC, methotrexate, vinblastine, doxorubicin, and cisplatin; MEC, methotrexate, epirubicin, and cisplatin; CISCA, cisplatin, cyclophosphamide, and doxorubicin; PAM, cisplatin, adriamycin, and methotrexate

The overall survival was defined as the time from radical cystectomy to any cause of death; all deaths from any cause were counted as events, and surviving patients were treated as censored at the date of last follow-up before December 31, 2001. Median follow up was 3.8 years (range, 0.1 to 11.8 years). The survival rates were estimated with the Kaplan-Meier method. The log-rank test was performed to test associations between perioperative chemotherapy and survival according to stage. Two-sided P values of less than 0.05 were regarded as statistically significant. All statistical analyses were done by using SAS version 8 (SAS Institute, Cary, NC, USA).

Results

A total of 913 TCC patients (760 men [83.2%] and 153 women [16.8%]), with a mean age of 64.8 years (range, 31 to 80 years), were treated with radical cystectomy with bilateral lymph node dissection. As shown in Table 1, pathological findings demonstrated that 369 patients (40.4%) harbored bladder tumors of pT1 or less, 171 (18.7%) had pT2a tumors, 120 (13.1%) had pT2b tumors, 185 (20.3%) had pT3 tumors, and 68 (7.4%) had pT4 tumors. Eight hundred and fifty-five patients (93.6%) were without evidence of lymph node involvement. Regarding treatment strategy, 341 patients (37.3%) were treated with perioperative chemotherapy. Neoadjuvant chemotherapy was adopted for 174 patients, adjuvant chemotherapy for 114 patients, and a combination of both chemotherapies for 53 patients. The chemotherapeutic regimens used included MVAC (methotrexate, vinblastine, doxorubicin, and cisplatin), MEC (methotrexate, epirubicin, and cisplatin), CISCA (cisplatin, cyclophosphamide, and doxorubicin), and PAM (cisplatin, adriamycin, and methotrexate), as well as other miscellaneous regimens.[6,14–16] The MVAC regimen was the most frequently used for neoadjuvant or adjuvant

Table 2. The effects of adjuvant chemotherapy on overall survival in 406 analyzable patients without neoadjuvant chemotherapy

Pathological stage	Adjuvant chemotherapy	No. of patients	No. of deaths	Overall survival (%)		*P* value
				3-Year	5-Year	
pT2a,pN0	Without[a]	119	21	89.9	81.9	0.655
	With	7	1	83.3	83.3	
pT2b,pN0	Without	49	18	64.9	56.5	0.016
	With	24	3	90.8	90.8	
pT3,pN0	Without	65	28	51.5	51.5	0.020
	With	22	4	90.9	75.0	
pT4,pN0	Without	24	6	69.3	69.3	0.033
	With	7	6	17.9	0.0	
pTany,pN+	Without	53	32	40.6	29.8	0.210
	With	46	21	50.7	40.5	

[a] Without neoadjuvant chemotherapy

Table 3. The effects of neoadjuvant chemotherapy on overall survival in 466 analyzable patients without adjuvant chemotherapy

Clinical stage	Neoadjuvant chemotherapy	No. of patients	No. of deaths	Overall survival (%)		*P* value
				3-Year	5-Year	
T2N0	Without[a]	169	36	83.9	76.4	
	With	42	9	84.8	80.9	0.993
T3N0	Without	123	47	56.5	56.6	
	With	77	19	80.0	73.5	0.015
T4N0	Without	18	5	67.3	67.3	
	With	22	11	47.8	47.8	0.345
Tany,N+	Without	5	3	60.0	60.0	
	With	10	4	60.0	60.0	0.979

[a] Without adjuvant chemotherapy

chemotherapy. The indications for perioperative chemotherapy varied among institutions, but, generally, adjuvant chemotherapy was adopted mainly for patients with pT2b or greater tumors and those with pathological lymph node involvement (pN+; Table 2), whereas neoadjuvant chemotherapy was used mainly for patients with T3 or greater tumors, or those with N+ (Table 3).

During follow up, 249 patients (27.2%) died, and the overall survival rates for all 913 patients at 3 and 5 years were 77.3% and 71.6 %, respectively. The overall survival decreased proportionally with more advanced pathological stage. The rates for 5-year overall survival were 85.0% for organ-confined disease (pT0-2, pN0), 58.0% for locally invasive disease (pT3-4,pN0), and 37.3% for disease with lymph node involvement (pTany,pN+) (Fig. 1a). To identify the patients who benefited from perioperative chemotherapy, the effects of adjuvant chemotherapy on survival were assessed in subgroups stratified by pT and pN stage in those without neoadjuvant chemotherapy. The overall survival of patients with adjuvant chemotherapy was significantly better than those without adjuvant chemotherapy in patients with pT2b,pN0 or pT3,pN0 ($P = 0.016$ or 0.020, respectively), whereas adjuvant chemotherapy had no or opposite effects on patients with pT2a,pN0, pT4,pN0, or pTany,pN+ (Table 2). On the other hand, in the analysis of those without adjuvant chemotherapy, neoadjuvant chemotherapy provided a statistically significant survival benefit only for patients with clinical T3N0 ($P = 0.015$; Table 3).

The response was assessed in 176 patients who received neoadjuvant chemotherapy for whom information was available. The response rate (partial response/complete response; PR/CR) was 56.3%, and CRs were achieved in 27 patients (15.3%). Regarding the regimens, MVAC and MEC demonstrated similar response rates, and these were superior to those of CISCA and the miscellaneous other regimens (Table 4). Although the survival rates for patients with PR and those with no change/progressive disease (NC/PD) were not significantly different, the overall survival rate for patients with CR in the high-risk subgroup (patients with T3 or more or N+) was significantly better than that of those with PR or NC/PD ($P = 0.043$; Fig. 1b). Furthermore, although differences did not reach significance, the 5-year-survival rates of patients treated with MVAC/MEC as neoadjuvant chemotherapy tended to be better than those of patients with other neoadjuvant chemotherapy regimens or those without neoadjuvant chemotherapy in the T2/3N0 subgroup (77.3%, 74.0%, and 67.7%, respectively).

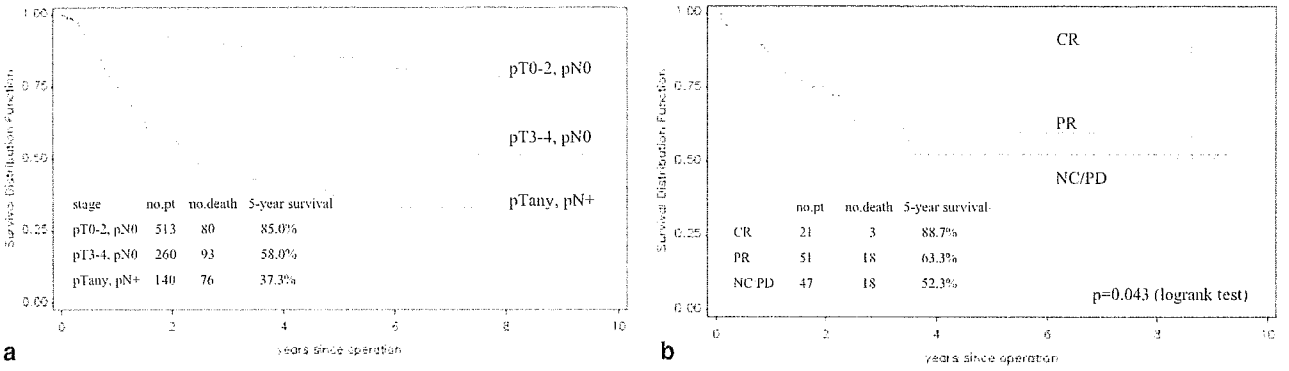

Fig. 1. **a** Estimated Kaplan-Meier overall survival curves for all patients (*pt*), stratified by alpathological stage. **b** Estimated Kaplan-Meier overall survival curves for patients with T3 or higher or N+, stratified by the response to neoadjuvant chemotherapy. *CR*, complete response; *PR*, partial response; *NC*, no change; *PD*, progressive disease

Table 4. Responses of 176 assessable patients to various regimens of neoadjuvant chemotherapy

Regimen of neoadjuvant chemotherapy	No. of patients	Average no. of cycles	Response to chemotherapy		
			CR	PR	NC/PD
MVAC	80	1.5	15 (18.8%)	33 (41.3%)	32 (40.0%)
MEC	34	2.1	7 (20.6%)	16 (47.1%)	11 (32.4%)
CISCA	30	1.2	1 (3.3%)	8 (26.7%)	21 (70.0%)
Other	32	2.3	4 (12.5%)	15 (46.9%)	13 (40.6%)
	176				

MVAC, methotrexate, vinblastine, doxorubicin, and cisplatin; MEC, methotrexate, epirubicin, and cisplatin; CISCA, cisplatin, cyclophosphamide, and doxorubicin; PAM, cisplatin, adriamycin, and methotrexate; CR, complete response; PR, partial response; NC, no change; PD, progressive disease

Discussion

In this multiinstitutional retrospective study, we demonstrated the current status of perioperative chemotherapy in the management of locally advanced TCC of the bladder in Japan and assessed the patients who could benefit from chemotherapy. In total, 341 patients (37.3%) were treated with perioperative chemotherapy, and the rates of neoadjuvant and adjuvant chemotherapy were similar. As for the regimen of perioperative chemotherapy, MVAC was the most frequently adopted in our series, and this is the global standard chemotherapy regimen for bladder cancer. Several modified regimens were developed on the basis of MVAC to reduce the toxicity of the chemotherapy or to enhance its effects. MEC (methotrexate, cisplatin, and epirubicin) was also used, and the response rates to MEC were almost the same as those of MVAC. Although the indications and regimens of chemotherapy varied among the institutions in this study, perioperative chemotherapy, in the form of cisplatin-based combination chemotherapy, was the main modality adopted for high-risk patients, and, therefore, we consider this series acceptable for analysis.

Adjuvant chemotherapy has been established for the treatment of other cancers, in which the response rates to chemotherapy are the same or lower than those in bladder cancer.[17,18] In invasive bladder cancers, two randomized trials revealed a benefit of adjuvant chemotherapy compared with radical cystectomy alone.[19,20] These two reports showed the apparent benefit of the chemotherapy in patients who had especially poor-risk cancers (pT3 or pN+), although each of these trials included fewer than 100 patients, and both were terminated prematurely, on the basis of an interim analysis favoring the chemotherapy group, without evaluating overall survival curves as an endpoint. In the present study, 5-year overall survival rates in patients with adjuvant chemotherapy were better than those in patients without adjuvant chemotherapy in the subgroup of patients with pT2b,pN0 or pT3,pN0 (P = 0.016 or 0.020, respectively), but adjuvant chemotherapy had no, or opposite, effects on patients with pT2a,pN0, pT4,pN0, or pN+. These results suggested that adjuvant chemotherapy may improve prognosis in locally advanced bladder cancer (pT2b or pT3 without lymph node metastasis), although retrospective studies do have a patient selection bias. On the other hand, 80% of patients with pT2a or lower stage bladder cancers survived for more than 5 years after radical cystectomy

alone; thus, an incremental survival benefit with chemotherapy may not have been detected in this subgroup. Conversely, massively advanced cancer, including extravesical invasion (pT4) or lymph node metastasis, may be beyond the therapeutic ability of adjuvant chemotherapy, because of the large residual tumor burden.

As for neoadjuvant chemotherapy, there were more studies than those of adjuvant chemotherapy in bladder cancer.[9–11,21,22] The neoadjuvant approach has several benefits: one is that it allows preoperative reduction of tumor size, which may make local therapy more effective. Second, patients may best tolerate chemotherapy before they have received potentially debilitating local treatment such as radical cystectomy. Furthermore, neoadjuvant chemotherapy can provide clinically important information about chemosensitivity. A recent metaanalysis[4] revealed a significant benefit of platinum-based neoadjuvant chemotherapy on overall survival in invasive bladder cancer, although several randomized trials have failed to demonstrate its effectiveness in invasive bladder cancer. Grossman and colleagues[23] reported the benefit of three cycles of neoadjuvant MVAC chemotherapy with radical cystectomy in a randomized trial enrolling 307 patients. Especially, the survival benefit of neoadjuvant MVAC appeared to be strongly related to downstaging of the tumor to pT0. In our study, the overall survival rate for patients with CR was demonstrated to be significantly better than that for those with PR or NC/PD, suggesting that neoadjuvant chemotherapy can provide a survival benefit only when tumors possess good chemosensitivity and the neoadjuvant chemotherapy achieves a CR.

When performing perioperative chemotherapy with radical cystectomy, important factors to consider, other than chemosensitivity, are: which regimens are suitable and how many cycles of chemotherapy are needed to improve survival. MVAC is the most cmmon regimen worldwide, and three cycles of MVAC have been adopted for randomized studies.[23] However, our data demonstrated that, in Japan, generally, MVAC or modified MVAC regimens (MEC or PAM) were used empirically for an average of two cycles, suggesting that three cycles of these regimens are not tolerable for Japanese. Recently, several novel cytotoxic compounds have been studied in the management of recurrent and metastatic bladder cancer. In particular, gemcitabine or paclitaxel are considered to be promising agerts for bladder cancer.[24,25] In the future, neoadjuvant chemotherapy, using these new compounds and molecular markers for predicting chemosensitivity, will need to be assessed by randomized prospective studies.

Conclusion

Our retrospective analysis indicated that perioperative chemotherapy may improve survival in patients with T3N0 or pT2b/pT3,pN0 bladder cancer who have a radical cystectomy. A significant survival benefit may be obtained in those who achieve pathological downstaging to a complete clinical response after neoadjuvant chemotherapy. To achieve maximum survival benefit from adjuvant chemotherapy and to avoid the administration of toxic chemotherapeutic agents to unresponsive patients, more reliable markers, and more attractive chemotherapeutic regimens should be carefully investigated by well-designed randomized trials.

Acknowledgments This work was supported in part by a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Culture, Sports, and Technology of Japan. We thank community hospitals associated with Kyoto University, Nara Medical University, and Nagoya University for the analysis of clinical data.

References

1. Raghavan D, Shipley WU, Garnick MB, et al. (1990) Biology and management of bladder cancer. N Engl J Med 322:1129–1138
2. Giuliani L, Giberti C, Martorana G, et al. (1985) Results of radical cystectomy for primary bladder cancer. Retrospective study of more than 200 cases. Urology 26:243–248
3. Pagano F, Bassi P, Galetti TP, et al. (1991) Results of contemporary radical cystectomy for invasive bladder cancer: a clinicopathological study with an emphasis on the inadequacy of the tumor, nodes and metastases classification. J Urol 145:45–50
4. Advanced Bladder Cancer Meta-analysis Collaboration (2003) Neoadjuvant chemotherapy in invasive bladder cancer: a systematic review and meta-analysis. Lancet 361:1927–1934
5. Hussain SA, James ND (2003) The systemic treatment of advanced and metastatic bladder cancer. Lancet Oncol 4:489–497
6. Sternberg CN, Yagoda A, Scher HI, et al. (1985) Preliminary results of M-VAC (methotrexate, vinblastine, doxorubicin and cisplatin) for transitional cell carcinoma of the urothelium. J Urol 133:403–407
7. Studer UE, Bacchi M, Biedermann C, et al. (1994) Adjuvant cisplatin chemotherapy following cystectomy for bladder cancer: results of a prospective randomized trial. J Urol 152:81–84
8. Freiha F, Reese J, Torti FM (1996) A randomized trial of radical cystectomy versus radical cystectomy plus cisplatin, vinblastine and methotrexate chemotherapy for muscle invasive bladder cancer. J Urol 155:495–499
9. Martinez-Pineiro JA, Gonzalez Martin M, Arocena F, et al. (1995) Neoadjuvant cisplatin chemotherapy before radical cystectomy in invasive transitional cell carcinoma of the bladder: a prospective randomized phase III study. J Urol 153:964–973
10. Malmstrom PU, Rintala E, Hellsten S (2001) The Nordic experience of cooperative urinary bladder cancer trials. Semin Urol Oncol 19:69–70
11. International Collaboration of Trialists (1999) Neoadjuvant cisplatin, methotrexate, and vinblastine chemotherapy for muscle-invasive bladder cancer: a randomised controlled trial. Lancet 354:533–540
12. Nishiyama H, Habuchi T, Watanabe J, et al. (2004) Clinical outcome of a large-scale multi-institutional retrospective study for locally advanced bladder cancer: a survey including 1131 patients treated during 1990–2000 in Japan. Eur Urol 45:176–181
13. Fleming ID, Cooper JS, Henson DE, et al. (eds) (1997) AJCC cancer staging manual, 5th edn. Lippincott-Raven, Philadelphia, pp 241–243
14. Kuroda M, Kotake T, Akaza H, et al. (1998) Efficacy of dose-intensified MEC (methotrexate, epirubicin and cisplatin) chemotherapy for advanced urothelial carcinoma: a prospective randomized trial comparing MEC and M-VAC (methotrexate, vinblastine, doxorubicin and cisplatin). Japanese Urothelial Cancer Research Group. Jpn J Clin Oncol 28:497–501
15. Sternberg JJ, Bracken RB, Handel PB, et al. (1977) Combination chemotherapy (CISCA) for advanced urinary tract carcinoma. A preliminary report. JAMA 238:2282–2287

16. Oshima S, Ono Y, Fujita T, et al. (1987) Three-drug combination chemotherapy for advanced urothelial tract carcinoma. Cancer Chemother Pharmacol 20 Suppl:S20–S23
17. Chau I, Cunningham D. (2002) Adjuvant therapy in colon cancer: current status and future directions.Cancer Treat Rev 28:223–236
18. Maehara Y, Baba H, Sugimachi K. (2001) Adjuvant chemotherapy for gastric cancer: a comprehensive review. Gastric Cancer 4:175–184
19. Skinner DG, Daniels JR, Russell CA, et al. (1991) The role of adjuvant chemotherapy following cystectomy for invasive bladder cancer: a prospective comparative trial. J Urol 145:459–464
20. Stockle M, Meyenburg W, Wellek S, et al. (1992) Advanced bladder cancer (stages pT3b, pT4a, pN1 and pN2): improved survival after radical cystectomy and three adjuvant cycles of chemotherapy. Results of a controlled prospective study. J Urol 148:302–306
21. Wallace DM, Raghavan D, Kelly KA, et al. (1991) Neo-adjuvant (pre-emptive) cisplatin therapy in invasive transitional cell carcinoma of the bladder. Br J Urol 67:608–615
22. Bassi P, Pappagallo GL, Sperandio P, et al. (1999) Neoadjuvant MVAC chemotherapy of invasive bladder cancer: results of a multicenter phase III trial (abstract). J Urol 161:264a
23. Grossman HB, Natale RB, Tangen CM, et al. (2003) Neoadjuvant chemotherapy plus cystectomy compared with cystectomy alone for locally advanced bladder cancer. N Engl J Med 349:859–866
24. von der Maase H, Hansen SW, Roberts JT, et al. (2000) Gemcitabine and cisplatin versus methotrexate, vinblastine, doxorubicin, and cisplatin in advanced or metastatic bladder cancer: results of a large, randomized, multinational, multicenter, phase III study. J Clin Oncol 18:3068–3077
25. Roth BJ, Dreicer R, Einhorn LH, et al. (1994) Significant activity of paclitaxel in advanced transitional-cell carcinoma of the urothelium: a phase II trial of the Eastern Cooperative Oncology Group. J Clin Oncol 12:2264–2270

0022-5347/05/1746-2138/0
THE JOURNAL OF UROLOGY®

Vol. 174, 2138–2143, December 2005
Printed in U.S.A.
DOI: 10.1097/01.ju.0000181214.32390.75

UROPLAKIN II AS A PROMISING MARKER FOR MOLECULAR DIAGNOSIS OF NODAL METASTASES FROM BLADDER CANCER: COMPARISON WITH CYTOKERATIN 20

XIUXIAN WU, YOSHIYUKI KAKEHI,* YU ZENG, RIKIYA TAOKA, HIROYUKI TSUNEMORI AND MASHASHI INUI

From the Department of Urology, Faculty of Medicine, Kagawa University, Kagawa, Japan

ABSTRACT

Purpose: Current methods used to determine pathological examination of the lymphatics after radical cystectomy are tedious and costly. We performed a systemic study of uroplakin II (UP II) and cytokeratin 20 (CK 20) expression in pelvic lymph nodes on multiple sides in patients with bladder cancer.

Materials and Methods: A total of 82 pelvic lymph node and 19 bladder tumor samples were obtained from 21 patients with bladder cancer by radical cystectomy with pelvic lymphadenectomy for reverse transcriptase-polymerase chain reaction assay.

Results: Of the 19 bladder tumor tissue specimens 19 (100%) and 13 (68.4%) were positive for UP II and CK 20 mRNA expression, respectively. UP II mRNA was detected in 15 of 16 pelvic lymph node samples (93.8%) with pathologically proven metastases, whereas 9 (56.6%) were positive for CK 20 mRNA. The reverse transcriptase-polymerase chain reaction assay for UP II was statistically more sensitive than that for CK 20 in detecting not only primary tumors, but also metastatic pelvic lymph nodes ($p = 0.0179$ and 0.0373, respectively). Of 66 pelvic lymph node samples without metastasis UP II was detected in 6 (10%), while CK 20 was not. In addition, UP II and CK 20 mRNA could be detected in at least 50 and 500 bladder cancer HT1197 cells, respectively.

Conclusions: These results indicate that UP II might be a more useful marker than CK 20 for detecting micrometastases of bladder cancer in the pelvic lymph nodes, although a greater number of patients and longer followup are needed to come to a definitive conclusion.

KEY WORDS: bladder, bladder neoplasms, neoplasm metastasis, uroplakin II, cytokeratin 20

The presence or absence of pelvic lymph node involvement is one of the most important prognostic factors after radical cystectomy for bladder cancer.[1,2] No less than half the patients with pathologically node negative, invasive bladder cancer could die of recurrence after radical cystectomy.[3] This indicates that there are not a few occult metastases that cannot be detected by conventional pathological examination. On the other hand, it has been shown that postoperative chemotherapy confers significant survival benefits in patients with pathologically progressive node metastases.[4] Therefore, an accurate determination of lymph node status is critically important for improving the clinical outcome after radical cystectomy. However, currently a pathologically thorough examination of excised lymphatic tissues is tedious and costly. Thus, an objective, rapid, reproducible and simple method to determine lymph node status is necessary in patients with bladder cancer after radical cystectomy.

Uroplakin II (UP II) is an urothelial differentiation related membrane protein that is expressed specifically in the urothelium and is also well preserved in transitional cell carcinoma of the urinary tract.[5,6] Recent studies using reverse-transcriptase (RT)-polymerase chain reaction (PCR) showed that UP II mRNA can be detected in bladder cancer cell lines and tumor tissues but not in prostate, skin, liver or ovary tissue specimens.[7,8] Furthermore, we and others have detected circulating cancer cells in the peripheral blood of patients with urothelial cancer using the RT-PCR assay for UP II.[7–9]

Cytokeratin 20 (CK 20) is a protein of the intermediate filament group that is selectively expressed in epithelial cells of the urinary tract, gastrointestinal tract and Merker cells.[10,11] CK 20 amplification has been extensively used to identify rare disseminated cancer cells in peripheral blood, bone marrow and lymph node samples in patients with several types of cancers, including bladder, colorectal and thyroid cancers.[12–15]

These 2 molecules may serve as biomarkers for detecting micrometastases in pelvic lymphatic nodes in bladder cancer cases. We analyzed the expression of UP II and CK 20 in pelvic lymph node and tumor tissue samples from patients with bladder cancer using RT-PCR assays.

MATERIALS AND METHODS

Patients and tissue samples. A total of 82 pelvic lymph node and 19 primary tumor tissue samples were obtained from 21 patients with bladder cancer by radical cystectomy with pelvic lymphadenectomy at Kagawa University Hospital between June 2001 and January 2005. All patients were pathologically diagnosed to have bladder transitional cell carcinoma. Table 1 lists patient clinicopathological characteristics. Primary tumors were graded and staged according to WHO criteria[16] and the TNM classification,[17] respectively. The mean number of examined lymph nodes in each patient was 4 (range 2 to 7). Of these lymph node tissue samples a total of 66 (80.5%) from 14 patients had no evidence of lymph node metastasis, whereas a total of 16 (19.5%) from 7 pa-

Submitted for publication February 9, 2005.
* Correspondence: Department of Urology, Kagawa Medical University, 1750–1 Oaza Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0793, Japan (telephone: +81–87-891–2202; FAX: +81–87-891–2203; e-mail: kakehi@med.kagawa-u.ac.jp).

TABLE 1. *Clinicopathological features in bladder cancer patients*

Pt No.—Age	Grade	TNM
1—74	3	T2
2—55	3	T2
3—49	2	T3a
4—76	2	T3b
5—76	3	T4
6—75	3	T3b
7—64	3	T2a
8—71	3	T2a
9—60	2	Tis
10—73	3	T3b
11—53	3	T2a
12—85	3	T3a
13—80	2	T3a
14—67	3	T3b
15—57	3	T3b
16—81	3	T3a
17—87	3	T3b
18—74	3	T3b
19—81	3	T2a
20—74	3	T3b
21—74	3	T3a

tients had lymphatic metastases on routine pathological examination. A hemisphere of each pelvic lymph node sample from each site was subjected to histopathological diagnosis and the other hemisphere was snap frozen in liquid nitrogen and stored at −80C until RNA extraction. Primary bladder tumor tissue samples were also prepared for RNA extraction.

Cell lines. We also analyzed the human bladder cancer cell line HT1197, the human prostate cancer cell lines LNCaP, DU145 and TSU-RP1, and the human renal cell carcinoma cell line ACHN as controls. These cells were cultured in RPMI-1640 supplemented with 25 mM HEPES, 100 U/ml penicillin, 100 μg/ml streptomycin and 10% fetal bovine serum at 37C in a humidified 5% CO_2 atmosphere. Total RNA was extracted and subjected to RT-PCR assay.

RNA extraction and RT-PCR assay. Total RNA was extracted from cell lines as well as tissue samples of primary bladder tumor and pelvic lymph nodes using TRIzol® reagent. The RNA concentration was determined using a GeneQuant™ pro RNA/DNA calculator. cDNA was synthesized using a First-Strand cDNA Synthesis kit (Amersham Pharmacia Biotech, Piscataway, New Jersey) at 37C for 60 minutes. Total RNA (1 μg) was added to the RT reaction.

Aliquots of the same cDNA were amplified with specific primers of UP II and CK 20. Primer sequences were 1) UP II sense 5′-TCCCCAGGGGCTGCAGACTT-3′ and UP II anti-sense 5′-GGTTTGTCACCTGGTATGCACT-3′, and 2) CK 20 step I sense 5′-CAGACACACGGTGAACTGG-3′ and CK 20 step I anti-sense 5′-GATCAGCTTCCACTGTTAGACG-3′, and CK 20 step II sense 5′-CTGTTTGTTGGCAATGAGAAAATGG-3′ and CK 20 step II anti-sense 5′-GATCTCTCTCAGTCTCATA-3′. UP II was amplified in single step PCR,[18] whereas Ck 20 was amplified in nested PCR,[19] as described previously. The size of amplified UP II and CK 20 products was 268 and 349 bp, respectively.

The 25 μl PCR mixture consisted of 25 pmol of each primer, 200 μM deoxynucleotide triphosphates, 0.2 U Taq™ polymerase, PCR buffer containing $MgCl_2$ and 1 μl template. PCR conditions for UP II were 35 cycles of 94C for 1 minute, 55C for 1 minute and 72C for 1.5 minutes. PCR reactions for CK 20 were done for 40 cycles for 20 seconds at 94C, 20 seconds at 55C and 30 seconds at 72C in each PCR step. Amplified products were separated on 2% agarose gel and bands were analyzed with ImageMaster™.

A sample without RNA and RNA extracted from HT1197 cells served as a negative and positive control, respectively, in every RT-PCR assay. In addition, amplified products of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers served as an internal control in all analyses.[20]

Determination of detection limit. RNA was extracted from HT1197 cells and serially attenuated with distilled water to determine the detection limit. The lowest dilution of RNA was that extracted from 50,000 cells and the highest dilution was from 0.05 cells, theoretically. RT-PCR was done using each specific primer for these serially attenuated samples.

Statistics. Comparison between UP II and CK 20 RT-PCR assays in pelvic lymph nodes was assessed by Fisher's exact test with $p < 0.05$ considered significant.

RESULTS

Sensitivity and specificity of the RT-PCR assay for UP II and CK 20. We evaluated the sensitivity of RT-PCR assay for UP II and CK 20 by testing RNA extracted from 0.05 to 50,000 HT1197 human bladder cancer cells. UP II and CK 20 mRNA expression was detected in RNA extracted from more than 50 and 500 HT1197 cells, respectively (fig. 1).

To verify the specificity of amplification products we then analyzed the prostate cancer cell lines LNCaP, DU145 and TSU-RP1, and the renal cell carcinoma cell line ACHN. Neither UP II nor CK 20 mRNA was detected in these nonbladder cancer cell lines (data not shown). We also examined 8 pelvic lymph node tissue samples from 4 patients with prostate cancer who underwent radical prostatectomy. All samples were negative for UP II and CK 20 mRNA (data not shown).

IUP II and CK 20 mRNA expression in bladder tumor and lymph node tissues. Table 2 shows the results of the UP II and CK 20 RT-PCR assay. Of the 19 bladder tumor tissue specimens analyzed 19 (100%) and 13 (68.4%) were positive for UP II and CK20 mRNA, respectively (table 3). The RT-PCR assay for UP II was more sensitive than that for CK 20 for detecting bladder tumors ($p = 0.0179$). Of 82 pelvic lymph node tissue samples 21 (25.6%) showed UP II mRNA and 11 of 82 (13.4%) were positive for CK 20 mRNA. In addition, we also examined UP II and CK20 mRNA expression in normal bladder tissue samples. In all 15 normal bladder tissue samples analyzed (100%) UP II and CK20 mRNA were positive. GAPDH expression was detected as the internal control in all samples studied.

Comparison of RT-PCR results with pathological diagnosis of lymph nodes. Tables 2 and 4 list the results of the RT-PCR

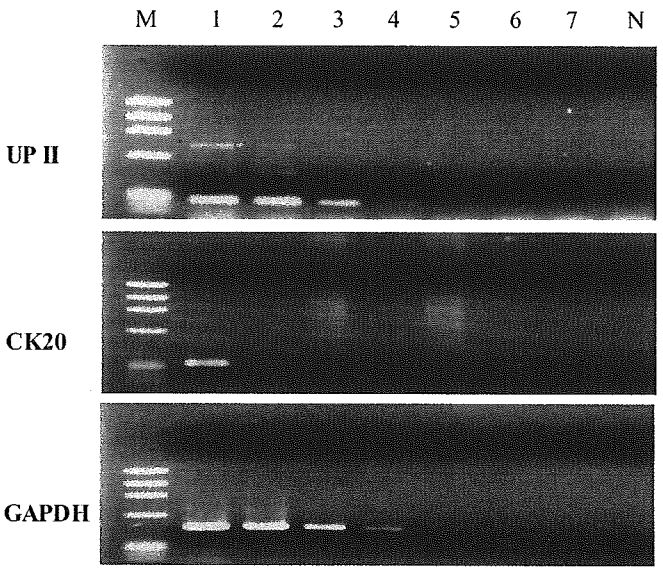

FIG. 1. UP II and CK 20 mRNA expression was detected in more than 50 and 500 cancer cells per sample, respectively, in HT1197 bladder cancer cell line under RT-PCR conditions. *Lane M*, DNA marker. *Lanes 1* to 7, 50,000 to 0.05 HT1197 cells. *Lane N*, negative control.